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Bioactive Molecules in Plant Defense

Signaling in Growth and Stress

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Preface

The sustainable production of foodstuff to nurse the increasing world population is a foremost challenge for plant scientists, especially due to the dynamic and unpredictable nature of global climatic conditions. Plants during their life cycles are generally encountered to a massive number of microbial pathogens, including bacteria, virus and fungi as well as nematodes, herbivorous and insects, in addition to numerous kinds of abiotic stressors such as drought, increasing soil salinity, and nonseasonal cold and heat waves. Plants are furnished with an array of defense mechanism to protect themselves against pathogen attack, and these defense mechanisms require a new state of cellular homeostasis be attained. Some of these plant defense mechanisms are preexisted even before pathogen attack, whereas other defense mechanisms are only inducted upon pathogen or insect invasion. Induced plant defense responses is usually a fitness cost, and thus, plants elaborate several regulatory mechanisms that efficiently organize the initiation of pathogen-specific defenses so that fitness costs are reduced, while optimal resistance is achieved. Plants are able to activate diverse types of defense mechanisms, depending on the plant–pathogen interactions. Understanding the main mechanisms by which plants tailor their responses to different pathogen and how plants manage the simultaneous interactions with multiple attackers is the key focus of plant defense-signaling research.

The present book entitled *Bioactive Molecules in Plant Defense* aims to present a detailed picture of the state-of-the-art research and latest development of plant defense mechanisms, including host plant–pathogen interactions and natural bioactive compounds involved in plant defense mechanisms, as well as new biotechnological aspects. The 12 chapters represented in this book will provide the readers with the recent advances in bioactive molecules in plant defense, including (1) bioactive molecules from *Bacillus* spp.: an effective tool for plant stress management, (2) plant growth-promoting fungi: diversity and classification, (3) microbial rhizobacteria-mediated signalling and plant growth promotion, (4) role of oomycete elicitors in plant defense signaling, (5) plant–microbe interactions: gene-to-metabolite network, (6) phytohormones in the modulation of plant cellular response to stress, (7) reactive oxygen species generation, scavenging and signaling in plant defense responses, (8) lipoxygenases and their function in plant innate mechanism, (9) alkaloid role in plant defense response to growth and stress,

(10) endogenous peptides: key modulators of plant immunity, (11) phytoanticipins: the constitutive defense compounds as potential botanical fungicides and final chapter discussing plant nutritional deficiency and its impact on crop production. This book attempted to maximize the involvement and collaboration between plant pathologist and crop research all over the world to improve the transfer of laboratory-based innovations to end-user practice for the enhancement of food sustainability.

Dharwad, India — Sudisha Jogaiah
Tottori, Japan — Mostafa Abdelrahman

Contents

About the Editors

Dr. Sudisha Jogaiah is an Assistant Professor at the Department of Studies in Biotechnology and Microbiology and is currently the coordinator of Karnatak University's "Plant Healthcare and Diagnostic Centre for Northern Karnataka". He has published more than 72 research papers in national and international journals and 11 book chapters on various aspects of the molecular communication between host and pathogen, and the development of novel strategies for ecology-based or sustainable agriculture. Dr. Jogaiah has a patent developed in the process of preparing material for seed coating for improved growth and disease immunity. He has received research grants from a number of national and state funding agencies, such as UGC, DST, JSPS, and VGST, to work on plant–microbe interactions and disease management. He has also received 15 Indian National Awards, including Young Scientist Award for Agriculture Microbiology from the Association of Microbiologist of India and two international awards, such a postdoctoral researcher award from the Japan Society for the Promotion of Science and the Japan International Award for Young Agricultural Researchers in 2012 from the Japanese Government. Recently, he was conferred with the Fellow of National Academy of Biological Sciences (FNABS). He is the Editor of Elsevier book *New and Future Developments in Microbial Biotechnology and Bioengineering*, 2019. He is a member of the editorial board of various journals, including *Scientific Reports* (Nature Publication), *BMC Plant Biology* (Biomed Central Publications) *PLOS ONE* (Plos Group), *Frontiers in Plant Science* (Frontiers Publication) and *Annals of Crop Sciences and Agriculture* (Austin Publishing group), and many more.

Mostafa Abdelrahman obtained his Ph.D. Degree from the United Graduate School of Agricultural Sciences, Tottori University, in 2015. His research focuses on understanding the transcriptomic and metabolomic variability of various cropping system in response to biotic stress, in particular, the Fusarium pathogen. From November 2015 to March 2018, he was a postdoctoral researcher at the Graduate School of Life Science, Tohoku University, Japan, where he used the RNA-Seq-based transcriptome to identify the genetics and molecular mechanisms of *Phomopsis asparagi* resistance in wild and cultivated asparagus plants. Since April 2018, Mostafa has been a JSPS Fellow at the Molecular Breeding Lab, Arid Land Research Center at Tottori University, where he is working on metabolomic and transcriptomic analyses to isolate the functional gene(s) associated with heat stress tolerance in wheat in order to understand cell responses under stress conditions and ultimately develop new crop varieties. He has published more than 31 research and review articles in peer-reviewed journals, and he is the Editor of Springer book *The Allium Genomes* published in the Compendium of Plant Genomes, 2018.

Bioactive Molecules from *Bacillus* spp.: An Effective Tool for Plant Stress Management

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S. Nakkeeran, S. Vinodkumar, P. Renukadevi, S. Rajamanickam and Sudisha Jogaiah

Abstract

The beneficial microbes are used to manage the plant diseases for sustainable agriculture production, which are alternatives to chemical fertilizers and pesticides. A key parameter in the effective management of plant disease is to protect the crop before the establishment of pathogen in an infection court. Recently, *Bacillus* species have been well demonstrated for its effectiveness against plant diseases, since it is most common bacteria found to colonize plants easily. *Bacillus* sp. inhibits the growth of phytopathogens directly or indirectly, through competition of space and nutrients. In addition, a wide variety of secondary metabolites are produced by *Bacillus* species that shows their ability as biocontrol agents against various plant diseases. Many potent *Bacillus* spp. possess secondary metabolites, including the difficidin, polyketides, and bacillaene. The *Bacillus* isolates produce several antimicrobial lipopeptides genes including fengycin, iturin, bacillomycin D, and surfactin. The resultant products of these molecules have been reported to inhibit fungal and bacterial diseases in crops. The indirect mode of action includes promoting plant growth and stimulation of the defense mechanism that trigger the first line of defense. These microbes have been known to facilitate a diverse mechanisms like quorum sensing (QS) for plant signal interference, production of volatile organic compounds (VOCs), displaying antimicrobial activity and induction of systemic resistance, thereby promoting beneficial plant–microbe interactions. Besides, the

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endospore-forming ability of the bacteria enhances their survival capability under diverse environmental conditions. Lastly, the antibiotic production induced resistance and growth promotion action mediated by *Bacillus* spp. as an effective management tool for plant disease control, since it is durable and environment-friendly alternative for chemical-based plant disease management.

Keywords
Bacillus spp. · Biomolecules · Volatile organic compounds · Iturin · Surfactin · Plant growth promotion

1.1 Introduction

Plant pathogens and plant have co-evolved over a million years. Susceptibility and resistance of the plant system against the microbes are a biological cycle, in between where the humans perish for food. Chemical agents play a vital part in the various plant disease management. The application of synthetic chemicals has been proven effective against various bacterial and fungal diseases (Jogaiah et al. 2007). Conversely, the microorganisms being the first tenants of the biological system evolve and develop resistance against them. Every year, new molecules are invented in order to replace the old chemical against which the microbes develop resistance. Benzimidazole, group of fungicides, ruled the history, with its ability to control various soilborne as well as foliar pathogens. However, in the recent decades, they have lost their efficacy, since the pathogens have modified the β-tubulin gene assembly in order to resist the effect of the chemical (Chen et al. 2009b; Wang et al. 2014). Similarly, many fungicides including organomercurials, phenylamides, morpholines, and strobilurins went ineffective due to the development of resistance (Hewitt 1998; Sudisha et al. 2005, 2010). Besides, non-selective activity of the chemical molecules affects non-targeted organisms, resulting in the loss of huge beneficial microflora. Carbendazim reduced the population of various beneficial microbes including azotobacter, azospirillum, and phosphate solubilizing bacteria (Mohiuddin and Mohammed 2013). These events symbolize the need to concoct an eco-friendly, credible, alternative approach for disease management. This spark ignited research, for biological control of plant diseases.

Biological control can be explained as the suppression of disease-producing activity or inoculum density of a pathogen in its dormant or active stage accomplished naturally or artificially by manipulating the environment or through mass introduction of potent microorganisms (Cook and Baker 1983). Diverse fungal (*Trichoderma* sp., *Chaetomium* sp., *Ampelomyces* sp., *Muscador* sp., etc.) (Jogaiah et al. 2013, 2018) and bacterial (*Bacillus* sp., *Pseudomonas* sp., *Ochrobactrum* sp., *Paenibacillus* sp. etc.) (Jogaiah et al. 2010), and actinomycetes organisms are utilized successfully as biocontrol agents (Jogaiah et al. 2016). Among them, *Bacillus* spp. play a distinctive role in the pests and diseases management and rule the kingdom of biological control.

Bacillus subtilis was first isolated in 1872 by Ferdinand Cohn which is a rod-shaped filament bacteria. *Bacillus* spp. are mainly focused on the frame of biological control due to their cosmopolitan distribution in diverse ecosystems, safety, combating ability against adverse environment (Earl et al. 2008; Nakkeeran et al. 2004, 2005; Montesinos and Bonaterra 2009). They curtail the growth of plant pathogens directly, through competition of space and nutrients, and secretion of volatile and non-volatile antimicrobial compounds. Indirect mode of action includes promoting plant growth (Kloepper 1992; Schroth and Hancock 1981; Sharma and Kaur 2010) and stimulation of the defense mechanism through MAMP molecules that trigger the first line of defense, which in turn activates lignification and synthesis of PR proteins, phytoalexins, phenolics and also triggers hypersensitive response/cell death through ROS production (Silipo and Mollinaro 2010; Vidhyasekaran 2007; Thomma et al. 2011). Amidst antibiosis have been proved momentous in the suppression of pathogen growth. *Bacillus* sp. producing antimicrobial peptides have been reported with effective inhibition properties against many phytopathogens (Romero et al. 2007; Chung et al. 2008; Joshi and McSpadden-Gardener 2006; Kloepper et al. 2004; Idris et al. 2007; Gonzalez-Sanchez et al. 2010).

1.2 Diversity of Anti Microbial Biomolecules

Bacillus sp. are utilized as effective biocontrol agents, and their potential has been proved during the last twenty years (Fiddaman and Rossall 1995; Sharga 1997; Campbell 1989). Besides, the endospore-forming ability of the bacteria enhances their survival capability under diverse environmental conditions. *Bacillus* sp. has been commercialized utilized (QUANTUM 4000) for its valuable properties which include biocontrol agent for plant pathogens and marketed as (Broadbent et al. 1971; Turner 1987). *Bacillus* sp. has been reported to produce diverse antibiotics with multifaceted mode of action that aids in the control of various plant pathogens (Kumar 1999; Asaka and shoda 1996). The antibiotic compounds are of diverse types and structures, viz. aliphatic hydrocarbons, fatty acids, phenolics, and lipopeptides (Silo-suh et al. 1994; Sathyaprabha et al. 2010; Mihailovi et al. 2011; Sadashiva et al. 2010; Musthafa et al. 2012; Mora et al. 2011).

Among the antibiotic compounds, antimicrobial peptides of short-chained amino acids display a remarkable performance in plant disease management. *Bacillus* spp. have been reported to secrete a antimicrobial compounds, namely bacillomycin, subtilin, iturins, mersacidin, surfactins, bacilysin, and fengycins

(Mora et al. 2011; Chung et al. 2008; Rajesh Kumar et al. 2014; Ramarathnam et al. 2007; Ongena and Jacques 2007; Vinodkumar and Nakkeeran 2015). Major antimicrobial peptides produced by *Bacillus* sp. can be grouped into three categories, viz., iturins, surfactins, and fengycins/plipastatins.

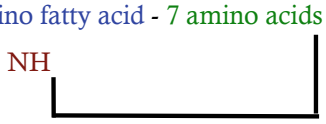
1.2.1 Iturin Family

Mycosubtilin, first antifungal lipopeptide popularly known as iturinic compound, was extracted and identified from *B. subtilis* (Walton and Woodruff 1949). Later, in 1950, another similar compound was identified and described by Delcambe (1950). The compound was identified to be produced by *Bacillus* species, isolated from the soil sample, collected from river basin of Ituri, Congo. Hence, the compound was named as iturin. The iturin family includes:

- Iturin A
- Iturin A_L
- Iturin C
- Iturin D
- Mycosubtilin
- Bacillomycin D
- Bacillomycin F
- Bacillomycin L
- Bacillomycin Lc.

All the members of the iturin family are heptapeptide molecules, composed of general structure with conserved LDDLLDL chiral sequence and variable peptide moiety. All the members of the iturin family are heptapeptide molecules, cyclized with an amide bond between α-COO group of the 7th amino acid and ß-NH_2 group of ß-amino fatty acid (ßAA). The seven amino acids are linked to each other through peptide bonds. The iturinic compounds exhibit diversity in the 1, 4, 5, 6, and 7 amino acid positions and the length of the ß-amino fatty acid (Peypoux et al. 1973, 1978, 1979, 1984; Besson et al. 1976; Winkelmann et al. 1983; Besson and Michel 1987).

General structure of iturinic compounds



Peptide moiety diversity of the amino acids in the iturin family

	1	2	3	4	5	6	7
Iturin A :	Asn	Tyr	Asn	Gln	Pro	Asn	Ser
Iturin C :	Asp	Tyr	Asn	Gln	Pro	Asn	Ser
Iturin D :	Asp	Tyr	Asp	Glu	Pro	Asp	Ser
Mycosubtilin :	Asn	Tyr	Asn	Gln	Pro	Ser	Asn
Bacillomycin D :	Asn	Tyr	Asn	Pro	Glu	Ser	Thr
Bacillomycin F :	Asn	Tyr	Asn	Pro	Glu	Asn	Ser
Bacillomycin L :	Asp	Tyr	Asn	Ser	Gln	Ser	Thr
Bacillomycin Lc :	Asn	Tyr	Asn	Ser	Glu	Ser	Thr

In addition to the diversity in the peptide moiety, the fatty acyl chain length varies among the members. Bacillomycin D, L display fatty acyl chain length of 14C and 15C, iturin A and C, whereas 16C and 17C are the length of bacillomycin F and mycosubtilin. Iturin AL was identified with a C16 of long fatty acyl chain (Winkelmann et al. 1983).

1.2.1.1 Mode of Action

Iturin A is a cyclic heptalipopeptide with strong antifungal property. The efficiency of iturin is comparable with that of other chemical pesticides (Phae et al. 1990). By their broader antibiotic spectrum and surface activity, iturin possess less toxicity, good biodegradability, and non-allergic effect on human and animals known as a environmentally safe biological pesticide (Phae et al. 1992). Moreover, the fatty acid chain length of iturins is well characterized for their antifungal activity (Bonmatin et al. 2003; Shai et al. 2006; Tabbene et al. 2011). *Bacillus* spp. producing iturin are employed as biocontrol agents against several plant diseases (Yoshida et al. 2001). Dose-dependent effect on disease suppression by iturin against damping-off of tomato by *Rhizoctonia solani* was reported by Mizumoto et al. (2007). Iturin has strong antagonistic activity against wide range of fungi yeast and retains biological activity up to 100 °C for a period of 30 min (Yu et al. 2002).

The mechanism of the antagonistic action of lipopeptides is dependent on the interaction with the cell membrane. At higher concentrations, solubilization of the membrane and pore formation is observed. Tyr residue in the peptide ring at position 2 plays significantly in the pore formation mechanism of target cells (Volpon et al. 2000). Pore formation results in the release of cytoplasmic components accompanied with cell death and other electrolytes. Besides, interaction with membranes of other sub-cellular organelles and nuclear membrane was also observed (Maget-Dana and Peypoux 1994). The application of antimicrobial peptides results in rapid enhancement of water and ion flow, osmotic dysregulation, and swelling (Juretic

et al. 1989; Ohta et al. 1992; Matsuzaki 1998). Steps involved in the formation of ion-conducting pores have been studied in detail by Brogden (2005):

- Attraction–electrostatic bonding
- Attachment interact with lipid bilayers
- Peptide insertion and membrane permeability transmembrane pores.

Antimicrobial peptides must be paying attention to the pathogen's surface. Later, electrostatic bonding is established between the surface structures, anionic, or cationic peptides of the pathogen. AMPs are hydrophilic and hydrophobic structures in nature. The molecules re-orient perpendicular to the membrane and get inserted into the lipid bilayer.

Barrel-stave model can be regarded as the model for peptide insertion for most of the pore-forming AMPs. The peptide helices form a bundle in the lipid membrane similar to a barrel poised of helical peptides as the staves.

1.2.2 Surfactin Family

The surfactin family mainly comprises 20 different lipopeptides (Bonmatin et al. 2003). In 1968, surfactin was identified as an extracellular compound, secreted by *B. subtilis* by Arima et al. (1968). Surfactin is an exceptional biosurfactant with antibacterial activity. Members of the surfactin family are cyclic lipo-hepta-depsipeptide molecules, with a general structure composed of LLDLLDL chiral sequence, and are interlinked with a fatty acid (ß-hydroxy). Amino acids belonging to aliphatic group constitute 2,4, and 7th positions of the peptide moiety (Peypoux et al. 1991; Itokawa et al. 1994; Bonmatin et al. 1995; Maget-Dana and Ptak 1995). However, several isoforms with varied peptide moiety have also been reported with 2D-NMR spectroscopy studies (Baumgart et al. 1991; Peypoux et al. 1991; Kowall et al. 1998). The heptapeptide sequence was linked by a lactone bond formed between ß-hydroxy fatty acid and –COO group of (Kakinuma et al. 1969).

General structure of surfactin compounds

Aliphatic chain of ß- amino fatty acid - 7 amino acids

Diversity of the amino acids in the peptide moiety of surfactin family

	1	2	3	4	5	6	7
Surfactin :	Glu	Val/Leu/Ile	Leu	Ala/Val/Leu/Ile	Asp	Leu	Val/Leu/Ile
Pumilacidin :	Glu	Leu	Leu	Leu	Asp	Leu	Val/Ile
Lichenysin :	Gln/Glu	Leu/Ile	Leu	Val/Ile	Asp	Leu	Val/Ile
Pumilacidin :	Glu	Leu	Leu	Leu	Asp	Leu	Val/Ile

1.2.2.1 Mode of Action of Surfactin

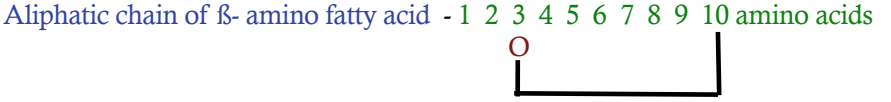
Surfactin, an lipopeptide biosurfactant, is produced by *B. subtilis* strains (Maget-Dana and Ptak 1995). Surfactin and iturin A are the popular lipopeptide compounds produced by *Bacillus* spp. The membrane-active and specific surface properties of the surfactin are helpful for the formation of biofilm. Hence, surfactin is utilized for their industrial functions for safer environment. Low concentrations of surfactin also deliver a remarkable membrane-destabilizing action and induce the pattern of ion channels in the lipid bilayers (Heerklotz and Seelig 2007). Asaka and Shoda (1996) observed that antibiotics like iturin A and surfactin, produced by *B. subtilis* RB 14, inhibit the damping-off disease in tomato. Phae et al. (1990) reported that under in vitro 23 range of phytopathogens were actively inhibited by *B. subtilis* that were capable of producing surfactin and iturin A. Due to excellent surface activities of surfactins, they are characterized as strong surfactants that can decrease the surface tension of water flow at a concentration of 10 M from 72 to 27 milli Newton/meter (mN/m) (Peypoux et al. 1999). In addition, surfactin also has antiviral and antibacterial properties (Beven and Wroblewski 1997). Surfactin displays antiviral, antimycoplasma, and antibacterial activity (Ongena and Jacques 2007). The mode of action of surfactin is almost same with the iturins; however, they are weaker molecules. Surfactin destabilizes the membrane integrity resulting in the efflux of internal contents finally leading to death (Bernheim and Avigad 1970). A series of mechanisms involved in the interactions of surfactin with membrane, such as insertion, chelation of mono- and divalent cations, and finally permeability of the membrane modification by solubilization.

Surfactin penetrates and inserts itself into the lipid membrane through hydrophobic interactions (Maget-Dana and Ptak 1995). Subsequently surfactin induce, dehydration of the polar head groups of phospholipids, this perturbs the packing of lipids and robustly compromise the stability of lipid bilayer. This structural fluctuation leads to membrane destabilization and leakage (Carrillo et al. 2003).

1.2.3 Fengycin Family

The fengycin family comprising plipastatin A, fengycin A, fengycin B, and plipastatin B is active cyclic lipo-deca-depsi-peptides produced by various *Bacillus* species (Vanittanakom et al. 1986; Williams et al. 2002; Sun et al. 2006; Romero et al. 2007; Bie et al. 2009; Pyoung et al. 2010). The peptidic moiety consists of an internal lactone ring between the hydroxyl group and carboxyl terminal amino acid (Ile) of the tyrosine residue. The C14 and C18 of β-hydroxyl fatty acid chains are tightly associated with the N-terminal amino acid (Glu) residue (Nishikiori et al. 1986; Vanittanakom et al. 1986). Fengycin A contains Ala at position 6, in fengycin B; it is replaced by Val (Vanittanakom et al. 1986; Nishikiori et al. 1986; Schneider et al. 1999). The tyrosine residues of D and L forms at 3 and 9 positions differentiates fengycins into two classes including fengycin and plipastatins.

General structure of fengycin compounds



Diversity of the amino acids in the peptide moiety of fengycin family

	1	2	3	4	5	6	7	8	9	10
Fengycin A :	^{L}Glu	^{D}Orn	^{D}Tyr	^{D}Thr	^{L}Glu	^{D}Ala	^{L}Pro	^{L}Gln	^{L}Tyr	^{L}Ile
Fengycin B :	^{L}Glu	^{D}Orn	^{D}Tyr	^{D}Thr	^{L}Glu	^{D}Val	^{L}Pro	^{L}Gln	^{L}Tyr	^{L}Ile
Plipastatin A :	^{L}Glu	^{D}Orn	^{L}Tyr	^{D}Thr	^{L}Glu	^{D}Ala	^{L}Pro	^{L}Gln	^{D}Tyr	^{L}Ile
Plipastatin B :	^{L}Glu	^{D}Orn	^{L}Tyr	^{D}Thr	^{L}Glu	^{D}Val	^{L}Pro	^{L}Gln	^{D}Tyr	^{L}Ile

1.2.3.1 Mode of Action of Fengycin

Fengycin is a lipopeptide, a biologically active compound produced by several isolates of *B. subtilis* which exhibits strong antifungal activities (Steller et al. 1999; Tao et al. 2010). Fengycin plays a key role in the protective effect afforded by the antagonist against apple gray mold and bean damping-off diseases (Ongena et al. 2005). Liang et al. (2007) reported the suppression ability of *Fusarium moniliforme*. Fengycin-producing *B. subtilis* isolates significantly inhibited the mycelium of *R. solani*, *Botrytis cinerea*, and *Fusarium oxysporum* (Ongena et al. 2005). Mixtures containing AMPs such as iturin, fengycin, and bacillomycin produced by *B. subtilis* are useful in management of powdery mildew disease of cucurbits (Romero et al. 2007). Some mutants of *Bacillus* that are not capable to produce secondary metabolites, surfactin bacillomycin, and fengycin failed to suppress the phytopathogens (Koumoutsi et al. 2004). Priming potato tuber cells with fengycins resulted in accumulation of phenolic and also displayed defense responses against the test pathogen (Dixon et al. 2000).

1.2.4 Other Antimicrobial Peptides

Apart from the above-mentioned compounds, various other lipopeptides secreted by *Bacillus* sp. are known to produce diverse antimicrobial peptides, including bacilysin, mersacidin, subtilin, and subtilosin. Studies indicate that they have potential antimicrobial activity and needed to be exploited (Chung et al. 2008; Mora et al. 2011; Vinodkumar and Nakkeeran 2015).

1.2.4.1 Zwittermicin

Zwittermicin, being an aminopolyol compound, has broad spectrum of action against wide range of pathogens. phytopathogens. *Bacillus thuringiensis* and *B. cereus* isolated from rhizosphere produce Zwittermicin A (He et al. 1994; Silo-Suh et al. 1994). Raffel et al. (1996) reported that each gram of soil consists Zwittermicin-producing organisms at a rate of 10^4 cfu. Zwittermicin exhibit

antibiotic performance against fungi and gram-positive and gram-negative bacteria (Silo-Suh et al. 1998). Zwittermicin inhibits the Oomycetes fungi *Phytophthora* and *Aphanomyces* (Silo-Suh et al. 1998). Fungal pathogens like *Alternaria alternata*, *A. panax*, *A. tagetica*, *B. cinerea*, *Colletotrichum trifolii*, *Cytospora cincta*, *Drechslera poae*, *Epicoccum nigrum*, *Fusarium graminearum*, *F. solani*, *F. sporotrichioides*, *Helminthosporium carbonum*, *H. sativum*, *Monilinia oxycocci*, *Phomopsis obscurans*, *R. solani*, *Sclerotinia sclerotiorum*, *Septoria musiva*, *Ustilago maydis*, *Venturia inaequalis*, *Verticillium albo-atrum*, and *V. dahlia* are most sensitive to ZmA (Silo-Suh et al. 1998). Similarly, plant pathogenic bacteria *Agrobacterium tumefaciens*, *Erwinia carotovora* and *E. herbicola* were also suppressed by zwittermicin under in vitro (Silo-Suh et al. 1998).

Zwittermicin is effective against various plant pathogenic bacteria and fungi in vitro (Silo-Suh et al. 1998). They are polycationic in nature and have antibacterial activity and phytotoxic at high concentrations. They disrupt cell walls and also inhibit RNA synthesis and DNA binding (Sudarshan et al. 1992).

1.2.4.2 Mersacidin

Mersacidin is a lantibiotic produced at the stationary phase by *Bacillus* sp. (Chatterjee et al. 1992). Mersacidin inhibits cell wall synthesis. Peptidoglycan biosynthesis is hindered by interaction with transglycosylation. Interaction with lipid II prevents precursors of peptidoglycan and prevents the cell wall synthesis (Brotz et al. 1995). Bierbaum et al. (1995) confirmed that biosynthesis of DNA, RNA, and proteins was not damaged, whereas D-alanine and glucose were inhibited; thereby, a reduced level of cell wall thickness was observed. The bactericidal action is exhibited by inhibition of peptidoglycan biosynthesis rather than other amps which are pore formers.

1.3 Biosynthesis of Lipopetides

The biosynthesis of major lipopeptides has been described in this section. Unlike other compounds, antimicrobial peptides are produced by non-ribosomal peptide synthesis pathway. In this chapter, biosynthesis of major antimicrobial peptides has been discussed in detail.

1.3.1 Non-ribosomal Peptide Synthesis

The possible synthesis of non-ribosomal peptide was first demonstrated by Gevers et al. (1968). He proved biosynthesis of gramicidin was not hindered in the presence of inhibitors or RNases that inhibit the mechanism of ribosome. Later, NRPS was found to be in function in most of the bacteria and fungi (Walsh 2004; Finking and Marahiel 2004; Sieber and Marahiel 2005). NORINE is a database of non-ribosomal peptides comprising 1184 peptides. The non-ribosomal protein

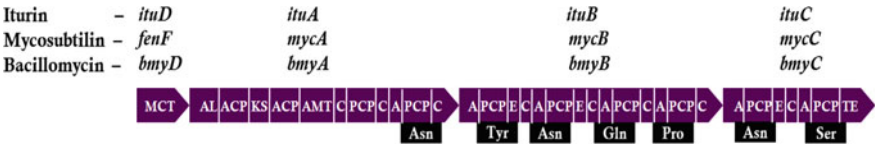
database illuminates knowledge on the source, structure, chemistry, and their bioactivity (Caboche et al. 2008).

Most of the antimicrobial peptides are synthesized through a gene cluster that comprises an operon. Each and every gene in the gene cluster contributes to the growth of the peptide chain. The biosynthesis is coordinated by various domains that catalyzes each and every step. Each and every ORF of the operon constitutes the specific domains. This differs with respect to the family of antimicrobial peptides (Sieber and Marahiel 2005; Tsuge et al. 2001).

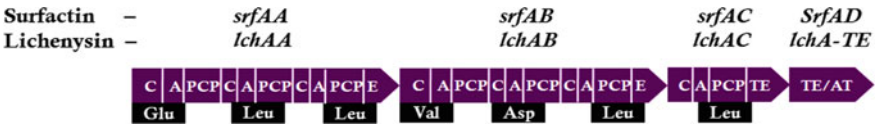
The adenylation domain activates the cognate amino acids as amino acyl adenylate which later transferred to the T-domain/peptidyl carrier protein (PCP). This domain transports the activated intermediate product. By phosphopantetheinyl transferase, the cofactor is transformed from inactive apoform of NRPS into its active holoform (Mofid et al. 2004). The condensation domain catalyses, peptide bond formation between PCPs of adjacent units to amino acyl substrate and terminated product is released by the thioesterase domain (Kopp and Marahiel 2007).

There are certain secondary domains involved in the biosynthesis of peptides. The secondary domains include oxidation, cyclization, epimerization, methylation, fatty acid chain, and glycosylation. Epimerization (E) and addition of fatty acid chain have a key function in the production of cyclic lipopeptides by *Bacillus* spp. (Du et al. 2001).

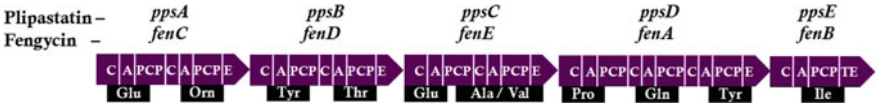
Iturin family



Surfactin family



Fengycin/plipastatin



Catalytic domains

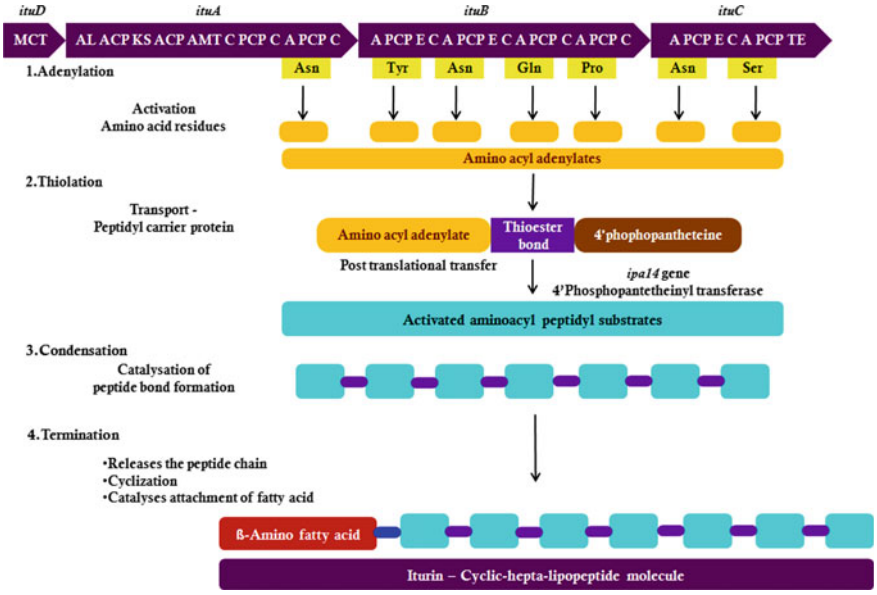
AL	Acyl-CoA ligase
A	Adenylation
C	Condensation
ACP	Acyl carrier protein
E	Epimerization
MCT	Malonyl-CoA transacylase
KS	β-keto acyl synthetase
PCP	Peptidyl carrier protein.

1.3.1.1 PKS/NRPS Complex in Iturin Biosynthesis

The iturin family operon comprises four ORFs/genes, viz. *fenF*, *mycA-C* encoding mycosubtilin compound (Duitman et al. 1999), *ituA-D* associated with iturin (Tsuge et al. 2001), and *bmyA-D* linked to bacillomycin compound (Hofemeister et al. 2004), while the remaining three genes of the iturin family are responsible for synthesis of all the seven amino acids. Thioesterase domain in the last ORF catalyzes the release of the synthesized product and is responsible for the cyclization of the lipopeptide molecule. Each and every domain synergistically aids in the biosynthesis of the lipopeptides (Jacques 2011; Roongsawang et al. 2011; Raaijmakers et al. 2010; Stein 2005; Finking and Marahiel 2004; Tsuge et al. 2001; Stein et al. 1996).

Adenylation: This domain selects the cognate amino acids of the peptide moiety and transforms them into stable amino acyl adenylate. The mode of action is similar to the ribosomal protein synthesis of the amino acylation of tRNA synthetases.
Thiolation: This is a peptidyl carrier domain that enables transport of the amino acyl adenylates. The activated amino acyl adenylates are attached to the 4′-phosphopantetheine (PPan) group by thioester bond (Lambalot et al. 1996).
Condensation: This domain forms the peptide bond between the activated aminoacyl adenylates, for the linear assembly of the heptapeptides.
Epimerization: This domain aids in the translation of L-amino acids to D-isomers.
Termination: Domains of thioesterase at the end of C terminal of the NRPS assembly terminate the synthesis and release the peptide molecules. Later, the fatty acid molecules are synthesized and attached to the peptide chains and results in cyclization of the lipopeptides.

The first ORF in the operon encodes malonyl-CoA transacylase. This along with acyl carrier protein (ACP domain), acyl-CoA ligase (AL-domain), amino transferase (AMT domain), and β-keto acyl synthetase (KS-domain) of *ituA*, *mycA*, and *bmyA* ORFs aids in the synthesis of last steps of the fatty acid chain. This type of hybrid PKS/NRPS is observed only in iturin family of lipopeptides (Aron et al. 2005; Hansen et al. 2007; Tsuge et al. 2001).



1.3.1.2 NRPS—Biosynthesis of Surfactin

Three operon codings for surfactin biosynthesis encompass three large ORFs *srfA-A*, *srfA-B,* and *srfA-C* and a short ORF *srfA-D* (Galli et al. 1994). The first three ORFs are involved in the formation of 7 amino acids. Adenylation domains of 3rd and 6th amino acid residues consist of an epimerization domain that transforms L-Leu into D-Leu. The fatty acid chain (β-hydroxylated) is attached to the first amino acid through starter condensation domain. PCP is the final domain which activates the thioesterase involved for the secretion of synthesized lipopeptide. The second thioesterase domain is encoded with the ORF, srfA-D, that initiates the biosynthesis.

The biosynthesis pathway of lichenysin is same as surfactin, except the variation in the incorporated amino acid residues (Konz et al. 1999). Replacement of amino acids in the positions 1, 2, 4, and 7 differentiates lichenysin from surfactin.

1.3.1.3 NRPS—Biosynthesis of Fengycin/Plipastatin

The synthesis of fengycin and or plipastatin by the operon consisting five ORFs *fenA-E* and or *ppsA-E* (Koumoutsi et al. 2004; Steller et al. 1999; Tosato et al. 1997). The initial three ORFs *fenC*, *fenD,* and *fenE* are responsive for the involvement of two amino acid residues each (Gln, orn—*fenC*; Tyr, Thr—*fenD*; Glu, Ala/Val—*fenE*). The fourth ORF (*fenA*) is meant for three Pro, Gln, Tyr residues, while last ORF (*fenB*) aids in incorporating the one last amino acid residue (Ile).

Similar to the biosynthesis of surfactin, the fatty acid chain (β-hydroxylated) is attached to the first amino acid residue through the condensation domain. The last ORF catalyzes the presence and release of thioesterase and the ester bond formation

between the carboxylic group Ile and the tyrosine in position 3. The epimerization is present in units 2, 4, 6, and 9 and is responsible for amino acid residues at D-form observed in the final product.

1.3.2 Antimicrobial Biomolecules Against Fungal Pathogens

Iturin produced by *B. subtilis*, was effective against several fungal pathogens. A significant decrease in seed mycoflora was noticed in the seed loads that were tested with iturin A with concentrations of 50–100 ppm. Treatment of corn seeds with iturin A @ 5 and 20 g/100 Kg showed a significant reductions in total microbial count was observed. *B. subtilis* strain RB14 that was capable of producing iturin B and surfactin that aided in suppressing damping-off disease of tomato (Asaka and Shoda 1996). In addition to disease control, *B. subtilis* strain BACT-O also promoted the plant growth and yield of cucumber (Utkhede et al. 1999). Liang et al. (2007) reported that seed priming with *Bacillus polymixa* increased the seedling height of safflower. A chitinolytic bacterial strain (YC300), isolated from a compost sample from Republic of Korea, produced an iturin-like compound. Later, this strain identified as *Paenibacillus koreensis* also provided a fair to good antifungal activities against *Colletotrichum lagenarium*, *F. oxysporum* and *S. sclerotiorum*, *B. cinerea* and *R. solani* (Chung et al. 2000). In an independent research, iturin A isolated from bacteria showed much stronger performance than surfactin against phytopathogens (Asaka and Shoda 1996). Three strains, *B. cereus* (L-07-01), *B. subtilis* (H-08-02), and *Bacillus mycoides* (S-07-01), exhibited significant antifungal activity against *F. graminearum* (Fernando et al. 2005; Ramarathnam et al. 2007). Soil application of *Bacillus sp.* was highly effective in the management of *Fusarium* wilt of carnation (Rajesh Kumar 2014). *B. subtilis*, *Bacillus amyloliquefaciens*, *B. licheniformis,* and *B. cereus* resulted in inhibiting the soil, air, and post-harvest plant diseases (Yoshida et al. 2002).

Zwittermicin A is an aminopolyol compound produced by *B. cereus* and is also known to possess good inhibitory action against pathogenic fungi including oomycetes group of pathogens (Silo-Suh et al. 1998; Fernando et al. 2005). Delivering of talc-based consortial formulation comprising of *B. subtilis* (S2BC-2) + *Burkholderia cepacia* (TEPF-Sungal) reduced vascular wilt and corm rot of gladiolus under protected cultivation. Besides, increase in cormel and corm production and flowering was promoted upon the application (Shanmugam and Kanoujia 2011).

1.3.3 Antimicrobial Biomolecules Against Bacterial Pathogens

There is a strong evidence that antimicrobial peptides, especially for antibacterial properties, are very less and depend on the environment for their activity.

Nevertheless, recent advancement in the isolation of newer compounds shows inhibitory actions against different bacteria of agricultural, environmental, and medical importance.

Surfactin like compounds isolated by *B. subtilis* (R14) and *Bacillus circulans* (Das et al. 2008; Fernandes et al. 2007) was found to suppress multidrug-resistant bacteria such as *Escherichia coli*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris,* and methicillin-resistant.

The four strains of *B. subtilis* which were effective against cucurbit powdery mildew also exhibited highest antibacterial activity against *Xanthomonas campestris* pv. *cucurbitae* and *Pectobacterium carotovorum* sub-sp. *carotovorum* (Romero et al. 2007). These strains produced lipopeptide antibiotics, viz. fengycins, surfactins, and iturins. Further, thin-layer chromatography studies and direct bioautography revealed that the antibacterial activity was correlated due to iturin compound. This result was further validated using defective mutants of lipopeptide, thereby elucidated the importance of AMPs in plant disease control.

1.3.4 Antimicrobial Biomolecules Against Mycoplasma

Mycoplasma causes several diseases in various crops all over the world. Surfactin is commercially utilized for refining of the contamination caused by mycoplasma (Boettcher et al. 2010). The mammalian cells contaminated with mycoplasmata were treated with surfactin-enhanced proliferation rates with low cytotoxicity, thus enabled their application in treating to mammalian infected by mycoplasma without any harmful effects on the metabolism of cells (Vollenbroich et al. 1997). A recent study confirmed the ability of surfactin to inactivate *Mycoplasma pneumoniae* at low concentration (MIC 25 μM) that can target cells independently. The synergistic effect of surfactin and enrofloxacin resulted in antimycoplasma properties.

1.3.5 Antimicrobial Biomolecules Against Virus

Surfactin is also positively used against many viruses, such as vesicular stomatitis virus, simian immunodeficiency virus, semliki Forest virus, herpes simplex virus 1 and 2, murine encephalomyocarditis virus, and feline calicivirus. This suggests that the antiviral properties exhibited by the application of surfactin are mainly because of the physicochemical interaction between the virus lipid membrane and the membrane-active surfactant. The number of carbon atoms in the acyl chain of surfactin is the deciding factor for the inactivation of the virus. The ability of virus inactivation increases due to the increase in the hydrophobicity of fatty acid chain (Vollenbroich et al. 1997). However, the effect of AMPs in suppression of plant viruses is not yet studied steadfast.

1.3.6 Synergistic Action of Antimicrobial Biomolecules

Antimicrobial compounds produced by microbial origin are the essential components for the success of biological control of phytopathogens. Among the microbes, *Bacillus* spp. have been widely cultivated for their potent secondary metabolites which can also biosynthesis genes responsive for antimicrobial properties (Gonzalez-Sanchez et al. 2010). The species of *Bacillus* secretes AMPs with diverse mode of action that elucidates the efficiency of biocontrol agents in disease control. The production of multiple compounds such as fengycin, bacillomycin, and iturin by *B. subtilis* has been broadly used in the management of cucurbits powdery mildew disease caused by *P. fusca* (Romero et al. 2007). Similarly, *B. subtilis* (ME488) inhibits the growth of cucumber *Fusarium* wilt and pepper *Phytophthora* blight disease through the production of bacilysin and iturin. Overall, this diverse action where one microbe can effective perform its action against two group of fungi Oomycetous and Dueteromycetous is unique in nature. Accordingly, various isolates of *Bacillus* that show positive upregulation of group of AMP genes are more effective in suppressing the growth of *Pyhthium ultimum* and *R. solani* as demonstrated by Joshi and McSpadden-Gardener (2006). In addition, the surfactin genes belonging to antimicrobial compound exhibited by *Bacillus* are useful for the attachment of pathogenic cell and later detach to the cell surfaces during the formation of biofilm through their swarming motility. Therefore, surfactin gene is essential for the sustainable performance against plant diseases (Ongena and Jacques 2007). In addition, *B. subtilis* also has the ability to colonize the host plant roots by its surfactin production and biofilm development, which resulted in reduction of susceptibility of the plant to *Pseudomonas syringae* pv. *tomato* (Bais et al. 2006).

The whole genome investigation of the *B. amyloliquefaciens* isolate (FZB 42) revealed several antimicrobial peptides possessing a broad spectrum of action against wider range of plant pathogens (Chen et al. 2009a). Such AMPs genes have also been reported in the isolates of *B. subtilis* (GB03, MBI 600 and QST713) which are commercialized available (Arguelles et al. 2009). Likewise, few AMP genes, namely *srrfAA*, *bmyB*, *bacA*, and *fenD* were found to be dominant in plant that has potentiality to boost the plant immunity toward resistance and can survive for long period environment (Mora et al. 2011).

Zwittermicin A is also popularly used as a broad spectrum of compound against different harmful microbes (Silo-suh et al. 1998). These groups of compound also exhibit diverse biological activity against Oomycetous plant diseases as well as insecticidal activity (Emmert et al. 2004). Moreover, there are several reports which demonstrated the biological activity against different groups of plant pathogens by *Bacillus* species (Kloepper et al. 2004; Correa et al. 2009; Jogaiah et al. 2010). *B. amyloliquefaciens* with 23 diverse AMP genes effectively inhibited *S. sclerotiorum* which causes stem rot of carnation. Further, it significantly enhanced the plant growth and yield (Vinodkumar et al. 2015). In another study, the synergistic action of iturin and surfactin against *Colletotrichum gloeosporioides* was performed successfully (Kim et al. 2010). The authors also demonstrated that lipopeptides

secreted by these combinations of compounds can provide significant reduction of phytopathogens in comparison with other agrochemicals available in the market (Kim et al. 2010). Similarly, the mixture of iturin and surfactin with low concentration can squeeze out the cell wall of *X. campestris* more effectively (Etchegaray et al. 2004).

1.4 Molecular Detection of Antimicrobial Biomolecule

The antimicrobial peptides genes of *Bacillus* spp. responsive for the biosynthesis of various potent antimicrobial compounds such as bacillomycin, ericin, iturin, bacilysin, mersacidin, fengycin, surfactin, subtilin, mycosubtilin, and subtilosin can be detected through molecular characterization (Vinodkumar et al. 2015). Chung et al. (2008) employed the polymerase chain reaction technique for the detection of genes that are involved in biosynthesis of 11 antibiotics produced by *B. subtilis* (ME488). They also reported that the isolate ME488 can produce potent broad antibiotics which are used as biocontrol agent to suppress cucumber and pepper pathogens. Mora et al. (2011) detected the presence of AMP genes including iturin, surfactin, bacillomycin, fengycin, bacilysin, and subtilin across various *Bacillus* sp. pertaining to various ecosystems.

1.5 Conclusion

Bacillus sp. can be exploited as versatile tool for the management of various plant diseases due to their diverse mode of action. So far, only 20% of their genome is involved in the biosynthesis of antimicrobial metabolites, emphasizing the value of these biomolecules (Gross 2007). Mostly all the antimicrobial lipopeptides are produced by different species of *Bacillus* and are especially synthesized by NRPS. In-depth analysis of the biosynthesis pathway will help to promote the pharmaceutical companies toward synthesizing artificial molecules with wide spectrum activity that aid in improving the plant health. Since microbes play a crucial role in agriculture, *Bacillus* sp. with diverse antimicrobial molecules can be explored well for the management of plant diseases.

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Plant Growth-Promoting Fungi: Diversity and Classification

2

S. Jahagirdar, D. N. Kambrekar, S. S. Navi and M. Kunta

Abstract

Crop diseases take a heavy toll on agriculture. An estimated annual loss due to various diseases ranged from 15 to 20% of the total production. Apart from the yield losses in commercial productions, the losses in storage and perishables are equally significant. Out of several management options of diseases in commercial production (row crops, vegetables, and horticultural crops), the potential use of Plant Growth-Promoting Fungi (PGPF) and its diversity is focused in this chapter. PGPF are integral part of sustainable management strategies in a holistic approach.

Keywords

Plant growth-promoting fungi (PGPF) · Disease suppression · Plant growth promotion · Biological control agent

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2.1 Introduction

The awareness about pesticides use and the adoption of improved agricultural practices has increased over the years due to advancement in agricultural science and technology. The indiscriminate use of pesticides has led to accumulation of residues on vegetables and fruits, insect pests and pathogens developing resistance and environmental hazards. Hence, use of Plant Growth-Promoting Fungi (PGPF) is gaining importance in organic agriculture.

Hossain et al. (2017) have reviewed phytostimulation and induced systemic resistance involving PGPF (Fig. 2.1). Further, they have compiled the nature and diversity of PGPF, their impact on plant growth and development. Similarly, we compiled crop-based use of PGPF, potential advantages in vegetables and challenges in row crops, preferences of farmers on liquid formulations than solid formulations, etc. (Jahagirdar et al. 2013).

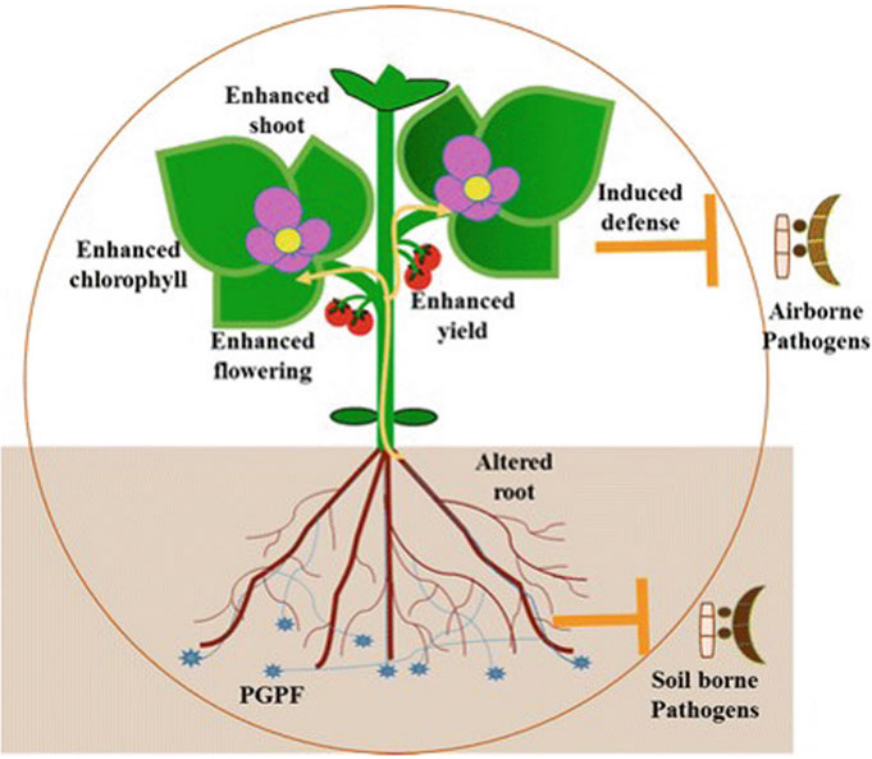

Fig. 2.1 Impact of Plant Growth-Promoting Fungi (PGPF) on plant growth promotion and disease suppression. PGPF stimulate shoot growth, root growth, photosynthetic efficiency, flowering, and yield. PGPF play a role in protection of plants against deleterious microorganisms by inducing systemic resistance. *Source* Hossain et al. (2017), reprinted with permission

PGPF are soil-borne filamentous fungi that have potential benefits on plants without causing any diseases. Species of *Trichoderma*, *Aspergillus*, *Penicillium* and some endophytes have been harnessed PGPF in agriculture. PGPF suppress plant pathogens in the rhizosphere through production of hydrolytic enzymes and plant hormones, and mineral solubilization (N, P, and Fe). Other mechanisms include mycoparasitism, competition for saprophytic colonization, and the induction of systemic resistance (Lewis and Papavizas 1991). PGPF often colonize the root system to enhance growth, development, and protection (Hyakumachi 1994). Some of significant subgroups in PGPF diversity listed in this chapter based on (a) *Trichoderma* spp. (b) Soil edaphic factors (c) Phylloplane survivability and (d) Rhizosphere competence.

The diversified traits of *Trichoderma* spp. such as plant growth promotion, nutrient utilization, rhizosphere modification, and suppression of phytopathogenic fungi by promoting defense mechanisms were previously reported (García-Garza et al. 1997; Harman et al. 2004; García et al. 2005). A wide-ranging *Trichoderma* spp. based biofungicide agents and agriculture products were listed by Verma et al. (2007) and Samuels and Hebbar (2015). Similarly, species of *Ampelomyces*, *Candida*, *Coniothyrium*, *Gliocladium*, *Talaromyces*, etc. based commercially available biocontrol products to manage fungal plant diseases were listed by Navi and Bandyopadhyay (2002).

Species of *Trichoderma* are a natural and potential boon to the farmers worldwide due to its occurrence in a wide variety of environments, easy availability, and extensive scope of activity (Ahmad and Baker 1987). Bissett (1991) described *Trichoderma* spp. as repeatedly branched conidiophores in dendroid fashion with phialides. Jash (2006) has divided the genus *Trichoderma* into four sections viz., (1) *Trichoderma* (*T. viride, T. atroviridae,* and *T. koningii*), (2) *Pachybassium* (*T. virens, T. polysporum, T. harzianum,* and *T. piluliferum*), (3) *Longibrachiatum* (*T. reesei, T. pseudokoningii, T. citrinoviridae,* and *T. longibrachiatum*), and (4) An unnamed section (*T. aureoviridae* and *Gliocladium viride*).

Kredics et al. (2014) in their book chapter have compiled biodiversity of the genus *Trichoderma* based on (i) natural soils, decaying wood and plants, (ii) agricultural habitats (iii) living plants, (iv) mushroom-related substrata, (v) human body, (vi) water-related environments, and (vii) air and settled dust. In addition, in this chapter, we tried to list the diversity of *Trichoderma* spp. (collected from various sources in the literature) based on geographical locations, soil types, and areas of rhizosphere and phyllosphere. It is necessary to understand and enhance our knowledge about the diversity of *Trichoderma* spp. to harness strains that are more effective to an area or a crop and genetically improve strains that can work in varied ecological conditions.

(i) *Trichoderma* diversity based on geographical distribution

The occurrence of *Trichoderma* spp. in different geographic regions, either have been studied or compiled by several researchers some are referred here (Turner et al. 1997; Shoresh et al. 2010; Kullnig et al. 2000; Druzhinina et al. 2005;

Kubicek et al. 2003, 2008; Zhang et al. 2005; Kredics et al. 2014). The predominance of *Trichoderma* spp. from section *longibrachiatum* worldwide confined to one or more geographic area (Kubicek et al. 2008). A comprehensive list of occurrence of species of *Trichoderma* or *Hypocrea* compiled by Kubicek et al. (2008) gives a better understanding of distribution of a particular species in one or more than one country/continent. Similarly, Kubicek et al. (2008) have compiled comprehensive list of occurrence of koningii clade of section *Trichoderma* and they have summarized data from "*Trichoderma koningii* morphological species" of Samuels et al. (2006). This list also gives a better understanding of distribution of species in koningii clade in one or more than one country/continent.

The distribution of *T. koningii* species complex frequently recorded from the soil and decaying plants. Samuels et al. (2006) reported 11 phylogenetic species using a multilocus sequence analysis. However, these studies failed to consider species diversity index in a specified geographical area in terms of its abundance and species diversity.

(ii) ***Trichoderma* diversity based on edaphic soil factors**

A little attention showed in assessing various edaphic soil factors affecting the growth of biocontrol agents (BCA) that limits the success under field conditions (Lewis and Papavizas 1991). Generally, higher population of *Trichoderma* spp. reported from soils rich in carbon, iron, bicarbonate (HCO_3), salt, and organic matter content. However, Lewis and Papavizas (1991) suggested that biological balance in soil can be achieved by altering soil organic matter, temperature, or pH. Most of the *Trichoderma* strains are mesophilic. Low temperatures during winter lead to reduced activity of the BCA. In addition, the large-scale applications of *Trichoderma*-based BCA cannot tolerate dry conditions. Therefore, there is a need of BCA that can withstand and improve efficiency either in dry soils or at low temperatures. In lab studies, Widden (1984) showed that *T. polysporum* and *T. viride* compete well for spruce litter (*Picea abies*) at lower temperatures compared to *T. koningii* and *T. hamatum* at higher temperatures.

(iii) ***Trichoderma* diversity based on Phylloplane**

Species of *Cladosporium*, *Penicillium*, *Candida*, *Cryptococcus*, and *Rhodotorula* were isolated from strawberry fruits (Jensen et al. 2013). Parikka et al. (2009) reported that species of *Mucor, Penicillium*, *Cladosporium*, *Alternaria*, *Acremonium*, *Fusarium*, *Trichoderma*, and *Botrytis* were the ample fungal epiphytes in organic strawberries. As *Trichoderma* showed diversified ecology, phyllosphere can be a habitat not as potent as soil for their survival.

(iv) ***Trichoderma* diversity based on Rhizosphere competence**

Hiltner (1904) coined the term rhizosphere. He proposed that microbial populations in the rhizosphere influence plant nutrient uptake and plant nutrition. Besides, some

root exudates produced by plants attract beneficial microbes for colonization and multiplication. The effectiveness of *Trichoderma* determined is by its biocontrol activities apart from their abilities to multiply and compete against other microflora for the efficient establishment in the rhizosphere. In Hungary, Körmöczi et al. (2013) identified 45 *Trichoderma* isolates from vegetable samples. These 45 isolates represented *T. asperellum, T. atroviride, T. citrinoviride, T. gamsii, T. hamatum, T. harzianum*, *T. koningiopsis/T. ovalisporum, T. longibrachiatum*, *T. pleuroticola,* and *T. virens.* Rao et al. (2016) reported a positive correlation between seed biopriming with *Trichoderma* spp. and root length, root-pulling strength. Isolates of International Rice Research Institute, Philippines, showed positive response to the increase in root length, in the presence of rhizosphere-competent endophytic strains of *Trichoderma.*

Rhizosphere competence is an important mechanism for the survival of BCA and their progeny with an enhanced rhizosphere competence through protoplast fusion (Harma and Sivant 1990).

2.2 The Other PGPF

Both biotic and abiotic factors influence the survivability, tolerance, competence, colonization, and expression of desirable traits in plant–soil system. Lubna et al. (2018) characterized *Aspergillus niger* CSR3 from *Cannabis sativa* which would support the production of siderophores, indole acetic acid (IAA), gibberellins, and phosphate solubilization. Similar studies were conducted by Usha and Padmavathi (2013), using species of *Aspergillus* in promoting growth and biocontrol ability on *Fusarium equiseticus* in black pepper (*Piper nigrum* L.). Mondal et al. (2000) isolated 2-carboxymethyl 3-n-hexyl maleic acid and 2-methylene-3-hexylbutanedioic acid from *A. niger* AN27 and showed that these compounds enhanced crop growth promotion activities such as germination, root and shoot lengths, vigor, and biomass in cauliflower seedlings.

Similar to species of *Aspergillus*, plant growth promotion features also observed in species of *Penicillium*. Some of the examples are plant growth promotion by the production of phytohormones and bioactive compounds, solubilization of minerals, especially, P solubility and uptake, and antagonism of phytopathogens (Radhakrishnan et al. 2013; Tiwari et al. 2007; Babu et al. 2015; Maity et al. 2014).

In this chapter, a classification or diversity of PGPF based on species of *Trichoderma*, *Aspergillus,* and *Gliocladium* provided in Table 2.1. In addition, we could not compile success stories anywhere else, but tried to provide some success stories or impacts of PGPF use on plant growth and development from an Indian perspective (Table 2.2).

Table 2.1 Classification of PGPF

Trichoderma	*Apergillus flavus*	*Asperigillus japonicus*	*Gliocladium virens*
Fungi	Fungi	Fungi	Fungi
Ascomycota	Ascomycota	Ascomycota	Ascomycota
Pezizomycotina	Pezizomycotina	Pezizomycotina	Pezizomycotina
Sordariomycetes	Eurotiomycetes	Eurotiomycetes	Sordariomycetes
Hypocreomycetidae	Eurotimycetidae	Eurotimycetidae	Hypocreomycetidae
Hypocreales	Euritiales	Euritiales	Hypocreales
Hypocreaceae	Asperigillaceae	Asperigillaceae	Hypocreace

Source http://www.mycobank.org/Biolomics.aspx

Table 2.2 Impact of PGPF on plant growth and development: an Indian perspective

Crop	Pathogen/disease	The specific effect of BCA/endophyte	Reference
Banana	*Fusarium oxysporum* f. sp. *cubense*/wilt	*Trichoderma viride, Pseudomonas fluorescens,* and *Bacillus subtilis* suppressed pathogens both in vitro and in vivo. Plant growth promotion (PGP) and disease suppression	Jahagirdar et al. (2000a)
Black pepper	*Phytophthora capsici*/ *Phytophthora* foot rot	Soil application of *T. viride* (75 g/plant) + metalaxyl spray (1.25 g/l) + Akomin (4 ml/l) or MPG 3 (10^1); PGP activity	Jahagirdar et al. (2000b)
Tomato	*F. oxysporum* f. sp. *lycopersici*/ damping off of tomato	MPG 3 as PGPR component and induced systemic resistance activity	Bhaskar (1994)
Tobacco	Tobacco mosaic virus (TMV)	Viroson @ 2% followed by *Bougainvillea* leaf extract @ 5% and neem @ 1500 ppm found effective in disease suppression. Panchaghavya @ 5% followed by cow urine @ 10% were effective in suppressing TMV	Jahagirdar et al. (2008)
Soybean	*Phakopsora pachyrhizi*/Asian soybean rust	*T. harzianum* @ 6 g/kg seed treatments or spray + cow urine spray @ 10% ± *T. harzianum* @ 0.5%. Upregulation defense genes reflected by isozyme studies and PGP activity	Jahagirdar et al. (2009, 2013)

2.3 Diversity of Endophytes

More and more evidences suggest that there is enormous biodiversity in endophytic fungi and bacteria in all plants including soybean for better plant growth and health (Impullitti and Malvick 2013; Bajaj et al. 2015; Arnold et al. 2003).

(i) **Based on geographical distribution**

Collado et al. (1999) conducted a systematic survey and studied the effect of geographic criteria on the endophytes of evergreen oak (*Quercus ilex*) in Spain. You et al. (2017) analyzed the distribution of endophytic fungi in three coastal environments of Korea.

(ii) **Based on seasonal factors**

Collado et al. (1999) reported the influence of season of sampling on the endophytes of *Q. ilex* where infection of the plants and the number of isolated fungi were higher in the spring. They have further confirmed that geographical dynamics influence the fungal communities more compared to the seasonal factors. Brunda et al. (2018) isolated 30 fungal endophytes from major soybean growing areas of two Indian states (northern Karnataka and Maharashtra).

Out of 30 endophytes, seven fungal endophytes (SFR-3, SFS-3, SFS-8, SFS-10, SFL-5, SFL-6, and SFL-13) and eight bacterial endophytes (SBR-6, SBR-1, SBS-6, SBS-9, SBS-11, SBL-1, SBL-2, and SBL-8) were characterized (Brunda et al. 2018; Brunda 2018).

(iii) **Based on edaphic soil factors**

You et al. (2017) studied the effect of various NaCl concentrations and pH values in salt-damaged environments on the growth of endophytic fungal biota. Compared to the haplophytes on the seacoasts of Korea, the halophytes native to the Dokodo Islands might absorb higher concentrations of Na^+. Fungal endophytes in the Dokodo islands might have acquired tolerance to high salts due to their symbiotic relationship with high salt-tolerant host halophytes.

(iv) **Based on host-associated factors**

Guo et al. (2008) showed that colonization and isolation rates of endophytic fungi were higher in the bark tissue followed by needle and xylem tissues in *Pinus tabulaeformis* (Pine). Out of 16,200 tissue segments of pine, 10,659 entophytic fungal isolates obtained. Similarly, Fisher and Petrini (1987) isolated 27 species of endophytes from the whole stem with higher frequency than from xylem segments alone of *Ulex europaeus* (gorse, common gorse, furze, or whin a flowering plant in the family Fabaceae).

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Microbial Rhizobacteria-Mediated Signalling and Plant Growth Promotion

3

G. Karthikeyan, L. Rajendran, M. Suganyadevi and T. Raguchander

Abstract

Bacteria are the most abundant microorganisms in soil compared to fungi and other microbes. They play a major role in maintaining soil fertility and plant growth. The rhizosphere is the region of soil that is directly influenced by root exudates of plants and associated with several soil microbes. The root exudates offer carbon-rich nutrients to the microbes, which in turn promotes plant growth indirectly and has a significant role in chemotaxis and biofilm formation. The relationship creates a symbiotic association between plants and microorganisms as a beneficial role such as atmospheric nitrogen fixation, increasing the availability of plant nutrients as well as water, root architecture modification, phytohormone production, microbial volatile production and induced systemic resistance (ISR). During the tripartite (plant-pathogen-rhizobacteria) interaction, different signalling pathways, viz. jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) are activated, which ultimately results in enhanced systemic resistance. JA regulates plant defence through intricate crosstalks with diverse signalling networks manipulated by other phytohormones such as salicylic acid (SA), ethylene (ET) and nitric oxide (NO). The role of non-secondary metabolites, volatile organic compounds and phytohormones in plant growth promotion and inducing resistance is discussed in the chapter.

Keywords

PGPR · Rhizosphere · Volatiles · Plant growth promotion · Rhizobacteria · Systemic resistance

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3.1 Introduction

Bacteria are the most abundant microorganisms in the soil and play a major role in soil fertility. They are approximately 10^{30} on earth, which contributes the biggest fraction of prokaryotic cells (Whitman et al. 1998). The stable temperature and relative humidity in soil matrices create a favourable niche for their survival (Lavelle and Spain 2001). A niche rhizosphere is a specific term coined by Hiltner (1904), found around root region (1–2 mm zone) with rich nutrients of root exudates and attracts microorganisms especially rhizobacteria, which promotes plant growth indirectly. Root exudates offer carbon-rich nutrients to the rhizosphere microorganisms. These organic acids such as citrate, malate, succinate, pyruvate, fumarate, oxalate and acetate and sugars such as glucose, xylose, fructose, maltose, sucrose, galactose and ribose constitute the main course, whereas variable amounts of α-amino acids, nucleobases and vitamins such as thiamin and biotin provide the entry or dessert (Baker and Snyder 1965; Lugtenberg and Bloemberg 2004). The establishment of microbial contact in the rhizosphere region has revealed the significant role of chemotaxis, flagellar motility, lipopolysaccharide (LPS) structure, the outer membrane protein OprF and pili (Lugtenberg and Bloemberg 2004). This relationship creates a symbiotic association between plants and microorganisms as a beneficial, neutral and also some have a harmful effect (Dobbelaere et al. 2003). In the harmful effect, phytotoxic substances as well as pathogenic activities can also be produced by microorganisms which may affect the growth of the plants. In case of beneficial role, atmospheric nitrogen fixation in leguminous plants, increase in the availability of plant nutrients as well as water, root architectures modification, phytohormonal production, microbial volatile production and induced systemic resistance have been documented. However, during the pathogen interaction, the resistance mechanism is developed, which was proved in various soil-borne and foliar pathogens. To enhance the plant yield and maintenance of immune system, plant growth-promoting rhizobacteria (PGPR) play an important role. This can be achieved by specific PGPR strains (Alstram 1991; Van peer et al. 1991; Wei et al. 1991). The PGPR interaction with plants has been commercially exploited in sustainable agriculture (Podile and Kishore 2006). The focus of this chapter is to provide an overview on rhizobacteria-mediated signalling and growth promotion in plants.

3.2 Soil Microflora and Rhizosphere

Soil matrix is relatively very stable in both temperature and humidity (Lavelle and Spain 2001), but different geographical location, soil structure, texture, particle size, composition, mineral nutrient and agricultural practices determine the microbial community. The microbes in the root zone are rich when compared to the bulk soil (Van Loon and Baker 2003), and the viability of cells strongly depends upon the water content of the soil matrices (Normander et al. 1999; Pedgley 1991). The population in the rhizosphere region (rhizocompetence) is determined by various factors which

include soil pH, water content, mineral and nutrient status of soil (Albareda et al. 2006). The PGPR are a group of saprophytic free-living bacteria that lives in the rhizosphere of plant and actively colonize the roots. These PGPR have been studied as plant growth promoters and used for increasing production in agricultural and horticultural crops and also used as biocontrol agents against plant diseases. They survive in seed or soil, multiply in the spermosphere in response to seed exudates rich in carbohydrates and amino acids (Kloepper et al. 1992) attach to root surface (Suslow 1980). PGPR, namely *Bacillus subtilis* (Ryu et al. 2003; Xie et al. 2009; Kwon et al. 2010; Zhang et al. 2009; Meldau et al. 2013), *Bacillus megaterium* (Gutiérrez-Luna et al. 2010), *Bacillus vallimortis* (Ann et al. 2013), *Bacillus amyloliquefaciens* (Hao et al. 2016; Asari et al. 2016), *Arthrobacter agilis* (Velázquez-Becerra et al. 2011; Castulo-Rubio et al. 2015), *Paenibacillus polymyxa* (Lee et al. 2012), *Burkholderia ambifaria* (Groenhagen et al. 2013), *Proteus vulgaris* (Bhattacharyya et al. 2018), *Pseudomonas fluorescens* (Park et al. 2015) and *Pseudomonas simiae* (Vaishnav et al. 2015), are involved in plant growth promotion (increase in root and shoot biomass as well as chlorophyll content). A root glycoprotein complex called agglutinin is involved in the short term adherence of pseudomonads (Glandorf et al. 1994). Intensive research work on the establishment of microbial contact with rhizosphere region has revealed the significant role of chemotaxis, flagellar motility, lipopolysaccharide (LPS) structure, the outer membrane protein OprF and pili in successful colonization (Lugtenberg and Bloemberg 2004).

3.3 Non-secondary Metabolites

The production of non-secondary metabolites has been linked to biocontrol (Maurhofer et al. 1994; Thomashow and Weller 1998; Paulsen et al. 2005). The non-secondary antifungal metabolites have been described in *Pseudomonas*. Nielsen and Sørensen (1999) identified a cell surface molecule with biosurfactant property and antifungal activity. Biochemical analysis of the compound showed it to be a newly described bacterial cyclic lipopeptide designated viscosinamide, which has subsequently been implicated in the control of *Pythium ultimum* (Thrane et al. 1999). HPLC analysis of antibiotics showed that small but significant amount of lipopeptide viscosinamide present in the rhizosphere soil inoculated with PGPR strains (Thomashow et al. 1997; Haas and Keel 2003). Paulsen et al. (2005) reported novel antibiotic compounds, namely cyclic lipopeptide or glutamic acid, polyketide and non-ribosomal peptides from the *P. fluorescens* strain Pf5.

3.4 Volatiles in Signalling

Microorganisms produce a wide range of volatile compounds to induce growth in plants directly or indirectly (Dotaniya and Meena 2015) upon the application of biological control agents (BCAs). Their secondary metabolites are important in

plant disease management. Ryu et al. (2003) first reported that the volatile organic compounds (VOCs) released by *B. subtilis* GB03 can regulate growth, nutrition and stress in *Arabidopsis thaliana*. These compounds act as a signal molecule having properties of low molecular weight, low boiling point and high vapour pressure and lipophilic nature. It has also been found that certain plants can release stress signals during pest attack, and these can cause defence responses in intact plants (Turlings et al. 1990). The induction of jasmonic acid enhanced the predation rates by triggering the release of airborne volatiles that attract the natural enemies of insect herbivores (Thaler et al. 2001). It can able to change physiological processes and carried through the water, air and soil (Kanchiswamy et al. 2015). Volatiles from attacked plants, microbes and herbivores can enhance plant defences. Depending on the living environment, through different metabolic pathways, VOCs are released from a different group of alcohols, alkanes, esters, alkenes, terpenoids, sulphur families and ketones (Audrain et al. 2015; Korpi et al. 2009; Schulz and Dickschat 2007). Identification of bioactive microbial compounds was done by gas chromatography coupled with mass spectrometry (Korpi et al. 2009). Volatile compounds involve four principles like hormonal balances, sugar concentrations, metabolism and inflection of essential nutrients in seedlings of the plant during cellular and physiological effects (Zhang et al. 2007). Iron element is a demanding essential micronutrient for the photosynthesis (Kim and Guerinot 2007; Waldvogel-Abramowski et al. 2014). Exposure of seedlings to dimethyl hexadecylamine (VOC of rhizobacteria) leads to uptake of iron which provides increased chlorophyll content and also photosynthetic activity as reported in *A. thaliana* by *B. subtilis GB03* (Zhang et al. 2009) and *Medicago truncatula* by *A. agilis* UMCV2 (del Carmen Orozco-Mosqueda et al. 2013) (Fig. 3.1). Additionally, VOCs of *B. amyloliquefaciens* strain BF06 activate gene at molecular level encoding for sulphate transportation and increase in Se accumulation (Wang et al. 2017). Six isolates of *B. subtilis* (B1, B6, B28, B40, B99 and B108) were evaluated against *Fusarium oxysporum* f. sp. *ciceris* of chickpea under in vitro and in vivo. Some isolates showed greater antifungal activity and were found to produce protease, siderophore, indole acetic acid, antifungal volatiles and other extracellular compounds (Karimi et al. 2012) (Table 3.1). It has been provided that volatiles can modulate different hormonal pathways, including jasmonate (JA), salicylic acid (SA), ethylene (ET) and auxin (IAA) signalling. Many of these pathways interact with each other through crosstalk mechanisms.

3.5 Jasmonates (JA)-Mediated Signals

Plants employ JA-mediated defence to defend against various microbial pathogens, and its synthesis was rapidly triggered both locally at the injured site and systemically in undamaged leaves throughout the plants upon pest chewing and wounding by the herbivory (Yan and Xie 2015). Exogenous methyl jasmonate (MeJA) application enhanced the host resistance to parasitic root-knot nematodes

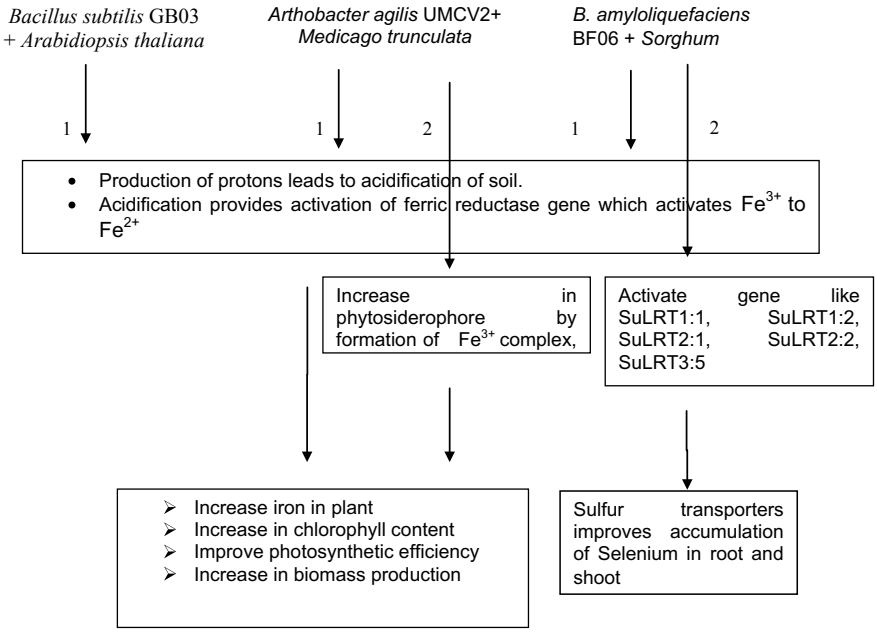


Fig. 3.1 Outline of action 1 and 2 involved in growth promotion of host. FRO1 = Ferric reductase, IRT1 = Iron-regulated transporter 1 and SULRT = Sulphur transporter

(RKNs) in tomato (Cooper et al. 2005). Some JAs can be released as VOCs to permit communication between plants in anticipation of mutual dangers. In addition, JA crosstalk occurs with salicylic acid (SA) which mediates systemic acquired resistance (SAR). JA, salicylic acid (SA) and ethylene signal pathways are integrated into the regulation of stress response and plant development.

3.6 Salicylic Acid (SA)-Mediated Signals

Salicylic acid (SA) is a hormone, mediates defence against pathogens by inducing pathogenesis-related genes and SAR mechanism. It is an essential signal elicitor for the induction of induced systemic resistance (ISR) and the orchestration of the events that occur during the hypersensitive reaction (HR). Shanmugam and Narayanasamy (2008) reported the production of SA in *Bacillus licheniformis* MML2501 from the experiments conducted under in vitro and in vivo. Under optimal pH, temperature, concentration of substrate and shaken conditions, *B. licheniformis* MML2501 showed maximum production of 18 µg/mL of SA, which is an important component in the induction of plant-mediated defence enzymes. Zhang et al. (2002) reported that plants treated with *Bacillus pumilus* strain Se34 had greatly increased levels of SA, compared with that of non-treated

Table 3.1 List of volatile organic compounds (VOCs) of PGPR and its function in the crop

Host	PGPR stains	Volatile compounds	Function(s)	References
Arabidopsis thaliana	*Bacillus subtilis* GB03	2,3-Butanediol	Surface leaf area	Ryu et al. (2003)
	B. megaterium XTBG-34	2-Pentylfuran	Fresh weight	Zou et al. (2010)
	B. pyrrocinia Bcc171	Indole 1-Hexanol pentadecanol	Fresh weight	Blom et al. (2011)
	Bacillus sp. B55	Dimethyl sulphide	Lateral root numbers	Meldau et al. (2013)
	B. ambifaria	Dimethyl disulphide acetophone	Biomass	Groenhagen et al. (2013)
Medicago sativa	*Arthrobacter agilis* UMCV2	Dimethyl hexadecylamine	Fresh weight, stem length, root length, lateral root density	Velazquez-Beceria et al. (2011)
Tobacco	*B. vallismortis* EXT-1	3-Hydroxy-2-butane	Fresh weight	Ann et al. (2013)
	Pseudomonas fluorescens SS101	13-Tetradecadien-1-ol, 2-Methyl-n-1 trideione, 2-Butanone	Fresh weight	Part et al. (2015)
Turmeric	*P. fluorescens*	Induced defence enzymes	Plant height, stem girth and number of leaves	Prabhukarthikeyan et al. (2018)
Tomato	*P. fluorescens* (Pf1)	Induced defence enzymes	–	Manikandan and Raguchander (2014)
Gerbera	*Bacillus subtilis* strain Bbv 57	Butanedioic acid, hexadecanoic acid, pentanedioic acid 2-oxo-dimethyl ester	Increase in number of flowers	Ramyabharathi et al. (2018)

plants or plants treated with two Gram-negative bacteria. *B. subtilis* produces a catecholate, trilactone, siderophore, bacillibactin (BB), under conditions of iron limitation (May et al. 2001).

3.7 Ethylene (ET) in Signalling

It is a simple two-carbon atom molecule, a gaseous plant hormone. Its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), can be metabolized using ACC deaminase by the bacteria, favouring plant growth and lowering the stress susceptibility (Van de Poel and Van Der Straeten 2014). ACC has been reported to function as a signal itself independently from ethylene. Some PGPRs are capable of converting plant-borne ACC into ammonia and α-ketobutyrate by ACC deaminase enzyme reported in *Pseudomonas chlororaphis* 6G5 (Klee et al. 1991),

Pseudomonas putida GR12-2 (Jacobson et al. 1994) and *P. putida* UW4 (Hontzeas et al. 2004). Saravanakumar and Samiyappan (2007) reported that the PGPR *P. fluorescens* strain TDK1 possessing ACC deaminase activity enhanced the saline resistance in groundnut plants, which in turn resulted in increased yield when compared with the groundnuts treated with *Pseudomonas* strains not having ACC deaminase activity. The bacterial ACC deaminase can reduce the endogenous ethylene levels of plant roots by limiting the amount of available ACC, which will in turn prevent ethylene-induced root growth inhibition, and thus promote plant growth (Glick 2014). Xu et al. (2014) reported that *B. subtilis* (HYT-12-1) exhibited multiple plant growth-promoting (PGP) traits, namely 37% of indole-3-acetic acid production; 37% of phosphate solubilization; 24% of siderophores production; 85% of potential nitrogen fixation; and 6% of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity.

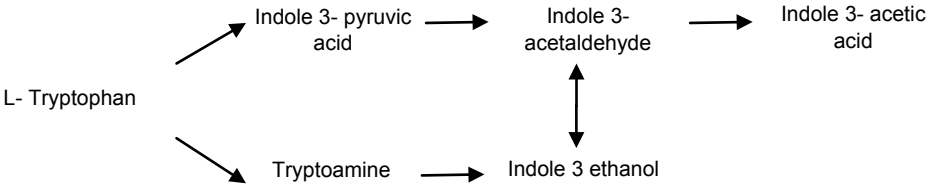
3.8 Auxin (IAA)

The auxin word has been derived from Greek means auxein, regulate a plant developmental process and best documented in PGPR, *Azospirillum* spp (Persello-Cartieaux et al. 2003; Spaepen et al. 2007), which regulates plant root development. This is chemically called as indole acetic acid (IAA) and act as signalling in defence response in the *Arabidopsis* against a foliar pathogen (Navarro et al. 2006). Almost, 80% of bacteria isolated from rhizosphere region can able to produce IAA (Patten and Glick 1996; Khalid et al. 2004) by the main precursor L-tryptophan, which is secreted in root region. The hormone acts as a signal molecule in bacteria and effector molecule in plants. Bacteria synthesize auxins in order to perturb host physiological processes for their own benefit (Yung 2010). PGPR possess different pathways for synthesis of (a) by indole-3-pyruvic acid in the case of *Rhizobium, Bradyrhizobium* and *Azospirillum* (Burdman et al. 2000; Costacurta and Vanderleyden 1995; Manulis et al. 1991; Patten and Glick 1996) (b) via tryptamine pathway by *B. subtilis*, *B. megaterium*, *B. licheniformis*, etc. Damodaran et al. (2013) reported that two stress-tolerant rhizobacteria, *B. pumilus* B-1 and *B. subtilis* B-3, had extensive zone formation for indole-3-acetic acid (>1 cm) and siderophore production with higher zone ranging from 0.6 to 0.9 cm. Mohite (2013) reported that the production of IAA was maximum in the tryptophan-amended medium. Indole-3-acetic acid stimulates cell elongation by modifying certain conditions like increase in osmotic contents of the cell, increase in permeability of water into the cell, decrease in wall pressure and an increase in cell wall synthesis and protein synthesis. It inhibits or delays abscission of leaves and induces flowering and fruiting (Zhao 2010). The microorganisms isolated from rhizosphere region of various crops have an ability to produce IAA as a secondary metabolite due to the rich supply of substrates (Table 3.2). IAA helps in the

Table 3.2 Illustrations of plant auxin signalling upon colonization by PGPRs

PGPR strain	Host plant	Phytohormone	Mechanism	References
Azospirillum	Wheat	Auxin-IAA	Increased rooting	Dobbelare et al. (1999)
Pseudomonas putida GR_{12-2}	Canola	Auxin-IAA	Root elongation	Xie et al. (1996)
Rhizobium	Legume-Pea	Auxin-IAA	Root nodule formation	Badenochjoner et al. (1983), Theunis et al. (2004)
Sphingomonas sp. LK11	Tomato	Gibberellin	Shoot elongation	Spaepen and Vanderleyden (2011), Khan et al. (2014)
Rhizobium phaseoli	*Graminaea*	GA1, GA3	Plant growth yield	Bastial et al. (1998)
B. pumilis, *B. licheniformis*	Alnus glutinosa	GA, GA3, GA4, GA20	Growth promotion	Gutierrez-Monero et al. (2001)
Acinetobacter calcoaceticus	Cucumber	GA	Higher shoot length, plant biomass, chlorophyll	Kang et al. (2012)

production of longer roots with an increased number of root hairs and root laterals which are involved in nutrient uptake (Datta and Basu 2000).



According to Dobbelaere et al. (1999), *Azospirillum* alters IAA production, which leads to increased rooting by the enhancement of root exudation and plant mineral, which in turn stimulates bacterial colonization (Steenhoudt and Vanderleyden 2000; Lambrecht et al. 2000). Similarly, *P. putida* GR12-2 stimulates root elongation was shown to the production of IAA (Xie et al. 1996). Root morphology was studied after the application of *Azospirillum,* and it mimicked by IAA (Morgenstern and Okon 1987) or mixtures of GA3, auxin and kinetin (Tien et al. 1979; Hubbell et al. 1979).

3.9 Crosstalks

The antagonistic effect of SA on JA signalling was shown to be controlled by a novel function of the defence regulatory protein NPR1 in the cytosol (Spoel et al. 2003). Based on the nature and cause of pathogen, the plant can decide which kind

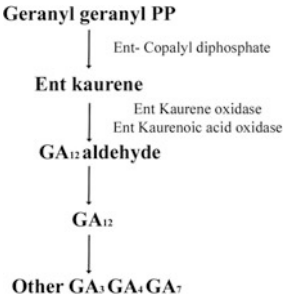
of strategy can be used for crosstalk between defence signalling pathways. De Vos et al. (2005) established the dynamics of SA, JA and ET signalling in a single plant species of *A. thaliana* in response to an attack by a range of microbial pathogens and herbivorous insects with very different modes of action. A complex set of transcriptional alterations are induced in all cases showing stress-related genes, and they are overlapped in response to a different mode of attack by pathogens and insects.

3.10 Phytohormones in Signalling

Plant hormones are usually found in small amount, can be natural or synthetic and defined as an organic substance synthesized in different organs could be translocated to other sites, where it alters morphological, physiological and biochemical processes. These are signal molecules, and chemical messenger in plants promotes its growth and development and regulates intrinsic genetic expression. They are generally classified as auxin, gibberellin, cytokinin, abscisic acid and ethylene. There was a positive correlation between phytohormones produced by PGPR and uptake of soil water and minerals.

3.11 Gibberellin (GAs)

Gibberellins are a major class of important diterpenoid acids (Martin et al. 2000) and are endogenous hormones play a vital role in developmental processes, viz. germination, elongation of stem, dormancy, sex expression and fruit senescence (Eleazar et al. 2000; Gelmi and perez-Correa 2000). Biosynthetic pathway of gibberellin involves precursor geranylgeranyl PP via copalyl diphosphates produce the kaurene and this will be converted into gibberellic acid 12 through a intermediate compound called GA12 aldehyde. Until now, four groups of gibberellic acid like GA1, GA3, GA4 and GA20 were identified from seven bacterial species (Hedden and Thomas 2012). When *Sphingomonas* sp. LK11 strain was inoculated in tomato plants, showed significant results in shoot elongation due to the production of gibberellin (Spaepen and Vanderleyden 2011; Khan et al. 2014). In red pepper, newly identified PGPR were evaluated for growth promotion but also increased endogenous gibberellin level (Joo et al. 2004, 2005). The gibberellin produced by *B. subtilis* strain HC8 (150 ng per 10^9 cells) significantly promoted plant growth and protected tomato against tomato foot and root rot. Gibberellin (approximately 200 ng per 10^9 cells) has been reported from *B. licheniformis* and *B. pumilus* by Manero et al. (2001).



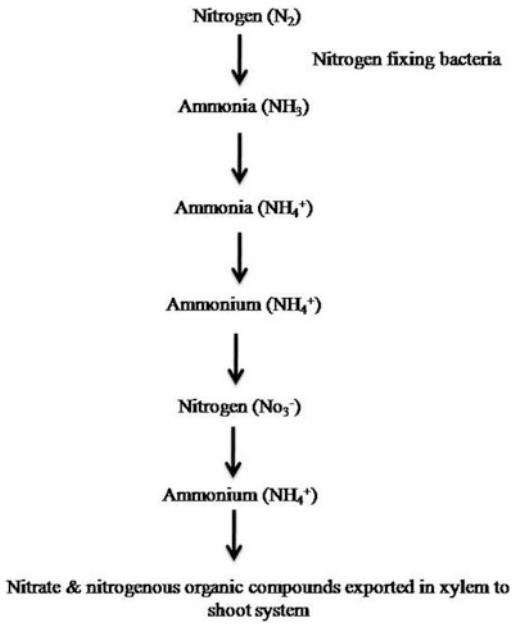
3.12 Cytokinin

In plant cells, cytokinesis process is regulated by one of the PGRs called cytokinin (Skoog et al. 1965). Naturally occurring kinetin (6-furfuryladenine) like compound was isolated from sunflower, maize and soybean (Miller 1961). Pure crystalline form of cytokinin was first isolated from *Zea mays* called as zeatin (Z) by Letham (1967). Plant growth has been directly promoted by PGPR, viz. *Azotobacter*, *Azospirillum* and *Rhizobium* through altering physiological process and production of metabolites (Arshad and Frakenburger 1993). PGPR like *Serratia* (Zhang et al. 1997), *Pseudomonas* (Arshad and Frankenberger 1993; Kloepper 1993) and *Bacillus* (Turner and Blackman 1991; Mariano et al. 1997) play a vital role in the promotion of plant growth. Growth promotion by *Pseudomonas* sp. was first reported by Lifshitz et al. (1987). The most studied PGPR, *Azospirillum,* was isolated from forage, grain and native crops as well as cultivated crops (Magalhães et al. 1983; Dobereiner and Pedrosa 1987; Reinhold et al. 1987; Khammas et al. 1989). When hydroponics media were amended with auxin and cytokinin, depicts morphology of roots were similar to the plants inoculated with *Rhizobium* (Skoog et al. 1965; Puppo and Rigaud 1978). At the root surface, the release of auxin and cytokinin can stimulate cell division in the cortex (Sequeira 1973). Some cytokinins production were observed in PGPR strains, including *Pseudomonas,* and *Serratia (*Kloepper et al. 1988) were screened for PGR production and a relationship between induction of root elongation and production (Young et al. 1990).

3.13 Atmospheric Nitrogen Fixation

Nitrogen is the most important gas present in the atmosphere even though it is available around 78%, cannot be utilized directly by plants. Using a complex enzyme system, known as nitrogenase (Kims and Rees 1994), atmospheric nitrogen is converted into ammonia by nitrogen-fixing microorganisms. Biological nitrogen-fixing microorganisms were widely distributed in the atmosphere and survives at mild temperature (Raymond et al. 2004), which serves as an alternative

to chemical fertilizer. These organisms are classified into a symbiosis and non-symbiosis group. Symbiosis group includes *Rhizobia* (Ahemad and Khan 2012; Zahran 2001) and *Frankia,* while non-symbiosis bacteria include *Cyanobacteria* (Anabaena, Nostoc), *Gluconacetobacter, Azospirillum* and *Azotobacter* (Bhattacharyya and Jha 2012).



Root exudates of *Alnus glutinosa* (black alder) contain flavonol (quercetin and kaempferol), which enhance the level of nodulation (Hughes et al. 1999). Curling of root hair is the primary event of the symbiotic process, when *Frankia* exposed to *A. glutinosa* root filtrate (Prin and Rougier 1987; Van Ghelue et al. 1997).

3.14 Rhizobacteria-Mediated Growth Promotion

The plant roots are highly influenced the presence of microbial diversity (Bais et al. 2006) that is the availability of nutrient compounds such as simple sugars, organic acids and amino acids in different stages of crop growth. Utilization of these substrates and compounds leads to increased microbial biomass, and activity around the root region is termed as rhizosphere effect (Hartmann et al. 2008). Moreover, the release of specific nutrients from the root zone, which created a fondness for specific bacterial strains, prefers selective colonization (Bowen 1991; Flores et al. 1999; Whipps 2001; Lugtenburg et al. 2002). The root-colonizing bacteria are termed as rhizobacteria, which are confined to root surface, and some of them enter

into host maintain symbiotic relationship called as endophytes (Sturz et al. 2000). According to Kloepper et al. (1980), artificial application of beneficial microbes which promote plant growth is called as plant growth-promoting rhizobacteria (PGPR). Physiological modifications as well as a revolution in the microbial communities in the root region create plant growth promotion (directly or indirectly) mechanisms in the host plant (Glick 1995; Glick et al. 1998). These rhizobacteria utilize root exudate which is a mixture of primary and secondary metabolites, cation, mucilaginous substances, enzymes, oxygen and water. In addition, 20–40% of the carbon produced from photosynthesis is utilized by underground root system (Philippot et al. 2013; Venturi and Keel 2016). Many PGPR strains have the ability to induce systemic resistance, produce volatile organic compounds, phytohormones, flavonoids as signals, fixation of atmospheric nitrogen, solubilization of available minerals and biofilm formation. Further, the antimicrobial compounds have other effects at sub-inhibitory concentrations, viz. (a) a role in intercellular signalling and (b) motility and biofilm formation (Davis et al. 2006). Voluminous intelligence between PGPR and plants is recognized by signal molecules of extreme different genera of which *Bacillus* and *Pseudomonas* spp. are predominant.

3.15 Mineral Solubilization

3.15.1 Phosphate-solubilizing Microorganism (PSM)

Minerals are naturally occurring inorganic chemical compound as a solid material and phosphorous play a vital role in the plant growth among 17 nutrients reported. Further, it plays a critical role in photosynthesis, energy transfer, transformation of sugars and starches and transformation of genetic material from one generation to other generation. Acquisition of plant nutrient was enhanced by soil microorganisms. Insoluble forms of phosphatic fertilizer like tricalcium phosphate $(Ca_3PO_4)^2$, aluminium phosphate (Al_3PO_4) and iron phosphate (Fe_3PO_4) were converted into available by microorganisms (Gupta et al. 2007; Song et al. 2008; Khan et al. 2013; Sharma et al. 2013). Wide range of biological process involved in transformation of insoluble nutrients into soluble nutrients (Babalola and Glick 2012). Two types of phosphate utilization were observed like direct application of phosphate fertilizer and microbial solubilization. In the soil, artificial application of phosphatic fertilizers leads to little amount of absorption by plant, and the remaining will be converted into insoluble complexes. These will be solubilized by microorganisms in higher-level conversion (Mckenzie and Roberts 1990) mediated by the enzymes released by the soil microbes called phosphatases (Yadav and Tarafdar 2003; Tarafdar et al. 1988; Aseri et al. 2009) and phytases (Maougal et al. 2014).

Phosphate-solubilizing activity was coupled with organisms which are termed as phosphate-solubilizing microorganism, which provides available forms of phosphorous to the plants (Khan et al. 2006). These bacteria are belonging to the genera,

Azotobacter, *Bacillus*, *Microbacterium*, *Erwinia*, *Pseudomonas*, *Rhizobium*, *Burkholderia*, *Flavobacterium*, *Beijernicka*, *Microbacterium* and *Serratia* (Bhattacharyya and Jha 2012). Gram-negative bacteria like *P. fluorescens, Chromobacterium violaceum* and *Pseudomonas aeruginosa* are phosphate-solubilizing bacteria, also secrete antibiotics (Lipping et al. 2008; Taurian et al. 2010) and act against soil-borne pathogens (Khan et al. 2002; Singh et al. 2010; Vassilev et al. 2006). Few genera of *Rhizobium* and *Bradyrhizobium* have also found to solubilize P and also secrete IAA (Badawi et al. 2011; Pandey and Maheshwari 2007). Glick et al. (2007) studied numerous phosphate-solubilizing bacteria which are having the ability to synthesize 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an immediate precursor for the plant hormone ethylene.

3.15.2 Potassium-solubilizing Bacteria (KSB)

Potassium (K) is considered as a major constituent and essential element in all living cells. Naturally, soils contain K in larger amounts than any other nutrients; however, most of the K is unavailable for plant uptake. Depending on soil type, 90–98% of potassium in the soil is in the unavailable form (Sparks and Huang 1985). This can be converted to soluble forms by potassium-solubilizing bacteria for the plant uptake (Etesami et al. 2017) and mostly belong to the genera *Bacillus spp.* having the capacity to solubilize K minerals like feldspar, muscovite, biotite, orthoclase, illite and mica. This can be possible by various processes in conversion of silicate minerals through the process like acidolysis, complexolysis, chelation and exchange of reaction.

Upon artificial inoculation of phosphate-solubilizing bacteria may lead to improve plant growth by increasing seed emergence, plant weight and yield. These include *Pseudomonas* spp., *P. chlororaphis*, *P. aureofaciens*, *P. solanacearum*, *P. syringae*, *P. fluorescens*, *Bacillus* spp., *B. pumilus*, *B. mucilaginous*, *B. amyloliquefaciens*, *B. fimus*, *B. megaterium*, *B. subtilis*, *B. licheniformis*, *Burkholderia cepacia*, *Delfitia acidovorans*, *Paenibacillus macerans*, *Pantoea agglomerans*, *A. lipoferum*, *Agrobacterium radiobacter*, *Azospirillum brasilense*, *Serratia entomophilia*, *Azotobacter chroococcum*, *Streptomyces* spp., *S. lydicus* and *S. griseoviridis* (Glick 2012).

Major mechanism involved in conversion of insoluble form into soluble form through formation of organic acids via production of protons (acidolysis mechanism) (Maurya et al. 2014; Uroz et al. 2009; Parmar and Sindhu 2013; Sheng et al. 2003; Meena et al. 2015b; Sheng et al. 2008; Meena et al. 2014). Various organic acids such as tartaric acids, oxalic acid, 2-ketogluconic acid, gluconic acid, succinic acid, citric acid, lactic acid, propionic acid, malic acid, malonic acid, fumaric acid, glycolic acid have been reported in KSB, which are effective in releasing K from K-bearing minerals (Hu et al. 2006; Krishnamurthy 1989; Liu et al. 2012; Prajapati et al. 2013; Keshavarz Zarjani et al. 2013; Saiyad et al. 2015; Prajapati et al. 2012; Sheng and He 2006).

According to Huang et al. (2013), the cation exchange complex was occurring in exchange of hydrogen ions by K^+, Mg^{2+}, Ca^{2+} and Mn^{2+}. In addition to decreasing soil pH, KSB produce organic acid can release K ions from mineral ore by formation of complex structure with Ca^{2+}, Al^{3+}, Si^{4+} and Fe^{2+} with K. Microbial decomposition of organic materials also produces ammonia and hydrogen sulphide that can be oxidized in the soil to form the strong acids such as nitric acid (HNO_3) and sulphuric acid (H_2SO_4). Hydrogen ions displace K^+, Mg^{2+}, Ca^{2+} and Mn^{2+} from the cation exchange complex in a soil (Huang et al. 2013). In addition to decreasing soil pH, organic acids produced by KSB can release K ions from the mineral K by chelating (complex formation) Si^{4+}, Al^{3+}, Fe^{2+} and Ca^{2+} ions associated with K minerals (Römheld and Kirkby 2010; Štyriaková et al. 2003; Meena et al. 2014). When seeds and seedlings were inoculated with KSB, enhancement of germination, increase in K uptake under field condition as well as greenhouse condition have been reported (Zhang and Kong 2014; Anjanadevi et al. 2016; Awasthi et al. 2011; Meena et al. 2014; Subhashini and Kumar 2014; Zhang et al. 2013; Meena et al. 2015a; Lynn et al. 2013). Parmar (2010) observed that inoculation of K-solubilizing isolate HWP47 in wheat cause increase in shoot dry weight as well as root dry weight.

3.16 Siderophores

Siderophore-mediated competition for iron by *Pseudomonas* sp. as well as induced resistance is primary mechanisms shown to be responsible for the suppression of *Fusarium* wilt (Lynch 1990). Vijendra Kumar and Ashok Kumar (2012) reported the production of siderophore in *B. subtilis* WR-W2 and *B. amyloliquefaciens* MR-AI strains under in vitro. Synthesis of siderophore was determined in the presence of iron-limited M9 medium. Siderophore production was inhibited above 20 μM concentration of Fe (III). *P. aeruginosa* strain FP7 was tested for its in vitro antagonistic activity against *Rhizoctonia solani* on King's B media, with and without $FeCl_3$, showed a significant reduction in *R. solani* growth with $FeCl_3$ supplementation compared to the control (without $FeCl_3$) (Sasirekha and Shivakumar 2016). A marine isolate of fluorescent *Pseudomonas* sp. having the ability to produce the pyoverdine type of siderophores under low-iron stress conditions was identified. This *Pseudomonas* culture and purified siderophore showed good antifungal activity against the plant deleterious fungi, viz. *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *F. oxysporum* and *Sclerotium rolfsii* (Manwar et al. 2004). Though siderophores are part of primary metabolism (iron is an essential element), on occasion they also behave as antibiotics which are commonly considered to be secondary metabolites (Haas and Defago 2005).

3.17 Conclusions

The evidence indicates that JA regulates plant defence through intricate crosstalks with diverse signalling networks manipulated by other phytohormones such as salicylic acid (SA), ethylene (ET) and nitric oxide (NO) (Yan and Xie 2015).

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Role of Oomycete Elicitors in Plant Defense Signaling

4

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Abstract

Plant pathogenic microbes, the oomycetes, have the remarkable ability to manipulate morphological, physiological, and biochemical processes in their host plants. The special adaptive responses of the oomycetes toward a host enable these pathogens to inflict devastating diseases on food crops with immediate impact on mankind. These manipulations are achieved through a diverse array of pathogenicity factors such as elicitors and effector molecules produced by the conidia/zoospores which have been accepted as the principal dispersive agents of all oomycete pathogens. These molecules can either promote infection or trigger defense responses. The elicitors are molecules which stimulate a defense response in the host plant. Most of them constitute pathogen-associated molecular patterns (PAMPs) since they are structurally conserved and very important product of pathogen life cycle. In order to establish an intimate association with the host plant, the pathogen must suppress immune responses triggered by their own elicitors by secreting effector proteins that can act in many different cellular compartments and alter the host physiological state which supports the colonization. The oomycete pathogen while interacting with the respective host system, both the host and the pathogen,

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is battling each other for control over the other. During the process, the plant cell membrane receptor or transmembrane pattern recognition receptors (TPRR) recognize the pathogen-associated molecular pattern domain in the apoplast and trigger PAMP-triggered immunity (PTI). The plant-resistant protein recognizes the pathogen effector entering the host cell and elicits effector-triggered immunity (ETI). However, research on oomycetes, especially in *Phytophthora,* is progressing at an interesting level due to tremendous improvement in host–pathogen interaction at genomic level. A variety of functional assays have been carried out to prove the role of elicitors in pathogen recognition and non-host resistance. These methods have identified important biochemical and molecular intermediates in elicitor-induced signaling responses in the host. Sequence analysis of the elicitor genes from oomycete pathogens shed light on the phylogenetic relationship of the oomycete pathogens and also demonstrates the importance of elicitors for pathogen recognition and development of host defense responses.

Keywords
Oomycete · Elicitor · Receptor · Molecules · Defense signaling · Plant resistance

4.1 Introduction

Plant diseases affect all the major food crops, and at least, 10% of the global food production is lost by the pathogens. Fourteen crops provide bulk of the food for human consumption, and all of them are devastated by oomycete pathogens. Oomycete pathogens have been known to inflict some of the most deadly diseases which have been known to alter the course of the civilizations (Russell 2006). The genus *Phytophthora* is perhaps the most notorious among all the oomycetes costing annually on a global basis in excess of five billion USD in terms of losses and control measures on potato crop alone. The latest available data suggest that pre-harvest pest damage by oomycetes accounts for 42% of the total attainable crop production or a production value of 300 billion USD (Anderson et al. 2004). *Pythium* is another genus that causes extremely serious disease problems on a variety of nutritionally important crop plants. Similarly, the members of the downy mildew group of pathogens such as *Pernosopora*, *Plasmopara*, *Pseudoperonospora*, *Sclerophthora,* and *Sclerospora* regularly cause severe diseases on a range of cereal crops including maize, sorghum, and pearl millet (Nutsugah et al. 2002). The downy mildew on cereal crops is of more serious concern because these crops are grown in the semiarid regions of the world where more than one-sixth of humanity survive on less than one dollar a day, and their problems are compounded by the fact that the region is plagued by unpredictable weather, long dry seasons, and inconsistent rainfall.

The cereal crops are the only crops that grow in such harsh conditions and can survive any kind of stress except the downy mildew (Oritz et al. 2002).

Oomycetes are the group of eukaryotic organisms that differ from true fungi in having cellulose and glucons as the main cell wall polymers also differ from fungi in their lysine biosynthesis pathway. The oomycetes organisms comprises two flagella in their zoospores, one of the flagella is typically ornamented with the tripartite hairs which are the key feature of the kingdom *Straminipila* (Dick 2001). The major cell wall component of true fungi is the chitin; however, oomycetes also possess chitin synthases that are activated during mycelia tip morphogenesis. Cells of oomycetes can be distinguished morphologically from true fungi by their mitochondria, and they possess tubular cristae as oppose to the flattened cristae of fungi or their hyphae which are always non-septate. The oomycetes are diploid during their vegetative mycelia stage, whereas fungi predominantly produce haploid thalli although, exceptions do exist (Emerson 1941). Another interesting aspect about oomycete genomes also exhibit variations in ploidy and can exist as either triploids or polyploids (Yoshida et al. 2013). The oomycetes are related to diatoms and seaweeds. Analysis of conserved DNA sequences such as mitochondrial COX2, large subunit ribosomal DNA, and small subunit rDNA has confirmed that oomycetes belong outside the fungal kingdom, within the *Chromalveolata* (Fawke et al. 2015). The *Chromalveolata* kingdom contains mainly photosynthetic species result of ancestral enslavement of the red algae but oomycetes have since lost their chloroplast.

4.2 Elicitors and Plant Resistance

Plants will be exposed to constant microbial attacks regularly. As a primary and non-inducible defense mechanism, plant cell wall, cuticles, and phytoanticipins are formed which provide physical and chemical barriers and prevent microbial attack (Underwood 2012; Newman et al. 2013). Apart from this, plants also possess signal-inducing compounds (Table 4.1) perceived by the innate immune system which can induce defense response called defense elicitors (Henry et al. 2012; Newman et al. 2013).

4.3 Oomycete-Specific Elicitor and Effector Molecules

Elicitors are molecules which stimulate a defense response in a host plant. These elicitor compounds can be biological in origin, derived from plant or microbe, or can even be synthetically generated (Walters et al. 2013). Most of them constitute pathogen-associated molecular patterns (PAMPs) since they are structurally conserved and thought to be very important components or products of a pathogen life cycle or infection process. In order to establish, intimate association with the host

Table 4.1 Plant immunity triggering plant-derived patterns (Raaymakers and Ackerveken 2016)

Elicitor	Type	Receptor	Receptor type	Source	Reference
Oligogalacturonides	Carbohydrate	Cell wall-associated kinase 1 (WAK1)	EGF-like (epidermal growth factor)	Cell wall	Ferrari et al. (2013)
Cutin monomers	Fatty alcohol	Unknown		Cell wall	Fauth et al. (1998)
Peps	Peptide	PEPR1/PEPR2 (PEP1 receptor1/PEP1 receptor2)	RLK (receptor-like kinase)	Cytosol	Bartels and Boller (2015)
Extracellular Adenosine triphosphate (ATP)	Nucleoside triphosphate	Does not respond to nucleotides 1/LecRK-I.9 (lectin receptor kinase clade 1.9)	LecRK (lectin receptor kinase)	Cytosol	Choi et al. (2014)

plant oomycetes must suppress immune responses triggered by their own elicitors by secreting effector proteins that can act in many different cellular compartments, pathogens alter the plant's physiological state to benefit colonization (Fawke et al. 2015). During the recognition of PAMPs, the pattern-triggered immunity (PTI) is activated, resulting in the production of immune elicitors. This defense response restricts the growth of the pathogen, enabling the systemic induced resistance and making the plant less susceptible to later infections (Henry et al. 2012). Systemic induced resistance can be categorized as systemic acquired resistance (SAR) or induced systemic resistance (ISR). In systemic acquired resistance, the defense response will be in terms of localized necrosis, involving the salicylic acid (SA) pathway or by the expression of pathogenesis-related (PR) protein genes, while induced systemic resistance is often initiated by plant growth-promoting rhizobacteria (PGPR) (Walters et al. 2013), involving the jasmonic acid (JA) and ethylene (ET) pathways and is not associated with necrosis (Walters and Heil 2007; Henry et al. 2012).

Non-host PAMPs activate resistance at the species or higher level, and many molecules have been identified that elicits resistance at the cultivar level. Development of such phylogenetically conserved cultivar-specific disease resistance is governed by avirulence determinants and regulated at the genetic level (Nurnberger and Brunner 2002). To date, avirulence determinants encoding PAMPs have been identified from viruses, bacteria, fungi, and oomycetes and the recognition of these PAMPs by the host will result in the induction of early defense responses like the transmembrane fluxes, cytosolic acidification, and kinase activity. The major difference between the oomycete avirulence determinants and PAMPs from other groups of pathogens is the ability of the oomycetes to cause necrosis and subsequently elicit a hypersensitive response in the host leading to the establishment of a long-lasting systemic acquired resistance (SAR). This ability of the oomycete elicitors to trigger plant protection toward a broad spectrum of phytopathogenic

microbes could be used to introduce disease resistance in plants of agronomic interests.

Elicitor-mediated nonself recognition and signal transduction are likely to activate an array of inducible defense responses that eventually lead to the interruption of attempted microbial invasion. The SAR induced by the oomycete elicitors was reported to be quite efficient against *Phytophthora* spp. in several plants including tobacco, tomato, and other crop plants. Therefore, oomycete elicitors are powerful activators long-term disease protection against various plant pathogens (Jones and Takemoto 2004). Different types of avirulence protein and other elicitors have been described and studied (Hardham and Blackman 2018). Plant cell wall components are dissolved by certian group of enzymes which are produced by the pathogen are referred as an elicitor. Many elicitor molecules are highly conserved across living organisms and are often referred to as pathogen or microbe-associated molecular patterns (PAMS/MAMPs).

4.4 Elicitors of Oomycetes and Host Response

Oomycetes from genus Phytophthora are one of the major causes for substantial yield loss in crops. The cell wall composition of oomycetes includes cellulose, glycan, and hydroxy proline-rich proteins. Necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLP) are majorly responsible for defense response in dicots (Qutob et al. 2006; Oome et al. 2014). Similarly, INF1 elicitin of *Phytophthora infestans* requires receptors like kinase SERK3/BAK1, required for multiple resistance responses to cause HR response in *Nicotiana benthamiana* (Kamoun et al. 1998; Heese et al. 2007). Other pattern-triggered immunity eliciting molecules include GP42 which acts on pep-13 (Nürnberger et al. 1994; Brunner et al. 2002) and cellulose-binding elicitor lectin (CBEL) associated with cell wall attachment (Gaulin et al. 2006; Hein et al. 2009).

4.5 Elicitor-Mediated Activation of Non-host Resistance

A potential plant pathogen has to overcome many preformed passive barriers before inflicting disease symptoms on the host plant. However, the majority of the plant pathogens do not overcome such barriers and fail to colonize the host. Such non-host resistance in addition to the preformed barriers requires activation of defense responses in order to prevent pathogen infection. Activation of non-host resistance is dependent on host recognition mediated by elicitors and avirulence determinants (Jones and Takemoto 2004) The elicitor- or PAMP-mediated activation of non-host resistance has been extremely well elucidated for NPP1 and Pep-13 PAMP which is a part of GP 42 surface-exposed molecule in *Phytophthora* (Brunner et al. 2002; Halim et al. 2004). The sensory systems for PAMPs share functional similarities

with that of mammals. Drosophila Toll and mammalian Toll-like receptors (TLR) have been identified in many host plants that recognize PAMPs through an extracellular leucine-rich repeat (LRR) domain and transduce the PAMP signal through a cytoplasmic TLR domain. Numerous DNA binding proteins of oomycete and other elicitors have been characterized. A soybean *Hg* (Heptaglucoside) protein was shown to bind to GP42 at the Pep-13 region and activate defense responses. Plants possess as many as 235 LRR-receptor-like kinases (LRR-RLKs), a significant number of which can be expected to be involved in the PAMP perception. Plant LRR-RLKs are known to interact with other LRR-RLK as well as non-LRR-RLK proteins which show that plants may have evolved a large capacity of pathogen perception through PAMP-specific complex formation (Shiu and Bleecker 2001; Morris and Walker 2003). Forward genetic screens have been used in *Arabidopsis–Phytophthora* system which has resulted in the identification of a set of mutant loci which shed more light on molecular basis of non-host resistance. A gene encoding '*Syntaxin SYP 21*' belonging to the SNARE family of proteins was found to be necessary for the development of Pep-13 induced non-host resistance. SNARE proteins are responsible for membrane fusion events during membrane trafficking. In addition, actin cytoskeleton and cell wall plasma membrane connectivity have been demonstrated to represent important preformed but inducible non-host responses due to treatment with elicitors and PAMPs. Other important components of elicitor-mediated non-host resistance in plants against oomycete pathogens include *NHO1* gene encoding a glycerol kinase and a BAX1 protein encoding a protease with caspase-like activity (Jones and Takemoto 2004). As advances in molecular biology take place more complex regulation, sensory and executive mechanisms comprising non-host resistance can be elucidated.

4.6 Oomycete Elicitors Induce Biological Activities in the Plant Hosts

Although oomycete pathogens can induce plant defenses, the biological role of elicitor detection in host–pathogen interactions is not yet properly elucidated because susceptible plants can also support the growth of pathogens capable of producing the elicitor without triggering a response (Cunha et al. 2006). However, application of purified elicitins to tobacco has revealed strong appearance of HR besides induction of programmed cell death, oxidative burst, and defense gene expression (Cordelier et al. 2003). Cryptogein application on the petiole of excised tobacco leaves induced necrosis that shows correlation with histological responses like the rapid chloroplast break down, collapse of cells leading to disorganization of the parenchyma tissues and ethylene/phytoalexin accumulation. Application of β elicitins triggers necrosis in tobacco plants which subsequently become resistant to further infection by the pathogens. This protection depends on a complex signaling network operating downstream of elicitor recognition (Edreva et al. 2002). The complex signaling events share conserved mechanisms with that of the

R-protein-mediated recognition. The MAPKs and their orthologues like the SIPK, WIPK are all known to be induced by recognition of number of PAMPs and elicitors like Pep-13 and NPP1 (Sasabe et al. 2000; Zhao et al. 2005). In addition to the activation of protein kinases, oomycete elicitors like cryptogein induce production of hydrogen peroxide which orchestrates the plant hypersensitive response. Cryptogein also induces lipid peroxidation mediated by enhanced lipoxygenase activities. Elicitins also trigger coordinated accumulation of SAR gene transcripts (Lebrun-Garcia et al. 2002). Capsaicin and Cryptogein were found to elicit an increase in gene transcripts of β subunit of proteasome. NPPI-mediated induction of PR proteins was shown to require functioning of NDR1 and PAD4 genes which are well-characterized signaling components involved in NBS-LRR-CC R gene-mediated resistance in non-host plants (Zhang and Klessig 2001; Jonak et al. 2002; Ren et al. 2002; Nürnberger et al. 2004).

Biological activities mediated by recognition of the elicitor in the host have been studied with a major focus on tissue-cultured cells. Cultured cells of clonal origin are physiologically more homogeneous than intact tissues, and after elicitor treatment exhibits many of the responses that occur when intact plant tissue interacts with the pathogen. Cultured cell suspensions are a valuable tool for studying elicitor-induced defense reactions in plants. They represent a model system with reduced complexity compared to the whole plant and offer the possibility to study early signaling events (Amanda et al. 2003). The responses include an oxidative burst, induction of phenylpropanoid pathway and altered peroxidase activity. The defense reactions activated in the tissue-cultured cells have been shown to stimulate closely the reactions that occur in the plant cells surrounding the site of pathogen attack (Hu et al. 2003; Ortmann et al. 2004).

When added to tobacco cells in sublethal concentrations, cryptogein was able to bring about rapid changes: a strong increase in pH and conductivity of the medium, followed by cytosolic acidification and transient production of ROS as early as 5–10 min after elicitor application (Kadota et al. 2004). Delayed cellular responses were ethylene production, lipoxygenase activities, proteinase inhibitor activities and increase in acylated steryl glycosides and sterol ester level. Cryptogein treated tobacco cells are also prone to extreme changes in gene expression (Suzuki et al. 2006). Northern blot hybridization showed that several plant disease proteins transiently accumulated. Proton ATPase, HMG reductase, PAL, PR1b, lipoxygenase, and a β subunit of proteasome demonstrated up-regulation patterns as early as thirty minutes post-elicitation (Zhao et al. 2005).

Cellular responses to elicitins depend on the specific binding of the elicitors to high-affinity binding sites. Binding sites for several oomycete elicitors have been identified from cell suspension cultures of various plants mainly tobacco. The binding of the elicitors to these sites or receptors have been shown to be saturable, reversible, specific and with an apparent K_d of 2 nm. These receptors are postulated to be glycoproteins with molecular mass ranging between 60 and 120 kDa (Wendeheene et al. 1996). Immediately after binding with receptors, elicitins induce phosphorylation cascade followed by calcium entry and demethylation of cell wall pectins as a consequence of the early events of host cell due to the recognition of

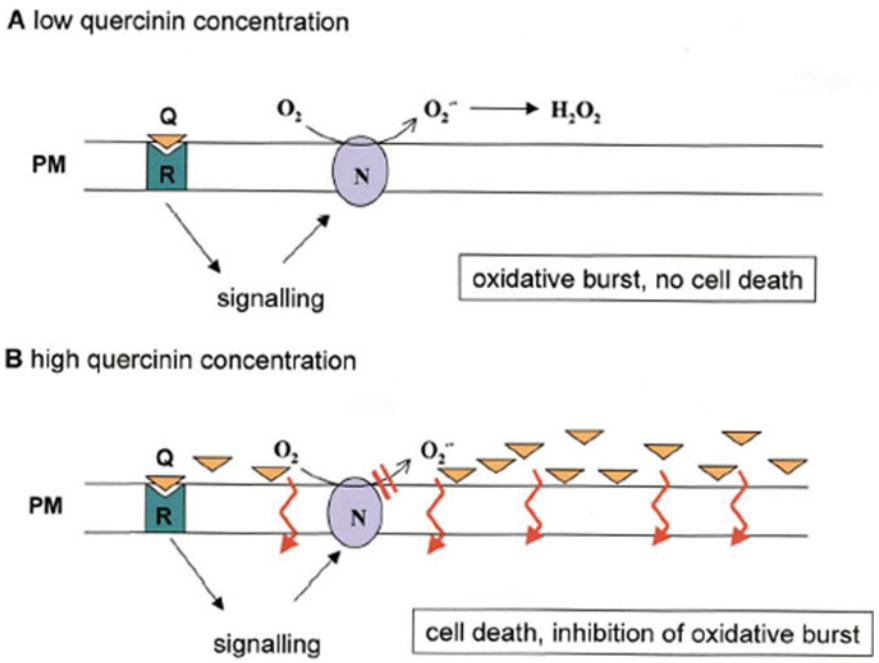

Fig. 4.1 Elicitation of induced defense response signaling by quercinin (Koehl et al. 2003)

active oxygen species (AOS) generation takes place. The AOS generation due to elicitor treatment has been speculated to be the activity of membrane-bound NADPH oxidase which in turn was regulated by a RAC2 encoded G protein. Thus, a complex web of cellular and physiological reactions is responsible for building up of non-host resistance after elicitor binding of the receptor (Tsukada et al. 2002) (Fig. 4.1).

4.7 Effectors in Host–Pathogen Interactions

In oomycetes, host–pathogen interaction with each side battling for control over the other, plant cell membrane receptor or transmembrane pattern recognition receptors (PRRs) recognizes the pathogen- or microbe-associated molecule patterns domain in the apoplast and trigger PAMP-triggered immunity (PTI). The plant-resistant protein recognizes the pathogen effector entering the host cell and elicits effector-triggered immunity (ETI). The defense response of the host aimed to interfere with the pathogen entry into the host cell and spread. During the process pH alkalinization, callose deposition and defense gene activation are considered as markers of PTI. In case of ETI, response controlled cell death or hypersensitive response (HR) is often visible. But in some of the cases, PAMPs can also trigger cell death responses such as in the case of *P. infestans* (INF1) infiltrated as a protein

or expressed inside *N. benthamiana* (Chaparro-Garcia et al. 2011). ETI provides race-specific resistance since different races of pathogen secrete a different range of effectors and may lack or may possess variant of the effectors necessary to trigger the response.

Many of the cytoplasmic oomycete effectors identified so for are characterized by an Arginine–any amino acid–Leucine–Arginine (RXLR) motif following an n-terminal signal peptide which allows translocation into the plant cell (Whisson et al. 2007). The RXLR motifs can be followed by an EER motif, and similarly, QXLR and RXLQ can replace the RXLQ motif or it can be absent such as in the case of ATR5 (Bailey et al. 2011). A second class of effectors is referred to as CRNs and characterized by their crinkling and necrosis inducing activity (Torto et al. 2003). In oomycetes, there are also common to see motifs such as LXLFLAK (Schornack et al. 2010). It has been reported that, RXLRs may be an adoption to facilitate biotrophy (Whisson et al. 2007), whereas certain other species like *Pythium* spp may employ CRNs as a result of their adoption to necrotrophy. Exception can be seen in many biotrophic oomycete pathogen to have both RXLRs and CRNs which indicate that a connection between effector class and lifestyle is not easily defined (Fawke et al. 2015). The interaction studies conducted using *Arabidopsis thaliana* with *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* revealed that a total of 137 proteins are potential targets of pathogen effectors (Mukhtar et al. 2011).

4.8 Agronomic Efficiency of the Oomycete Elicitor-Induced Resistance in Plants

In devising the strategies for crop disease management, the use of elicitors and effectors of great use and which in turn will increase the yield and quality of the crop. During the application of elicitors to elicit defense response in plants, it will become part of PTI which can be very costly for plants but should be less than the potential loss caused by disease if no defense was offered by the plant. By enhancing the efficacy of PTI, a quicker and more effective response can be obtained if the actual pathogen is encountered (Wiesel et al. 2014).

4.9 The Path Ahead

Non-host resistance mediated by recognition of oomycete elicitors or PAMPs still remains an unresolved issue in many host–pathogen interactions. However, advances in functional genomics have led to some exciting progress on the function of PAMPs and its associated responses. Oomycete elicitors have evolved in a dynamic environment, and new combination of such oomycete elicitors may be generated by mobile elements in a '*mix and match fashion*' and this results in some

pathogen encoded proteins being recognized in a physiologically specialized manner and produces striking phenotypic responses in the host plants leading to disease resistance (Randall et al. 2005). Recent molecular and functional genomic investigations have revealed that specific lipid-binding proteins secreted by the oomycetes play a key role in communication between the pathogen and the plant. The balance between the specificity and the non-specificity of sterol carrier proteins has an obvious evolutionary advantage for the plant and for the oomycetes in offering a versatile signal recognition system in oomycete host interaction. Elicitins probably deregulate a complex natural equilibrium established between constitutive and lipid loaded LTPs, and advanced crystallization studies should be used to determine accurately the parameters of sterol capture by the elicitins (Takenaka et al. 2006). Novel technological innovations like the laser capture microdissection allow sampling of biological material at the cellular or even subcellular level. This technique may enable scientists to recover, at medium to high-throughput, macromolecules (e.g., RNA and/or protein) from individual infected plant cells or even microbial infection structures such as infection hyphae or haustoria. RNA and protein samples obtained by microdissection of infection hyphae or haustoria may be used to generate cDNA libraries, as probes for cDNA microarrays, or to perform proteomic studies. This technique may thus represent a powerful means to obtain information about gene expression patterns and/or protein complements of oomycete elicitors (O'Connell and Panstruga 2006). Further research on oomycete elicitors could benefit from a host of improved genomic tools like differential in-gel two-dimensional proteomics and high-throughput functional assays of host defense genes after elicitor recognition using VIGS will enable identification of as yet unidentified elicitor molecules from oomycetes. Field analysis of elicitor effects on host plant resistance has largely benefited from the development of new methods for elicitor delivery like the nano-infusion which allows real-time analysis of elicitor effects on host plant (Hanstein and Felle 2004). Elucidation of mechanisms controlling the evolution of plant–oomycete interactions will be greatly impacted by new technologies that include rapid genome sequencing and the development of computational methods to analyze the wealth of genomic information including the development of exclusive oomycete secreted protein database. Parallel studies which employ post-genomic technologies that include systems biology approaches will ultimately allow us to understand the expression of all genes and elicitor proteins and understand the fine balance existing between pathogenicity and resistance. Thus, a complete understanding of the molecular basis of plant disease resistance, mediated by elicitors, will allow the application of these discoveries to develop plants with novel combinations of disease resistance pathways, resistant against a wide spectrum of potentially devastating crop pathogens.

4.10 Future Perspectives in Oomycete Elicitors and Effectors in Crop Disease Management

Different strategies have been employed to control oomycete infection even though their mode of infection is diverse. The pathogenicity factors of the pathogen like elicitors and effectors and their involvement in the host–pathogen interaction have become important tools to apply in oomycete pathogen caused diseases in crop plants. The use of elicitors and effectors has been used in plant protection measures with different strategies.

These strategies mainly include (a) use of R genes, (b) knockouts or mutation of S genes, and (c) transgenic hairpin RNA silencing of essential pathogen transcripts. The use of R genes may provide short-term success over the pathogen, but this resistance will be overcome by the pathogen due to the presence of vast range of effectors. Another drawback of this approach is, apart from being laborious and expensive, an epistatic interaction may also be encountered between the resistance genes. An alternative to this marker-assisted screening for the identification of R-proteins and effector-based high-throughput expression assays has been developed (Vleeshouwers et al. 2011). In such expression assays when combined with plant disease epidemiology and comparative genomics, the effectors present in virulent strains and also in numerous other isolates could be better managed and prioritized (Kamoun et al. 2015). With the aid from structural biology, the researchers have begun the investigation of functional relationships between plant proteins and pathogen interactions. The knowledge of immune receptor functions at molecular level can lead to the development of better techniques to detect a broad range of oomycete effectors (Segretin et al. 2014; Chapman et al. 2014). The specificity and functioning of R gene on a given effector could be validated by transient co-expression with effectors in plants that do not carry resistant genes. Once identified, the techniques of R gene stacking, variety mixing, or using multilines, the durability of the resistance could be extended. Currently, the implementation in a large-scale agriculture field has some limitations which can overcome by using stably engineered R-proteins with extended recognition spectra providing an alternative solution (Segretin et al. 2014; Chapman et al. 2014).

Apart from using R Genes, resistance can also be achieved by removing the key plant genes required for infection. Susceptibility gene mutation-based resistance should provide better effect and durability when compared with the use of R genes since they involve a component crucial for pathogen survival. S gene-mediated resistance has shown promising results to economically significant oomycetes like *ram2*-mediated resistance to *Phytophthora palmivora* and *Aphanomyces euteiches* (Wang et al. 2012; Gobbato et al. 2013). Knockout of S genes may cause some serious implications on essential host processes. For example, knockout of DMR1 gene resulted in lethal phenotypes (van Damme et al. 2009), and mutation of RAM2 in *Medicago truncatula* resulted in altered water permeability of seed coat (Wang et al. 2012). Hence, to utilize such S genes, first different alleles must be identified which encode proteins reduced but not abolished activity. It can be achieved by

'artificial evolution' where targeted mutagenesis, or assessment of natural variations based on haplotype-specific markers (Bhullar et al. 2010) could be utilized. The genome sequences of a number oomycete species including *H. arabidopsidis*, *Pythium ultimum*, *P. infestans*, *Phytophthora ramorum*, *Phytophthora sojae*, and *Phytophthora capsici* are currently available (Table 4.1) (Lévesque et al. 2010; Haas et al. 2009; Baxter et al. 2010; Tyler et al. 2006). This knowledge could help in understanding the oomycete interactions with their hosts, and ultimately which gene encodes effectors, resistance protein, or susceptible proteins.

Apart from the above two strategies, a third approach of 'host-induced gene silencing,' a technique utilizes the transgenic plant's hairpin RNA, constructs targeting pathogen transcripts essential of virulence be used. This principle has been demonstrated in fungi, and evidences have suggested its transferability to *Phytophthora* and *Bremia* spp. (Vega-Arreguín et al. 2014; Govindarajulu et al. 2014; Jahan et al. 2015).

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Plant–Microbe Interaction: Gene-to-Metabolite Network

5

Sonia Chadha

Abstract

Plants and microbes interact with one another in a beneficial/neutral or unfavorable manner. These plant–microbe associations affect plant physiological processes, where plants maintain balances between plant fitness costs and defense responses. For the establishment of effective plant–microbe relationship/invasion, a microbe has to first pass through the plant preformed barriers and defense machineries. To limit the microbial entry and pathogen propagation or kill pathogens, plant cells trigger immune response. Plant immune signaling consists of two defense cascades: microbe/pathogen-associated molecular pattern (MAMP/PAMP)-triggered immunity (MTI/PTI) and effector-triggered immunity (ETI). Both MTI/PTI and ETI networks comprise of structurally and functionally diverse genes, proteins, and/or small molecules that are tightly regulated via feedback loop(s). The signaling cascade involves number of events such as ion fluxes, mitogen-activated protein kinases (MAPKs), biosynthesis/regulation of plant hormones, calcium protein kinase, lipids, proteins, transcriptional programming, stomatal closure, callose deposition, lignification, along with calcium burst and generation of reactive oxygen species. The present chapter addresses plant and microbe metabolites with pivotal roles in plant–microbe interactions, plant perception systems for pathogen recognition, and how these defense molecules interact to activate defense networks in plants.

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5.1 Introduction

Plants in their natural habitats are surrounded by a large number of microbes that directly interact with plants in a mutually beneficial manner or colonize the plant only for their own benefit. Based on their interaction and impact on plant health, microbes can be classified as saprophytic, pathogenic, and beneficial. The plant association with beneficial microbes helps in the improvement of host plant resistance toward biotic and abiotic stresses and thus helps in the improvement of the crop quality and yield at low-cost expenses. Seed-associated microbes including endophytic microbes and microbes present on the seed surface can influence plant development, health, and productivity. Beneficial microbes are classified as biofertilizers (such as rhizobia), phytostimulators (such as auxin-producing, root-elongating *Azospirilium*), rhizoremediators (pollutants degraders which use root exudate as their carbon source), and biopesticides (Lugtenberg Ben et al. 2002). Root microbes protect root tissues from soilborne pathogens via the production of antibiotics and competition for nutrients and niches. For example, arbuscular mycorrhizal fungi (AMF) which live within the plant roots, helps in the phosphate absorption from the soil and improves crop yield. Another widely studied example is of nitrogen-fixing bacteria which survives in the root nodules of legumes and form a mutually beneficial relationship (Oldroyd 2013). Unlike legume-specific rhizobium, AM fungi establish symbiosis with more than 80% of land plants in all plant lineages. The rhizobia and AM fungi induce different symbiotic responses to host plants but have conserved, early signaling pathway in legumes and non-leguminous plants (common symbiotic pathway, CSP), essential for symbiosis (Parniske 2008). Plant interactions with beneficial microbe increase fitness, yield, and quality of plants and hence have a direct influence on agricultural practices. On the other hand, plant pathogenic microbes can significantly reduce the plant health, yield, and quality. Selected plant–microbe pairs are extensively studied and represented as most suitable pathosystem. One of the extensively studied bacteria and plant pair is of *Pseudomonas syringae pv. Tomato* and *Arabidopsis thaliana*. Biotrophic bacterial pathogen such as *Xanthomonas oryzae pv. Oryzae* is also well studied for its interaction with *Oryza sativa*. Among fungi, a number of pathogen–plant pairs have been well investigated and reported such as *Ustilago maydis* and *Zea mays*, *Melampsora larici-populina* and *Populus trichocarpa*, *Sclerotinia sclerotiorum* and *Glycine max*, *Magnaporthe oryzae* and *Oryza sativa*. Examples of oomycetes–plant pairs include *Hyaloperonospora parasitica* and *A.*

thaliana and *Phytophthora infestans* and *A. thaliana*. The successful colonization and establishment of pathogenic or beneficial plant–microbe interactions require modulation of plant immune system by microbes. The immensity of microbe–plant interactions and their multidimensional functional interactions is difficult to investigate and disentangle. The advancement of molecular, biochemical, bioanalytical, bioinformatics, and system biology tools and technologies has resulted in the plethora of the information in the fields of genomics, transcriptomics, metabolomics, phenomics, and interactomics of plant–microbe interactions and their influence on plant immune signaling networks. This has led to the elucidation of the several molecular and chemical components of both plants and microbes, and complex interactions and networks. These defense network studies provide new insights on plant–microbe interactions and have immense potential to improvise plant cultivation and provide food for an ever-growing population.

5.2 Plant Immune Response

Plant defense relies on a multi-layered system involving several lines of defense. In order to establish an effective relationship or infection between plants and microbe, and to get access to nutrients from the plant, a pathogen has to first pass through the plant preformed barriers and defense system (Fig. 5.1). These physical barriers include cuticle, cell wall, and constitutively produced antimicrobial compounds. Failure hereof leads to the activation of plant innate immune system. Plants perceive pathogens by two different recognition systems that initiate the so-called pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), both of

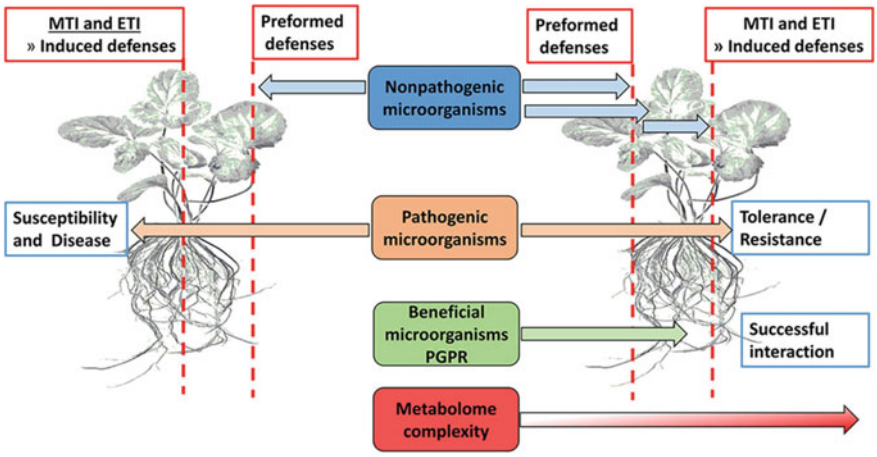

Fig. 5.1 Plant physical barriers and defense responses (MTI and ETI) that presents obstacles to the establishment of potential microbial beneficial interaction with plant roots. Figure originally published by Mhlongo et al. (2018); https://doi.org/10.3389/fpls.2018.00112

which are accompanied by a set of induced defenses that usually repel pathogen attacks. In PTI, resistance is conferred against a broad group of microorganisms, whereas in ETI, a specific response is produced to a given effector produced by isolates of microorganisms. In the first layer of plant defense, plants perceive pathogen by recognizing conserved pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs). The plants can also detect damage-associated molecular patterns (DAMPs), which are plant degradation products released by the plants resulting from the action of invading pathogens and endogenous molecules (Boller and Felix 2009). DAMPs arise from necrotic, damaged, or stressed cells, e.g., cutin monomers, small peptides, and cell wall fragments. PAMPs/MAMPs are conserved microbial molecules such as bacterial flagellin or fungal chitin, that are recognized by plant surface-exposed receptors called as pattern recognition receptors (PRRs) (Macho and Zipfel 2014). Some of the classic and well-studied MAMP-PRR pairs are listed in Table 5.1. The perception and recognition of PAMPs or MAMPs by PRRs induce a complex PTI or

Table 5.1 Examples of classic MAMP-PRR and DAMP-PRR pairs

MAMP/DAMP	Corresponding plant receptor PRRs	References
Flagellins	FLS2 (*Arabidopsis*) LeFLS2 (Tomato) OsFLS2 (Rice)	Felix et al. (1999), Gomez-Gomez and Boller (2000), Robatzek et al. (2007), Takai et al. (2008)
Elongation factor Tu (EF-Tu; elf18/26)	EFR *Arabidopsis; Brassicaceae*	Kunze et al. (2004), Zipfel et al. (2006)
Ax2	Xa21	Song et al. (1995); Lee et al. (2006)
Chitin	CERKs' *Arabidopsis* CEBiP and OsCERK1	Miya et al. (2007), Shimizu et al. (2010), Liu et al. (2012), Shinya et al. (2015)
Xylanase	E1X (Tomato)	Bailey et al. (1990), Ron and Avni (2004)
INF1	NbLRK1 *N. benthamiana*	Kanzaki et al. (2008)
Heptaglucoside	Binding site soybean	Cheong and Hahn (1991)
Pep13	Binding site parsley	Nennstiel et al. (1998)
Glycopeptide	Binding site tomato	Basse et al. (1993)
DAMPs		
Systemin	SR160	Pearce et al. (1991), Scheer and Ryan (2002)
AtPep1	PEPR1	Yamaguchi et al. (2006)
Oligogalacturonides	WAK1 *Arabidopsis*	Ridley et al. (2001), Brutus et al. (2010), Ferrari et al. (2013)

MTI signaling cascades to resist pathogen attack. Recognition of DAMPs also triggers immune responses similar to the PTI response. Some pathogens are capable of down-regulating PTI or MTI by the secretion of pathogen effector molecules and deliver these molecules in the extracellular matrix or the plant cell, thereby causing host colonization and disease, referred to as effector-triggered susceptibility (ETS). To overcome this, plant resistance (R) proteins recognize these molecules and activate a second line of defense which is a rapid and robust response, termed as effector-triggered immunity (ETI) (Dodds and Rathjen 2010). The ETI response is often accompanied by a rapid hypersensitive response (HR) and induced systemic resistance (ISR). The ISR can be defined as a phenomenon by which a plant exhibits an increased level of resistance to pathogen infection after the appropriate stimulation by avirulent or non-pathogenic microbes. The onset of immune signaling triggers an induced resistance in tissues distal from the site of infection and involves one or more long-distance signals that propagate an enhanced defensive capacity in still undamaged plant parts (Shah and Zeier 2013). This pathogen-induced resistance is known as systemic acquired resistance (SAR). SAR is accompanied by the activation of pathogenesis-related (PR) genes, many of which encode PR proteins with antimicrobial activity. PR-1 is among the best-characterized PR genes and often used as a marker for SAR (Ryals et al. 1996; van Loon et al. 2006).

5.3 Metabolites in Plant–Microbe Interactions

Plants accumulate and release a diverse array of metabolites into the surrounding, many of which are well known to function in defense. Metabolite content refers to all small molecules that are the products or intermediates of metabolism (metabolites) present within a biological organism. Upon contact with pathogens or with non-pathogenic microbes or microbial elicitors, plants produce the signaling small molecules like phytohormones which regulate and induce defense response such as the strengthening of cells wall, and the accumulation of phytoalexins and pathogenesis-related (PR) proteins (Dangl and Jones 2001; Garcia-Brugger et al. 2006). The secondary metabolites of plants play an important role in defense activities such as deterrence/anti-feedant activity, toxicity or may act as precursors to physical defense systems. In plants, a complex array of defense response is induced after the detection of microorganism via recognition of elicitor molecules released during plant–pathogen interaction. Different types of elicitors have been discovered that can induce plant defense responses such as lipopolysaccharides, siderophores, pyocyanin, iron-regulated metabolites, flagella, antibiotics, biosurfactants, and volatile organic compounds (VOCs). These metabolites are secreted by diverse bacterial and fungal species such as *Pseudomonas*, *Bacillus*, *Penicillium simplicissimum, Phoma*, *Piriformospora indica*, *Fusarium oxysporum*, and AM fungi.

5.3.1 Phenolic Compounds

Phenolic compounds are produced by both plants and microbes, but they differ in chemical structures. The phenolic compounds released from roots and seeds of plants often show high antifungal, antibacterial, and antiviral activities against soilborne pathogens. Phenolic group of metabolites include terpenoids, phenylpropanoids, cinnamic acids, lignin precursors, hydroxybenzoic acids, catechols, coumarins, flavonoids, isoflavonoids, and tannins. In plant–microbe interactions, phenolic compounds also play a role in signaling. In the rhizosphere, flavonoids are important signaling molecules in the rhizobia–legume symbiotic interaction and regulators of root nodule development (Reddy et al. 2007). The rhizobial outer membrane protein NodD (the LysR-type transcriptional regulator) perceives specific flavonoids and initiates transcription of *nod* genes that encode the biosynthetic machinery for a bacterial signal, the Nod factor (Faure et al. 2009). Nod factors (NFs) are lipo-chitooligosaccharides (LCOs), consisting of β-1, 4 linked *N*-acetylglucosamine (GlcNAc). Rhizobia produce a diversity of modified Nod-LCOs that differ in the length of their chitin–oligosaccharide chain, lipid acylation, and the presence of modifications such as sulfation, acetylation, and fucosylation, which probably contribute to plant host specificity (Denarie et al. 1996; Maillet et al. 2011). LCOs are also important signal molecules in the rhizosphere with a role in plant growth enhancement in legumes and non-legumes alike.

5.3.2 Volatile Organic Compounds

Plants emit a plethora of volatile organic compounds (VOCs) in response to beneficial microbes and necrotrophic/biotrophic pathogens from their above-ground organs (Ryu et al. 2004; Sharifi et al. 2018). Initial microbe–plant interactions activate large number of genes involved in various defense pathways including induction of synthesis and emission of volatiles. The volatile compounds belong to broad classes of volatile isoprenoids, metabolites of shikimic acid pathway, carbohydrate and fatty acid cleavage products including methyl salicylate, phenylpropanoids, benzenoids, indole, monoterpenoids, homoterpenes, and sesquiterpenes (Niinemets et al. 2013). Release of plant volatiles can directly participate in defense by reducing or inhibiting growth and biological activity of pathogens. VOCs also act indirectly by serving as signals for elicitation of plant systemic responses (Heil and Silva Bueno 2007), promoting resistance/susceptibility to subsequent pathogen attack. Pathogenic microbes and their elicitors induce VOCs emissions in plants, which is completely dependent on the virulence status of the pathogen (Huang et al. 2003). Plant VOCs upon exposure to susceptible pathogen can prime the expression of defense-related genes such as PR1, PR2, and PR4 similar to the inoculated resistant plants (Quintana-Rodriguez et al. 2015).

5.3.3 Plant Hormones

Plant hormones, also known as phytohormones, are small molecules that play important regulatory roles in various plant processes. Plant hormones are functionally classified based on their primary role in diverse physiological growth processes and in defense and immune responses. The most important classes involved in plant immune signaling include hormones such as salicylic acid (SA), jasmonate (JA), and ethylene (Pieterse et al. 2012). Plant hormones SA, JA, and ET are essential signaling molecules for both local and systemic responses and play important roles in disease resistance. These hormones are synthesized in plants as primary signals in the regulation of plant immunity to activate effective defense responses. Plant hormones involved in growth or stress processes such as abscisic acid (ABA), auxins (AUX), gibberellins (GA), cytokinins (CK), brassinosteroids (BR), and strigolactones (SL) also play crucial roles in plant defense (Shigenaga and Argueso 2016). These hormones are recognized by their receptors and convey different signaling and defense responses. Due to their prominent role in immune signaling, plant hormones and their networks are discussed separately in Sect. 5.5.8.

5.3.4 Extracellular Polysaccharides

Surface polysaccharides are important for bacterial interactions with plants, and some also act as virulence factors in pathogens. Bacterial extracellular polysaccharides (exopolysaccharides, EPSs; lipopolysaccharides, LPSs; capsular polysaccharides, CPSs; and cyclic β-glucans) are usually accumulated on cell surfaces and/or secreted into the cell surroundings. They form an adaptable dynamic interface and have diverse roles, for example, in cell-to-cell interactions, in protection against stress, attachment to surfaces, and inhibition of the plant defense response in plant–microbe interactions (Kyungseok et al. 2008). Bacterial exopolysaccharides (EPS) are essential for the development of infected root nodules in the legume–rhizobium symbiosis (Fraysse et al. 2003). The role of bacterial EPS in this infection process has been most extensively studied in the *Sinorhizobium meliloti–alfalfa* and *Rhizobium leguminosarum*–pea symbioses.

5.3.5 *N*-Acyl-L-Homoserine Lactones (AHLs)

Microbial compounds such as *N*-acyl-L-homoserine lactones (AHLs) are a class of signaling molecules involved in bacterial quorum sensing, which helps bacterial population to adhere plant tissues and biofilm formation, thereby resulting in beneficial plant–microbe interactions. AHLs can also prime plant defense response through modification of secondary metabolites (Schenk et al. 2014).

5.4 Plant–Microbe Interaction Networks

Plant–microbe interaction network-based analysis is a holistic approach that can enable a detailed understanding of the relationships between plants and microbes. A biological network can be defined as a group of multiples biological entities connected to each other via biochemical interactions. Plant immune system and the arsenal of virulence factors used by pathogens can be considered as robust and complex biological network that controls inducible responses to pathogen attack (Peyraud et al. 2017). The first plant–pathogen network-1 (PPN-1) was constructed using effectors from two pathogens spanning the eukaryote–eubacteria divergence, three classes of immune system proteins and ~8000 other *Arabidopsis* proteins. In this network, effectors were converged onto highly interconnected host proteins. The plant pathogens from different kingdoms deploy independently evolved virulence proteins that interact with hub proteins to facilitate their diverse life-cycle strategies (Mukhtar et al. 2011). Hub proteins are highly connected proteins in a protein–protein interaction network. Signaling networks are represented by several genes, proteins, and/or small molecules, which correspond to the vertices connected by directed edges (arrows representing signal flows between them). A tree-like network with only diverging and no converging vertices is not a complex network (Katagiri 2018). There are a number of tools which can be employed in the analysis of large genomic and metabolomics data, generic or pathway-based analysis, and network visualization (Table 5.2).

Immune networks are tightly tuned system in temporal and quantitative terms (Schwessinger and Zipfel 2008). For example, MTI/PTI and ETI networks are tightly regulated via feedback loop(s) that ensures the transient and appropriate levels of molecular components. It also suggests that only a part of the signaling network is usually used. The network model revealed that the components of the network are highly interconnected and negative regulatory relationships are common among signaling sectors. In the immune signaling network, pathogens trigger the network response and produce effectors that attack the networks. Sato et al. (2010) predicted novel regulatory immune signaling networks in *Arabidopsis* upon challenge with *P. syringae* expressing the effector protein AvrRpt2. Pathogen PAMPs/effectors can negatively affect plant growth and developmental processes by inhibiting the transcriptional expression of genes, negative regulation of signaling pathways, etc. One of the common strategies implemented by effectors is the modulation of the plant hormone homeostasis, resulting in the deactivation of the appropriate PTI and ETI networks. Pathogen-induced changes in the plant hormone homeostasis lead to the transcriptional reprogramming of defensive genes (Fig. 5.2). Such tightly regulated organization of immune signaling network balances its robustness and minimizes its negative impacts on plant fitness.

Table 5.2 List of the pathway and networking tools

Name	Description	References
BioTapestry	Interactive tool for building, visualizing and stimulating genetic regulatory networks	Longabaugh et al. (2009), Paquette et al. (2016)
Cytoscape	Data integration, network visualization, and analysis	Shannon et al. (2003)
Impala	Integrated pathway-level analysis from gene or protein expression and metabolomics data; identification of additional pathways from the combined datasets	Kamburov et al. (2011)
InCroMAP	Tool for generic or pathway-based analysis and visualization of heterogeneous, cross-platform datasets	Wrzodek et al. (2012)
iPEAP	Integrate multiple omics and genetic data for pathway enrichment analysis	Sun et al. (2014)
PaintOmics 3	Pathway analysis and visualization of multi-omics data. A tool for a comprehensive pathway enrichment and analysis workflow and interactive heatmaps	Hernandez-de-Diego et al. (2018)
Pathview	Pathway-based data integration and visualization	Luo et al. 2017
PathVisio	A pathway analysis tool that allows you to draw, edit, and analyze biological pathways	Kutmon et al. (2015)
pwOmics	Compute consensus networks between signaling molecules (genes, proteins, and transcription factors)	Wachter and Beissbarth (2015)
MetaboAnalyst 4.0	For comprehensive metabolomic data analysis, interpretation, and integration with other omics data	Chong et al. (2018)
MetScape	Gene, enzyme, and metabolite networks analysis with emphasis on metabolic pathways; correlation networks; pathway enrichment analysis based on gene expression data	Karnovsky et al. (2012)
SAMNetWeb	Tool generates biological networks for genes, proteins, and transcription factors representing changes in protein and gene expression levels. It covers integrated network and pathway enrichment analysis	Gosline et al. (2015)

For metabolites, the first plant genome-scale metabolic model was constructed for *Arabidopsis* to study heterotrophic cell suspension culture (Poolman et al. 2009). In the last decade, several genome-scale models or specific metabolic pathways models are constructed for different plant species (Pilalis et al. 2011; Yuan et al. 2016) utilizing various system biology tools (Table 5.2).

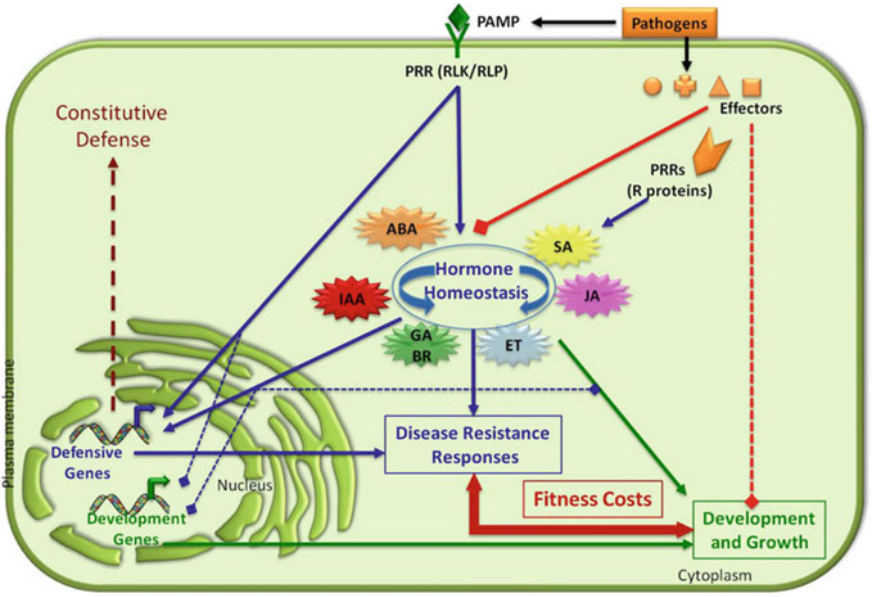

Fig. 5.2 Pathogen PAMPs/effectors modulate plant hormonal homeostasis, defense responses and fitness costs. Positive and negative interactions are indicated by arrows and squares, respectively. Figure originally published by Denancé et al. (2013); https://doi.org/10.3389/fpls.2013.00155

5.5 Plant Immune Signaling Networks

Immune signal transduction events comprise complex and overlapping networks that mediate and integrate the induction of PTI and ETI defense responses in plants. PTI and ETI interact with each other at early stages and form immune signaling networks and share many molecular and biochemical sections that differ in the intensity of the host responses. These plant defense signaling networks involve plant receptors present on cell surface, phytohormones, release of chemical signals such as reactive oxygen and nitrogen species, lipids such as phosphatidic acid (PA), production of secondary metabolites, and phosphorylation cascades along with changes in expression of defense genes (Boller and Felix 2009; Cui et al. 2015).

5.5.1 Immune Receptor Networks

5.5.1.1 LRR-RK and RLP Networks

Plant–microbe interactions activate immune receptors that serve as a trigger for diverse cascades of immune responses and signaling. The recognition of PAMPs or MAMPs involves complexes of cell-surface receptor kinases, which are localized on

the plant cells' surface (Jones and Dangl 2006). The recognition of DAMPs by plants also occurs in a similar manner (De Coninck et al. 2015). Various selective forces contribute to the expansion and diversification of the PRRS involved in MAMP perception (Lehti-Shiu et al. 2009). In comparison to strain-specific pathogen effectors, MAMPs are considered as evolutionarily conserved. Plant PRRs can be categorized into two major classes that include plasma membrane-localized receptor-like kinases (RLKs) and receptor-like proteins (RLPs) with functional domains. *Arabidopsis* genome encodes more than 600 RLKs and 57 RLPs. Other plant species also have a similar number of RLKs/RLPs (Fritz-Laylin et al. 2005). Class 1 PRRs consist of leucine-rich repeat (LRR)-receptor-like kinases (RLKs) referred to as LRR-RLKs. RLKs contain an extracellular domain (ECD), a single-pass transmembrane (TM) domain, and an intracellular kinase domain. A number of PRR/MAMPs have been identified to date (Table 5.1). For example, *Arabidopsis* flagellin-sensitive 2 (FLS2) receptor recognizes the 22 amino acid long epitope flg22, the N terminus of flagellin from *Pseudomonas aeruginosa* (Gomez and Boller 2000). Similarly, rice Xa21 perceive Ax21, which is a conserved peptide in *X. oryzae* and is considered as MAMP (Song et al. 1995; Lee et al. 2006). Another well-studied PRR/MAMP pair is of *Arabidopsis* EF-Tu receptor (EFR), which perceives elongation factor Tu (EF-Tu) via elf18, 18 amino acid long eliciting epitope from *Escherichia coli* (Zipfel et al. 2006). As compared to MAMPs, fewer DAMPs have been identified in plants to date (Table 5.1).

Class 2 PRRs are receptor-like proteins (RLPs) which have similar structure to RLKs but lack kinase domains. RLPs often exhibit a short cytoplasmic domain with no signaling signature and have different molecular mechanisms of receptor activation. Examples of RLPs include receptors for the fungal MAMP xylanse (ethylene (ET)-inducing xylanse, EIX) in tomato named as LeEIX (Ron and Avni 2004) and chitin-binding site CEBiP (chitin elicitor-binding protein) in rice (Kaku et al. 2006). The receptor kinase gene family has undergone huge expansion in plants; for example, *A. thaliana* genome contains about 610 members; and many of these are involved in plant defense signaling (Lehti-Shiu et al. 2009). The expansion of both RLKs and RLPs in plants suggests that these are the preferred systems for non-self-perception in plants.

Most known PRRs require the leucine-rich repeat (LRR) receptor kinase BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (BAK1) for function (Heese et al. 2007). BAK1 belongs to family of five somatic embryogenesis receptor kinases (SERKs) and is also known as SERK3 (Shiu and Bleeker 2003). In *Arabidopsis*, BAK1 does not have a direct role in elicitor perception, but FLS2 rapidly forms complex with BAK1 after elicitation. This interaction results in the phosphorylation of both proteins (Chinchilla et al. 2007). BAK1 also has a role in the perception of other elicitors, probably also through hetero-dimerization with PRRs in the LRR-receptor kinase family (Schulze et al. 2010). In response to necrotrophic pathogens, BAK1 controls host PCD where its functions are independent of a brassinosteroid (Kemmerling et al. 2007). Various studies suggest that BAK1 functions as a general regulatory adapter protein and controls signaling triggered by several LRR-RKs (Schwessinger and Zipfel 2008).

The fungal cell wall contains chitin as a key component. The release of short chitin fragments, known as chitooligosaccharides in plants, acts as a general elicitor of plant innate immunity. In *A. thaliana*, fungal chitin is perceived by a ligand-induced heteromeric complex that is composed of the lysin motif-containing receptor-like kinase 1 known as chitin elicitor receptor kinase 1 (CERK1)/LysM RLK1(Miya et al. 2007; Wan et al. 2008). It has a LysM motif in the external domain and cytoplasmic protruding kinase domain. The mechanism of chitin perception differs in rice where CERK1 associates with the glycosyl phosphatidylinositol-anchored LysM-receptor-like protein CEBiP and does not form a ligand-induced complex with a LysM-receptor kinase (Shinya et al. 2015). The Nod-LCOs having biochemical similarities with chitin are also perceived by pairs of LysM-receptor kinases in legumes, similar to the perception of chitin in *A. thaliana* (Liang et al. 2014). These receptors have an extracellular LysM-receptor-like kinase that binds with its cognate NF. This binding sets off a cascade of signaling events that includes calcium burst and cytokinins accumulation, followed by root hair curling, development of an infection thread, and rhizobial infection. The CERK1 role in the perception of chitin, peptidoglycans, and LCOs has a dual function in both immunity and symbiosis as it is used as a common co-receptor by various high-affinity ligand-binding LysM-containing receptor kinases and receptor-like proteins (Oldroyd 2013).

5.5.1.2 NLR Networks

The ETI response involves pathogen perception via the recognition of pathogen effectors by plant immune receptors or R proteins which resides primarily inside plant cells and consist of nucleotide-binding (NB) leucine-rich repeat-containing proteins (NLRs). NLRs are intracellular immune receptor that can directly detect the pathogen effector proteins or indirectly sense the pathogen's virulence activities (Cui et al. 2015). The activation of NLRs or NLR pairs by one or more effector molecules induces a rapid and robust ETI response (De Coninck et al. 2015) that leads to the termination of pathogen growth. NLRs can be subdivided into three anciently diverged classes distinguished by their N-terminal domains: Toll–Interleukin 1 receptor (TIR) NLRs (TNLs), coiled-coil (CC) NLRs (CNLs), and NLRs containing an N-terminal RPW8 domain (RNLs). NLRs form a complex network where direct interactions between effectors and R proteins are relatively rare. In NLR networks, pathogen effectors are recognized by a sensor NLR and subsequent action by a helper NLR, which act downstream of a sensor NLR. In *A. thaliana*, three different sensor NLRs detect pathogen effectors, whereas the helper NLRs such as ADR1, ADR1-L1, and ADR-L2 contribute redundantly to ETI response. Several sensor NLRs such as Prf, Rpi-blb2, Mi-1.2, and R1 have been identified in *Nicotiana benthamiana*. These sensors recognize pathogens and are followed by the action of helper NLRs such as NRC2, NRC3, and NRC4 to elicit HR response (Wu et al. 2017).

The indirect NLR-effector detection is through detection of effector activities. In this mode, the activation of NLR receptors by the pathogen effectors relies on the modification of a host factor that is bound to and monitored or guarded by the NLR

proteins in the plant cell. NLRs monitor the status of other plant proteins called as "guardees," which are the direct targets of pathogen effector proteins (Dangl and Jones 2001). In *Arabidopsis*, CNL receptor such as RPM1 (resistance to *P. syringae* pv. *maculicola)* and RPS2 (resistance to *P. syringae)* constitutively guards a host protein known as RIN4 (RPM1-interacting protein) for interference by the *P. syringae* effectors (Kim et al. 2005). Pathogen effector induces phosphorylation and cis/trans isomerization coupled with conformational changes of RIN4. These RIN4 changes are sensed by RPM1 which then activates an immune network. RIN4 forms a node with a large number of connections (designated as "hubs"). In the absence of RPM1 and RPS2, RIN4 acts as a negative regulator of basal resistance (Kim et al. 2005; Liu et al. 2009) and in that capacity appears to be targeted for manipulation by multiple bacterial effectors (Wilton et al. 2010). Another indirect NLR-effector recognition involves plant decoy strategy. In this strategy, plant factor serves as bait to trap pathogen effectors, thereby triggering ETI (van der Hoorn and Kamoun 2008).

In plants, NLR genes show distinct expansion and gene loss patterns. In the plant species genomes such as in a grapevine, potato, rice, and soybean, NLR genes are often expanded to hundreds of genes (Shao et al. 2016). The functional diversification of NLR genes is attributed to the strong selection pressure exerted by pathogens. The extensive knowledge about NLR receptors has been generated; however, NLRs activation and signaling mechanisms and its correlation with phylogeny remain unclear (Wu et al. 2017).

5.5.2 Calcium Burst

The perception of MAMPs/PAMPs leads to a series of signaling events such as ion fluxes, MAPKs activation, Ca^{2+}-dependent protein kinase (CPKs or CDPKs), transcriptional reprogramming, callose deposition, lignification, and ROS production (Kadota et al. 2015). An influx of extracellular Ca^{2+} in the cytosol is one of the earliest known physiological responses. Calcium influx starts at ~30 s to 2 min after perception of MAMPs/DAMPs and reaches a peak around 4–6 min. BIK1 family proteins positively regulate calcium burst which induces the opening of other membrane transporters. In addition, there is an influx of H+ and efflux of potassium, chloride, and nitrate ions, which lead to depolarization of the plasma membrane (Jeworutzki et al. 2010; Nomura et al. 2012).

5.5.3 ROS Burst

One of the early plant defense reactions to pathogen is called as "oxidative burst," which results in the production of ROS, a common feature of plant defense response. ROS plays a central role in plant immune signaling and includes singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radicals (OH). Among them, H_2O_2 is most stable and often acts as an intercellular and intracellular signal

to trigger downstream responses. In *Arabidopsis*, respiratory burst oxidase homolog D (RBOHD) is responsible for MAMP-induced ROS burst. RBOHD is a NADPH oxidase, localized at plasma membrane. The plasma membrane ROS acts as an antimicrobial molecule and plays a role by limiting the pathogen entry in host cell through reinforcement of plant cell wall and callose deposition. In addition, ROS also acts as local and systemic secondary messengers and triggers other defense responses including gene expression, posttranslational modifications, and stomatal movement. ROS burst also induces calcium influx and has a positive feedback effect on cytosolic calcium levels. Low molecular antioxidants such as ascorbate, glutathione, and tocopherol form redox buffers that determine the lifetime and specificity of ROS signal (Foyer and Noctor 2005; Ranf et al. 2011; Camejo et al. 2016).

5.5.4 Reactive Nitrogen Species

Nitric oxide (NO) and its derivatives are collectively referred to as reactive nitrogen species. In plants, the enzymatic source of NO is the NAD(P)H-dependent nitrate reductase (NR), a cytosolic enzyme (Yamasaki and Sakihama et al. 2000). NAD(P)H-dependent NR further reduces nitrite to NO by a mitochondrial electron transport-dependent reductase (Planchet et al. 2005). Pathogen induces influx of Ca^{2+} into the cytosol that activates calmodulin (CaM) and/or similar proteins, which then triggers the NO synthesis. Nitric oxide (NO) contributes to ROS and redox regulation (Besson-Bard et al. 2008). NO plays an important role as a signaling component in immune networks of PTI and ETI. Several studies showed that the cooperativity between NO and ROS burst is essential to fully activate the HR in plants (Delledonne et al. 1998). It regulates the expression of many defense genes including those involved in plant hormones SA and JA pathways. NO also induces the endogenous levels of SA hormone. The increase in SA and NO molecules leads to the regulation of the conformation of NPR1 (nonexpressor of pathogenesis-related) gene (Tada et al. 2008).

5.5.5 Phosphatidic Acid (PA)

In addition to calcium, ROS, and nitrogen reactive species, lipids such as phosphatidic acid (PA) also function as signaling molecules in plant immune network. PA is a universal lipid second messenger and is the essential intermediate for the de novo biosynthesis of all glycerolipids. The PA involved in cell signaling is generated via two distinct phospholipase pathways. It is formed directly by a phospholipase D (PLD), which hydrolyzes structural phospholipids to generate PA and a free head group. It is also produced via the sequential action of phospholipase C (PLC) and diacylglycerol (DAG) kinase (DGK). The pathogenic elicitors activate the PLC–DGK pathway. The basal levels of signaling lipids PA are usually maintained at low levels, but it rapidly increases in response to pathogen infection.

Their accumulation in cells is transient as the signal is rapidly downregulated. PA targets and modulates activities of different components of immune network (Testerink and Munnik 2005) such as CDPKs (Farmer and Choi 1999), 30-phosphoinositide-dependent protein kinase 1 (PDK1) (Anthony et al. 2004), constitutive triple response 1 (Testerink et al. 2007), RBOHD/F (Zhang et al. 2009), phytohormone JA and ET (Wang et al. 2000), and enzymes of protein kinase pathways (Testerink et al. 2007). PA also plays a crucial role in SA-mediated signaling cascade, basal defense, and non-host resistance against plant pathogens (Kalachova et al. 2013).

5.5.6 14-3-3 Proteins

In plants, 14-3-3 proteins are one of the most highly connected nodes in the plant interactome. Among 300 predicted 14-3-3 proteins identified, only 40 have been further characterized (de Boer et al. 2013). 14-3-3 proteins play positive role in pathogen defense and participate in both PTI and ETI signaling networks. 14-3-3 proteins act as phosphosensors, bind phosphorylated client proteins, and modulate their activity. 14-3-3 protein complexes associate with defense-related proteins such as BRI1-associated receptor kinase 1 (BAK1), which is a co-receptor kinase of PAMP receptors, several WRKY transcription factors, and R proteins (Chang et al. 2009). Other examples of 14-3-3 proteins interacting partners include maize plasma membrane H+-ATPase (Jahn et al. 1997), NtrbohD (Elmayan et al. 2007), tomato MAPKKK alpha, and MKK2 (Oh et al. 2010; Oh and Martin 2011). These proteins are also potential targets of pathogen effectors. The conserved *P. syringae* effector HopM1 binds *Arabidopsis* 14-3-3κ in addition to its well-known target AtMIN7/BEN1/BIG5 and promotes its proteasomal degradation (Nomura et al. 2006), thus significantly contributing to *P. syringae* pathogenesis. The disruption of interactions between 14-3-3 proteins and their client proteins leads to a drastic reduction in the MAMP-triggered ROS burst and stomatal closure in *Arabidopsis* and *N. benthamiana* (Lozano-Duran et al. 2014). Numerous evidences suggest that 14-3-3 proteins also interact with many phosphorylated proteins involved in immune signaling in plants; however, the full extent of their role and action mechanisms still needs to be investigated.

5.5.7 Mitogen-Activated Protein Kinases (MAPKs)

Protein phosphorylation mediated by protein kinases is an essential posttranslational modification that is fast and reversible. Protein phosphorylation occurs mainly on serine, threonine, and tyrosine residues. Phosphorylation regulates plethora of different processes including signal transduction, cell cycle regulation, metabolism, and transcriptional and translational control. Mitogen-activated protein kinase (MAPK) cascade activation is one of the earliest signaling events after plant sensing of PAMPs/MAMPs and pathogen effectors. MAPK cascades play the

crucial role in the signaling of multiple defense responses such as defense gene activation, reactive oxygen species (ROS) generation, production and signaling of plant stress hormones, cell wall strengthening, and stomatal closure. To promote pathogenesis, pathogen's effector molecules inactivate plant MAPK module or suppress signaling of plant MAPK module by targeting upstream signaling components (Meng and Zhang 2013). MAPK cascades consist of three-kinase cascades that function downstream of sensors or receptors that transmit extracellular stimuli into intracellular responses. MAPKs are activated by upstream kinases, MAPK kinases (MAPKKs) also known as MAPK and ERK kinases (MEKs). MAPKKs phosphorylate a threonine and tyrosine residues in the Thr-X-Tyr activation motif of MAPKs. MAPKKs are regulated by their upstream kinases, MAPKK kinases, or MEK kinases (MAPKKKs or MEKKs), through the phosphorylation of two Ser/Thr residues in the Ser/Thr-X3−5-Ser/Thr motif of the MAPKK activation loop (Widmann et al. 1999; Meng and Zhang 2013). *Arabidopsis* genome contains around 20 MAPKs, 10 MAPKKs, and approximately 60 MAPKKKs (Hamel et al. 2006). The perception of Flg22 by FLS2 PRR induces the formation of immune receptor complex at the plasma membrane and subsequent auto- and trans-phosphorylation by MAPK of different proteins involved in defense signaling. In *Arabidopsis*, two MAPK modules MKK4/MKK5-MPK3/MPK6 and MEKK1-MKK1/MKK2-MPK4 are involved in plant defense signaling. These are present downstream of BAK1/FLS2. MEKK1-MKK1/MKK2-MPK4 module is guarded by the R protein SUMM2 through a mechanism of control of MEKK2. Pathogen effectors such as HopAI1 result in the deactivation of MPK4 and thus reduce the control of MEKK2, which activates SUMM2, leading to cell death and other defense responses (Kong et al. 2012). The activation of MPK3, MPK4, and MPK6 depends on BIK1/PBLs and the calcium burst. Most of MAPK substrates are transcription factors including WRKY33 and VIP1. These factors play an important role in the defense responses of MPK3, MPK4, and MPK6. The effector AvrB leads to the activation of MPK4, an event promoted by the Hsp90 chaperone and its co-chaperone RAR1 (Cui et al. 2010). This leads to the subsequent activation of RIN4 which is negative regulator of PTI defense. Some pathogen effectors target many components of defense signaling including upstream components of defense signaling and inhibit immune MAPK module.

5.5.8 Plant Hormone Interplay

Microbial invasion and proliferation activate various defense signaling networks including those connected with plant hormones. Plant hormones such as SA, JA, and ET are recognized as key players in the regulation of the signaling pathways involved in SAR and ISR (van Loon et al. 2006; von Dahl and Baldwin 2007; Pieterse et al. 2012). Phytohormone signaling varies considerably in timing, quantity, and composition, depending on the interacting microbe. SA plays a key role in defense against biotrophic pathogens that feed on live tissues, whereas the combination of JA and ET hormones is critical to defense against pathogens feeding

on dead tissues, i.e., with a necrotrophic lifestyle (Glazebrook 2005). In *Arabidopsis,* SA contributes to flg22-triggered immunity against *P. syringae* (Tsuda et al. 2008). SA production is triggered by several regulatory proteins such as EDS1 (enhanced disease susceptibility 1), SID2 (SA INDUCTION-DEFICIENT 2), EDS4, EDS5, and PAD4 (phytoalexin-deficient 4), where EDS1 is a major node for SA-dependent resistance against pathogens. EDS1 is involved in the ETI activation, mediated by the TIR–NB–LRR proteins. EDS1 interacts with PAD4, RPS4, RPS6, SAG101 (senescence-associated gene101), SRFR1 (suppressor of RPS4-RLD1), and SNC1 (Feys et al. 2001, 2005; Heidrich et al. 2011). The formation of EDS1 and PAD4 complex is necessary for basal resistance and activation of SA-defense response (Rietz et al. 2011). In *Arabidopsis*, histone deacetylase HDA19 is involved in the over-accumulation of SA and upregulation of SA marker genes including PR1, PR2, ICS1, EDS1, PAD4 (Choi et al. 2012). NPR1 is a master regulator of SA-mediated responses that controls gene expression (Cao et al. 1997). SA-binding receptors NPR3 and NPR4 function as E3 ligases and degrade NPR1, hence regulating NPR1 levels (Fu et al. 2012; Yan and Dong 2014). SA inhibits the interaction between NPR4 and NPR1 at low levels, thus allowing NPR1 accumulation, whereas high SA levels during pathogen infection promote the association between NPR3 and NPR1 leading to degradation of NPR1. SA causes redox changes in NPR1 protein that results in translocation of NPR1 from a cytosol to the nucleus. In nucleus, NPR1 activates the transcription of defensive genes and interacts with transcription factors such as TGA (TGACG sequence-specific binding protein) which then induces PR proteins (Robert-Seilaniantz et al. 2011). In addition to NPR-1-dependent signaling, SA receptors mediated NPR1-independent signaling pathways are also present in plants (Yan and Dong 2014).

The pathogen attack also leads to the accumulation of JA which triggers the activation of a subset of immune genes and the production of defensive secondary metabolites such as benzophenanthridine alkaloids, anthocyanins, nicotine, terpenoid indole alkaloids (TIA), glucosinolates (GS), benzophenanthridine alkaloids, or flavonoids. In response to pathogen attack, bioactive JA-Ile is synthesized that promotes the JA receptor complex, which consists of F-box protein coronatine-insensitive 1 (COI1), jasmonate ZIM domain (JAZ) proteins, and inositol pentakisphosphate (Xie et al. 1998; Katsir et al. 2008; Sheard et al. 2010). The core signaling module consists of the ubiquitin–proteasome degradation machinery where the SCFCOI1 (Skp–Cullin–F-box–COI1) E3 ubiquitin ligase complex interacts with a JAZ protein (Xu et al. 2002), which leads to the degradation of JAZ proteins (Sheard et al. 2010). JAZ proteins are repressors of positively acting transcription factors (TFs) such as MYC2, MYC3, and MYC4 which bind to JA-responsive elements of JA-inducible genes. The degradation of JAZ repressor proteins leads to the release of the JAZ-mediated repression of TFs and to subsequent induction of JA-responsive gene expression. Several JA-dependent genes encode pathogenesis-related proteins that are also used as JA marker genes, for example, chitinase B (CHIB), hevein-like protein (HEL), plant defensin 1.2 (PDF1.2), and thionin 2.1 (THI2.1) (Reymond and Farmer 1998). In addition to SA and JA, in the last few years ET networking model has evolved from a linear

cascade to a more complex pathway that involves different feedback loops. In plants, ET is perceived by five receptor proteins ETR1, ERS1, ETR2, ERS2, and EIN4, which are localized in the endoplasmic reticulum (ER) membrane. These receptors are grouped into two classes based on the presence (ETR1, ETR2, and EIN4) or absence (ERS1 and ERS2) of the receiver domain (Merchante et al. 2013). CTR1 protein kinase is the primary negative regulator of ET signaling (Kieber et al. 1993). Upon ethylene binding, the receptors transmit the signal to the CTR1 and inhibit its ability to phosphorylate EIN2. In its inactive form, CTR does not phosphorylate the C-terminal domain (CEND) of EIN2 receptor. The translocation of dephosphorylated CEND to the nucleus assists in the stabilization of EIN3, followed by the release of MKK9. The translocation of MKK9 to the nucleus activates MPK3 and MPK6, which promotes the stability of the ET-dependent transcription factors (EIN3 and EIL1) (Merchante et al. 2013). Phosphorylation of EIN3 blocks its proteasomal degradation and enables it to activate ET-responsive genes (Jagodzik et al. 2018).

The cross talk between various hormonal pathways can have antagonistic or synergistic effects and is largely multidimensional. SA is a strong antagonist of the JA/ET-signaling pathway, while a marked synergy was reported between the JA- and ET-signaling pathways. During PTI and ETI, the accumulation of both SA and JA increases. According to PTI signaling network, PAD4 together with JA accounts for activation of SA signaling during PTI. A substantial cross talk has been observed between the SA and JA response pathways in case of multiple infections, where SA pathway suppress the JA pathway. SA and JA signaling pathways share negative cross talk (Gimenez-Ibanez and Solano 2013). In *P. syringae*, the negative cross talk between SA and JA leads to the productions of phytotoxin coronatine (COR) for suppression of SA-mediated signaling (Zheng et al. 2012). The mediator subunit 16 (MED16) is a positive regulator of SA-induced defense response and a negative regulator of JA/ET-signaling pathway (Zhang et al. 2012). In addition, NPR1 also regulates SA-mediated suppression of the JA/ET-signaling pathway. A negative regulation of NPR1 by posttranslational mechanisms leads to the activation of JA signaling cascades (Spoel et al. 2003), whereas the transcription factor WRKY33 which is a positive regulator of JA-related genes represses SA-signaling pathway. The absence of WRKY33 results in the increased SA accumulation and induced expression of SA-regulated genes including SID2/ICS1, EDS5/SID1, PAD4, EDS1, NIMIN1, PR1, PR2, PR3 (Gimenez-Ibanez and Solano 2013). Recent studies have shown that in addition to classical plant defense hormones SA, ET, and JA, other plant hormones including ABA, BR, GA, CK, and auxin are gaining attention as important players in the cross talk that modulates plant–microbe interactions. For example, ABA antagonizes SA pathways, leading to inhibition of plant defenses (Cao et al. 2011). Similarly, BR can also antagonize SA and JA pathways by interfering downstream and upstream of hormone synthesis.

5.6 Conclusions and Perspectives

The plant defense cascades are crucial for the development of durable defense against plant pathogens as well as for the utilization of beneficial microbes for crop improvement. With the advancement of molecular and bioanalytical high-throughput technologies, the understanding of the diverse cellular, physiological, and molecular responses of plant interactions with microbes has taken a big leap forward. Progress made in the understanding of direction of the identification and characterization of genes, proteins, and metabolites has led to the consolidation of knowledge on plant receptors, pathogen perception/recognition, and immune signaling networks. The understanding about plant immune signaling networks is further enhanced by the availability of complete genome sequences of microbes, identification of microbial metabolites, and cellular components along with greater understanding of microbial genome adaptation and evolutionary mechanisms. The ongoing research in plants has demonstrated the overlap in microbe recognition receptors and immune signaling networks. Biochemical and molecular analyses along with system biology approaches have led to the elucidation of immune signaling networks, hubs, and cross talk involved in the compatible and incompatible host–microbe interactions. The identification of hubs, cross talk, and network analysis offers considerable potential for the development of crop cultivars resistant to multiple stressors; however, available knowledge is partial and several aspects still need more attention. The complex immune signaling network, molecular and chemical components involved in plant–microbe interactions remain to be fully discovered. The application of this knowledge is crucial to understand how these responses are integrated in space and time. Moreover, the outcome of the plant immune networks relies not only on the host, but also on the microbial genotype, phylogeny, and lifestyle. The complete understanding of the immune signaling networks involved in plant–microbe interactions needs further research and requires the integration of various biotechnological and system biology approaches, and their continuous advancement. These integrated studies will provide new insights into the field of immune signaling networks of plant–microbial interactions and may lead to unexpected discoveries. These challenging tasks are poignant for the implementation of better strategies to solve the global food security problem for an ever-growing population.

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Phytohormones in the Modulation of Plant Cellular Response to Stress

6

Mostafa Abdelrahman

Abstract

Brassinosteroids (BRs) are important class of steroidal hormones that play critical roles in monitoring broad spectrum of plant growth and developmental processes. The maintenance and regulation of endogenous level of BR is important for different biological functions in plant, including cell division, cell elongation, vascular-differentiation, senescence, reproduction photomorphogenesis, and seed germination as well as respond to various abiotic and biotic stresses. Recent studies highlighted the importance of plant BR homeostasis as a critical step in the establishment of plant immunity. In this chapter, we review the recent progress in deciphering the immune-regulatory role of plant hormones, with a special focus on the cellular components and BR hormone signaling involved in regulation of plant defense. We will also discuss the possible approaches of manipulating BR hormone homeostasis to enhance crop resistance to pathogen as well as other abiotic stressors.

Keywords

Brassinosteroids · Microbe-associated molecular patterns · Plant defense · Stress tolerance

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6.1 Introduction

In their natural environments, plants are exposed to different biotic (fungi, bacteria and viruses, oomycetes, and insects) and abiotic (drought, heat waves, increase soil salinity, and heavy metals) stressors that compromise plant survival and offspring (Denancé et al. 2013; Kazan and Lyons 2014; Abdelrahman et al. 2017a, b, 2018a, b). To prevent the effects of these stressors, plants elaborate different signaling networks and defense mechanisms that are activated whenever changes in metabolism are encountered. Plant hormones are small signaling molecules that antagonistically and/or synergistically play various roles during the lifespan of plants (de Zelicourt et al. 2013; Grover et al. 2013; De Bruyne et al. 2014; Abdelrahman et al. 2019). Plant hormones not only control essential developmental process in plant but also convey environmental inputs and regulate immune responses to biotic and abiotic stresses, and a fine-tune regulation of these immune responses is required to avoid negative impacts on other physiological processes such as seed and biomass production, as a trade-off survival (Jaillais and Chory 2010; Kempel et al. 2011; Denancé et al. 2013). Plant hormones such as jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) are playing a key regulatory role in plant immune responses (Cao et al. 2011; Pieterse et al. 2012; Abdelrahman et al. 2018c; Jogaiah et al. 2018). In addition, other plant hormones, such as gibberellins (GAs), auxins (AUs), cytokinins (CKs), abscisic acid (ABA), and brassinosteroids (BRs) have been thoroughly reported to mediate plant growth and development and have recently emerged as key regulators of plant immunity (Hauvermale et al. 2012; Zhu et al. 2013a). Upon infection, plants synthesize a specific mixture of these hormones, depending on the pathogen type. For instance, in *Arabidopsis thaliana*, resistance to necrotrophic pathogens tend to be resisted through a mixture of ET and JA signaling, whereas resistance to biotrophic pathogens is usually dependent on SA (Pieterse et al. 2009; Jogaiah et al. 2018). In addition, interaction between these two pathways is most often antagonistic, which has led many authors to propose that plant immunity follows a dual model with JA/ET, and SA having opposite effects (Bari and Jones 2009; Jogaiah et al. 2018). However, this classical view is overlay generalize, and accumulating clues in different plant systems indicate more complex reality (Pieterse et al. 2012; Van der Ent and Pieterse 2012; De Vleesschauwer et al. 2013; Riemann et al. 2013). For instance, depending on plant species, the pathogen type, and timing of infection, ABA can act as both a negative or positive regulator of disease resistance, by feeding into the SA-ET-JA backbone of the plant immune system (Asselbergh et al. 2008; Cao et al. 2011). In case of AU, it is now well established that the SA and AU pathways act in a jointly antagonistic manner during plant defense. Moreover, a growing body of evidence indicates that some pathogens either increase plant AU biosynthesis or produce AU by themselves upon infection to control the plant's defensive and developmental machinery (Valls et al. 2006; Abdelrahman et al. 2016; Jogaiah et al. 2018).

Understanding the mechanisms underlie the plant interactions with the abiotic and biotic stressors is fundamental to both plant sustainable agriculture and biotechnology. Employment of a plant hormone biosynthesis and signaling pathways can enhance resistance to a specific pathogen type, but it can also has a robust negative impact on plant growth and resistance to other type of pathogens. Thus, to develop hormone-based breeding strategies to enhance crop resistance to pathogen attacks, we need to have a comprehensive view of how pathogens interfere with this hormone regulation and the complex regulation of hormone homeostasis during plant–pathogen interactions. In this chapter, we review the recent progress in deciphering the immune-regulatory role of plant hormones, with a special focus on the cellular components and BR hormone signaling involved in regulation of plant defense. We will also discuss the possible approaches of manipulating BR hormone homeostasis to enhance crop resistance to pathogen as well as other abiotic stressors.

6.2 Brassinosteroids (BRs) Signaling in Plant Innate Immunity

6.2.1 Pathogen- or Microbe-Associated Molecular Patterns

To contest against pathogens, plants have developed a multifaceted protection system that is triggered upon pathogen attacks (Bajguz and Hayat 2009; Pieterse et al. 2012). Plant first innate immunity is activated by the perception of the conserved microbe-specific molecules of many pathogens, named pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) by pattern-recognition receptors (PRRs) at the plant cell surface. General elicitors like peptidoglycans (PGN), bacterial flagellin (Flg), lipopolysaccharides (LPS), elf18, an 18-aa peptides from the elongation factor Tu (EF-Tu), Ax21 (Activator of XA21-mediated immunity in rice), β-glucans from oomycetes, and fungal chitin are recognized by plant surface localized PRRs (Jones and Dangl 2006; Boutrot and Zipfel 2017). The recognition of PAMPs or MAMPs by PRRs activates downstream signaling cascades, deliberating resistance to a wide range of microbial pathogens, which is known as PAMP/MAMP-triggered immunity (MTI or PTI) (Chinchilla et al. 2007; Boller and Felix 2009) (Fig. 6.1). When the first defense PTI system defeated, cells become exposed to pathogenic threats. At this stage of infection, pathogens release their special proteins named effectors to carry on invasion. These pathogenic proteins, when recognized in the cytoplasm by the nucleotide-binding leucine-rich repeats (NB-LRR) proteins encoded by *RESISTANCE* (*R*) genes, trigger the second layer of plant defense known as effector-triggered immunity (ETI) (Greenberg and Yao 2004; Cunnac et al. 2009). Although, both ETI and PTI can induce a suite of defense responses including increased expression of pathogen-response genes, a reactive oxygen burst, and mitogen-activated protein kinase (MAPK) signaling (Cui et al. 2015). However, ETI is stronger, quicker, and usually induces the

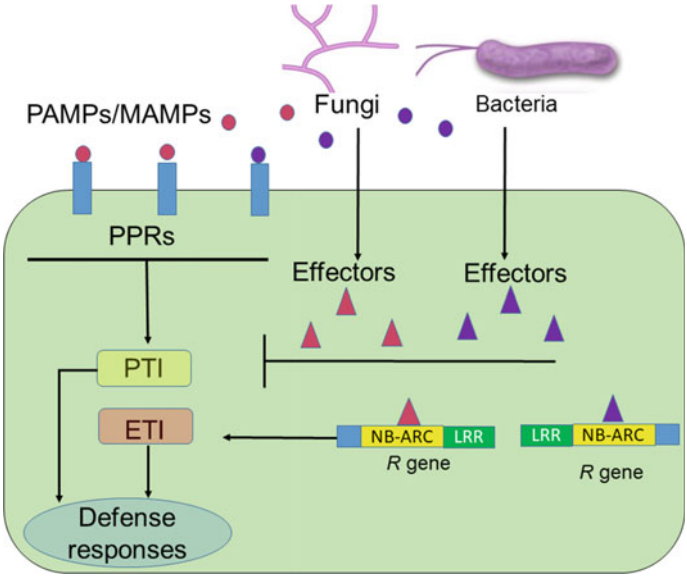

Fig. 6.1 Schematic diagram showing the PAMP/MAMP-triggered immunity and effector-triggered immunity (ETI) system. Pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs), pattern-recognition receptors (PRRs), nucleotide-binding leucine-rich repeats (NB-LRR), resistance (R)

hypersensitive response (HR) that causes localized cell death to inhibit pathogens from spreading further (Yang et al. 2017; Yu et al. 2018).

6.2.2 Brassinosteroid Biosynthesis and Signaling

Both PTI and ETI are modulated by plant hormones, and the three stress-related hormones ET, SA, and JA are considered to be the primary signals involved in the two immune system responses. However, recent advances indicated that growth hormones BRs and GAs are also involved in a complex molecular interaction network that steers host defense responses upon pathogen attacks (Choudhary et al. 2012; De Bruyne et al. 2014). BRs are a group of plant-specific class of polyhydroxylated steroidal phytohormones with essential roles in regulating myriad developmental and physiological process, such as photomorphogenesis, senescence, flowering, pollen tube elongation, and seed germination. BRs also play a prominent role in plant-environment interactions, actively involved in shaping plant fitness, and the growth-defense trade-offs (Lozano-Durán and Zipfel 2015; Yu et al. 2018). BR biosynthesis and degradation are important components of BR homeostasis and for keeping the endogenous level of BR. With sufficient synthetic BR compound, research on the determination of BR physiological effects in a wide range of biological systems to enhance crop yield was carried out (Cutler 1991; Yokota et al. 1991). Clouse and Zurek (1991) released the

early report regarding the effects of BR on *Arabidopsis* growth, followed by a description of BR-insensitive mutants in *Arabidopsis*, demonstrating that BRs are regulating the gene expression in that species (Clouse et al. 1993). After that, four key studies described the characterization and identification of BR-deficient and BR-insensitive mutants in *Arabidopsis* plants (Clouse et al. 1996; Li et al. 1996; Kauschmann et al. 1996; Szekeres et al. 1996). The mutant plants exhibited dwarf phenotype, which could be rescued through BR treatment of the deficient, but not the insensitive, mutants, concluding that BRs are important regulators for normal plant growth and development. However, excessive treatment of active BR compounds leads to upregulation of BR-inactivation gene and downregulation of BR-specific biosynthesis genes, hindering normal development of plants (Zhu et al. 2013b; Saini et al. 2015). The conversion of the membrane sterol campesterol to brassinolide (BL) occurs via a series of reductions, epimerizations, hydroxylations, and oxidations that have been extensively studied in several species. Endogenous levels of BRs differ across plant tissue age, organ type, and species, where immature seeds and pollen containing the highest levels. According to current studies, active BRs such as BL and castasterone (CS) bind directly to BRASSINOSTEROID-INSENSITIVE1 (BRI1) which encodes the extracellular domain of the leucine-rich repeat receptor-like kinase (LRR–RLK). This binding induces a series of biochemical responses, including BRI1 separation from the negative regulator BRI1 KINASE INHIBITOR 1 (BKI1), subsequent heterodimerization of BRI1 with co-receptor membrane-bound BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE1 (BAK1), phosphorylation of the BRI1-interacting signaling kinase (BSK1), and activation of the protein phosphatase BSU1. These events eventually culminate in inhibition of the shaggy-like kinase BIN2 and resultant activation of the homologous transcription factors (TFs) BZR1 and BES1/BZR2. Finally, activated BZR1 and BES1 migrate to the nucleus where they bind BR-responsive promoters, triggering transcriptional changes that ultimately shape BR-signaling outputs (Tang et al. 2011; Wang et al. 2012) (Fig. 6.2). BR application demonstrated that BRs had a strong positive effect on growth of mesocotyls and coleoptiles in monocots and induced stem elongation, including promotion of hypocotyl, epicotyl, and peduncle elongation in dicots (Mandava 1988). BRs were shown to accelerate senescence and stimulate ATPase activity and increased rates of cell division, particularly under AU and CK limitations (Clouse 2011; Yu et al. 2018). BRs also were shown to mediate abiotic and biotic stresses, including salt and drought stress, temperature extremes, and pathogen attack (Clouse and Sasse 1998; Krishna 2003; Sasse 2003; Hacham et al. 2011).

6.2.3 Brassinosteroids Modulate the Efficiency of Plant Immune Response

One merging point that could link MAMP signaling responses to plant growth-promoting hormone is the LRR-RK BRI1-BAK1 interaction (Vert 2008; Pieterse et al. 2009; Belkhadir et al. 2012). For example, a recent study by Belkhadir et al. (2012) suggested that the activation state of $BRI1^{sud1}$ induced

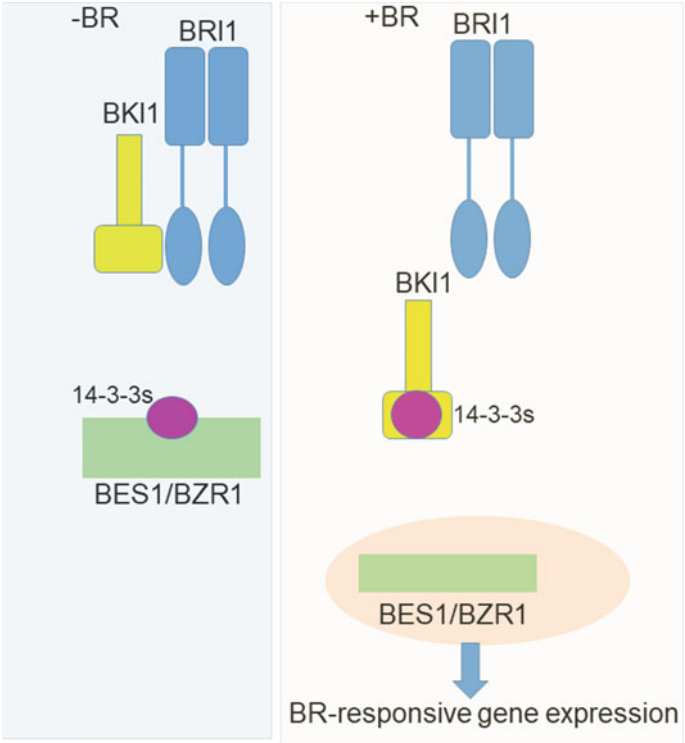

Fig. 6.2 Brassinosteroid signaling pathway. BRASSINOSTEROID INSENSITIVE 1 (BRI1), is inactive in the absence of brassinosteroid (BR), due to the negative regulator, BRI1 KINASE INHIBITOR 1 (BKI1). In the presence of BR, plasma membrane BKI1 dissociated and interacted with a subset of 14-3-3 proteins to release inhibition of BRI1 by BKI1. The cytosolic BKI1-14-3-3 s interaction enhances the accumulation of BRI1 EMS SUPPRESSOR 1 (BES1)/BRASSINAZOLE RESISTANT 1 (BZR1) in the nucleus to regulate BR-responses

susceptibility to hemibiotrophic pathogens, and the enhanced susceptibility was not observed in plants, in which BR signaling was attained through increased BRI1 dosage. On the other hand, very slight enhancement of the BR pathway induced both BAK1-independent and BAK1-dependent changes during interactions with an obligate biotrophic pathogen. These results were in line with the result of Albrecht et al. (2012), who suggested that BR signaling modulates plant immunity in a BAK1-independent manner. These MAMPs act via explicit pattern-recognition receptors to activate the mitogen-activated protein kinase (MAPK), leading to oxidative burst that stop microbe proliferation (Segonzac and Zipfel 2011). Also, flg22 binding to FLS2 induces rapid association and transphosphorylation with BAK1, and activated FLS2 phosphorylates the receptor-like cytoplasmic kinase BIK1 to transduce the signal to BR-induced BRI1 signaling (Lu et al. 2010; Segonzac and Zipfel 2011; Wang 2012). The data suggested that, BAK1 is working as co-receptor for both BRI1 and FLS2, by competing each other for BAK1 or

enhance each other by increasing the cellular pool of active BAK1 (Chinchilla et al. 2009; Wang 2012). Tobacco (*Nicotiana tabacum*) plants treated with BL displayed improved resistance against the bacterial pathogen *Pseudomonas syringae* pv. tabaci, tobacco mosaic virus (TMV), and the fungal pathogen *Oidium* sp (Nakashita et al. 2003). In addition, the measurement of SA level in BL-treated tobacco plants and SA-deficit line using NahG transgenic plants indicated that BL-induced resistance does not require SA biosynthesis, and BL application did not trigger the expression of basic or acidic pathogenesis-related (PR) genes, suggesting that BL-induced resistance is distinct from wound-inducible disease resistance and systemic acquired resistance (Nakashita et al. 2003). The overexpression of *Arabidopsis* BR biosynthetic gene *AtDWF4* in *Brassica napus* displayed an increase in root biomass and root length, improved seed yield leading to increased overall oil content per plant, significantly better tolerance to heat stress and dehydration, and enhanced resistance to necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Leptosphaeria maculans* (Sahni et al. 2016). These results indicated that BR can simultaneously enhance plant performance and productivity under stress conditions. In recent study, transgenic creeping bentgrass (*Agrostis stolonifera* L.) overexpressing *Arabidopsis BR-related acyltransferase 1* (*AtBAT1*), a BR-inactivating enzyme, which catalyze the conversion of BR intermediates to inactive acylated conjugates, exhibited BR-deficient phenotypes, including shortened internodes and reduced plant height (Han et al. 2017). In addition, the *AtBAT1* transgenic plants exhibited delayed senescence as well as drought tolerance (Han et al. 2017). Disease resistance approaches using the components of the BR-signaling pathway have the capacity to be versatile and effective. However, their achievement relays on managing the trade-offs between pathogen defense and plant growth. There is mounting evidence that hormone signaling crosstalk plays significant roles in survival trade-offs, thus by cracking this crosstalks through targeting a precise molecular mechanism, it will become possible to find more robust disease resistances without negatively affecting the crop yield. Therefore, it is essential to manipulate and identify the molecules that play direct roles in regulating crosstalks. These are probably among the most critical challenges for enhancing the disease resistance of crop species in the near future.

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Reactive Oxygen Species Generation, Scavenging and Signaling in Plant Defense Responses

7

Abbu Zaid and Shabir H. Wani

Abstract

Plants grow in an environment of abiotic stresses such as drought, high light (HL) intensity, heat, salinity, metal/metalloid, or a combination of these environmental stresses requires a delicate balance between energy production and consumption, to mention normal energy. Reactive oxygen species (ROS), a by-product of aerobic metabolism, are key signaling molecules which play a significant role in plants' responses to myriad of abiotic and biotic stresses. ROS initially evidenced as only damaging factors in plants further were found to play an important role in numerous signaling pathways that mediate plants' acclimatory and defense responses. The production and scavenging of ROS are accomplished in various cellular compartments such as the apoplast, cell membrane, mitochondria, chloroplasts, peroxisomes, and endoplasmic reticulum. Under abiotic stresses, an imbalance between ROS biosynthesis and scavenging and elimination in favor of biosynthesis with certain consequences for plant cell physiology has been termed as "oxidative stress." Regulation of redox environment and ROS signals via the cross talk of ROS with various signaling agents within plants' cell requires a high degree of coordination in different cellular compartments. In this present chapter, we provide an update on ROS generation, scavenging, and redox signaling in the context of plant abiotic stress tolerance. Unraveling destabilizing and stabilizing factors of ROS homeostasis and signaling in plants under biotic and abiotic stress environment

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may provide a detailed exploration of ROS/antioxidative signature-related kinetics that can help in designing new and sustainable innovative ways and means for (a) mounting proper acclimation response, (b) monitoring/increasing overall plants' fitness in improving health and productivity of plants under the influence of various stress conditions, and (c) identification and characterization of new targets and key regulator ROS-signaling transduction pathways which may provide excellent future candidates for breeding/engineering stress-resilient crop plants.

Keywords
Reactive oxygen species · Oxidative signaling · Oxidative stress · Plant defense responses

7.1 Introduction

Various abiotic pressures orchestrate the formation of reactive oxygen species (ROS) in plants, thus leading to severe alterations in plants' physiological, biochemical, and molecular process (Mahmud et al. 2017; Wani et al. 2018b). The ROS homeostases are most crucial events during oxidative stress-related metabolism in plants because ROS play a dual role in plants in a dose-dependent fashion, by acting as signaling molecules at low levels, and inducers of oxidative stress at the high levels (Chen et al. 2015; Requena et al. 2017; Mohanta et al. 2018; Wani et al. 2018a). As ROS accumulation poses negative impacts on plant cells, however, they regulate processes like systemic acquired resistance (SAR) and systemic acquired acclimation (SAA) during acclimation and defense responses in plants (Abdelrahman et al. 2016, 2017b; Czarnocka and Karpiński 2018). Thus, it cannot be ruled out that ROS are involved in diverse facets of development and metabolism of plants by regulating a plethora of oxidative and reductive signals and by acting as potential regulators of metabolic and energy fluxes in living organisms.

Superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydroperoxyl radical ($HO_2^{\cdot}$), alkoxy radical ($RO^{\cdot}$), peroxy radical ($ROO^{\cdot}$), excited carbonyl (RO^{*}) are free radical and hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\blacktriangle g$ or $^1O_2$), are non-radical molecular forms which are partially reduced or activated forms of atmospheric molecular oxygen (O_2), and are considered as ROS, and their high concentrations are considered as cytotoxic to plants tissues (Gill and Tuteja 2010; Vellosillo et al. 2010; Karuppanapandian et al. 2011; Abdelrahman et al. 2017a; Del Río 2015; Choudhury et al. 2017) (Fig. 7.1). ROS are regarded as unavoidable biochemical by-products of normal aerobic life that appeared on the surface of the Earth about 2.2–2.7 billion years ago, and their production is generally confined to cellular organelles having high flow of electrons like chloroplast, mitochondria, and peroxisomes (Choudhury et al. 2013) in addition to the apoplast

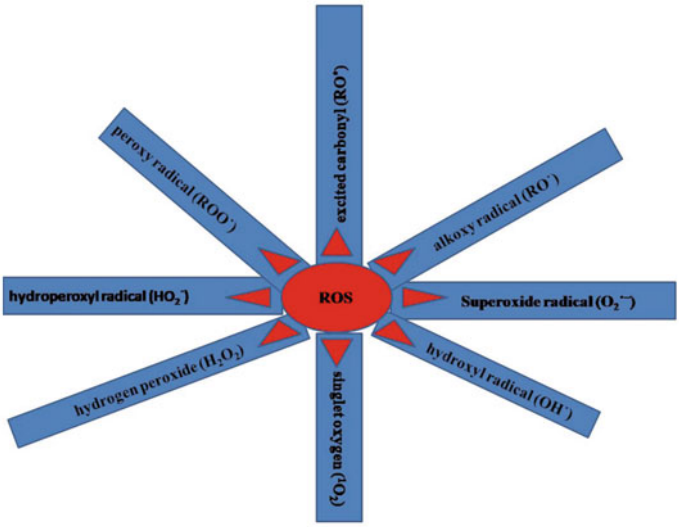


Fig. 7.1 A schematic representation showing free radical and non-radical forms of reactive oxygen species

(Roychoudhury and Basu 2012). About 1–2% of the molecular O_2 which is utilized by plants is sidetracked to lead the generation of ROS (Bhattacharjee 2005; Banerjee and Roychoudhury 2017). O_2 is a free radical and strong oxidant molecule because it contains two unpaired electrons having the same spin quantum number. This property makes it able to accept electrons, consequently leading to the generation of ROS in aerobic organisms. Anaerobic organisms also produce ROS and are thus having a well ROS detoxification system (Ślesak et al. 2012). Thus, we can say that both prokaryotic evolution and eukaryotic evolution took place in the presence of ROS-rich environment. In addition to various abiotic and biotic stress conditions, ROS are also produced under controlled conditions which lead to oxidative signaling in plants if they are consequently sequenced by antioxidants and osmolytes (Wani et al. 2018a, b). Under physiological circumstances, ROS are indispensable players for maintaining proper cellular metabolism, regulation of essential processes like proliferation, differentiation, and development of cells, light acclimation, cytoplasmic signaling reactions, pathogen resistance, hormonal signal transduction, and programmed cell death (Swanson and Gilroy 2010; Karpiński et al. 2013; Foyer and Noctor 2013; Gilroy et al. 2016; Mittler 2017).

However, biotic and abiotic perturbations such as high salt concentrations (Rasool et al. 2013; Ahmad et al. 2018), incidence of UV radiation and ozone (Yu et al. 2004; Yan et al. 2016; Chen et al. 2018), occurrence of drought (Huseynova et al. 2016; Sezgin et al. 2018), high and low temperature

(Li et al. 2015; Sailaja et al. 2015; Tahmasebi and Pakniyat 2015; Muneer et al. 2017; Abdelrahman et al. 2017a), heavy metal/metalloid accumulation (reviewed by Wani et al. 2018b; Kohli et al. 2018; Shi et al. 2018; Zaid et al. 2019), deficiency of mineral nutrients (Gill and Tuteja 2010; Liu et al. 2018), air pollution (Lodovici and Bigagli 2011; Lakey et al. 2016), hazardous gases (Muneer and Lee 2018), herbicides (Islam et al. 2016), and pathogen attack (De Gara et al. 2003; Torres et al. 2006; Torres 2010) lead to abrupt increase in endogenous ROS levels which in turn can lead to a state of "oxidative stress," thereby altering normal activities and causing cell death (Fig. 7.2) by damaging genetic makeup, oxidation of proteins, peroxidation of lipid bilayer, and leakage of ions. ROS accumulation due to various environmental stresses is a principal factor of decrease in global crop productivity (Khan and Singh 2008; Tuteja 2010; Khan and Khan 2017). In the complete sequence of events, ROS can lead to the initiation of new responses by triggering the expression of new genes. However, plants employ a sessile lifestyle and for countering oxidative stress-induced ROS bioaccumulation, they have evolved antioxidant defense systems that include enzymatic antioxidants which include battery of scavenger proteins, such as superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), and peroxiredoxin (PRX) and non-enzymatic antioxidants, such as ascorbic acid (vitamin C), reduced glutathione (GSH), tocopherols (vitamin E), carotenoids, and phenolic compounds (Ahmad et al. 2010; Rejeb et al. 2014; Inupakutika et al. 2016; Dar et al. 2017; Pandey et al. 2017; Abdelrahman et al. 2018; Mohanta et al. 2018). Also, osmolytes such as proline and glycine betaine present in microbes, animals, and plants are known to alleviate the inhibitory effects of ROS (Kumar and Yadav 2009; Iqbal et al. 2015; Noreen et al. 2018) (Fig. 7.2).

7.2 Types of ROS, Their Chemistry, and the Underlying Detoxification Systems

As mentioned in the above section, there are various types of ROS which are generated under various stressful environments. According to Temple et al. (2005), the presence of atmosphere O_2 enabled metabolism of respiration and energy transfer systems to use O_2 as terminal oxidant. This leads to ROS formation in cells. Atmospheric O_2 can relatively give rise to various intermediate ROS by the univalent reduction reactions, which otherwise is non-reactive in its ground state (Scandalios 2005). Also, the availability of d block elements such as copper and iron, which further catalyze the reactions through the Haber-Weiss mechanism or the Fenton reaction, gives rise to the formation of $OH^{\cdot}$, which is regarded as the most reactive chemical species in the biological systems. In the accompanying section, we are schematically representing the formation of various ROS in biological world.

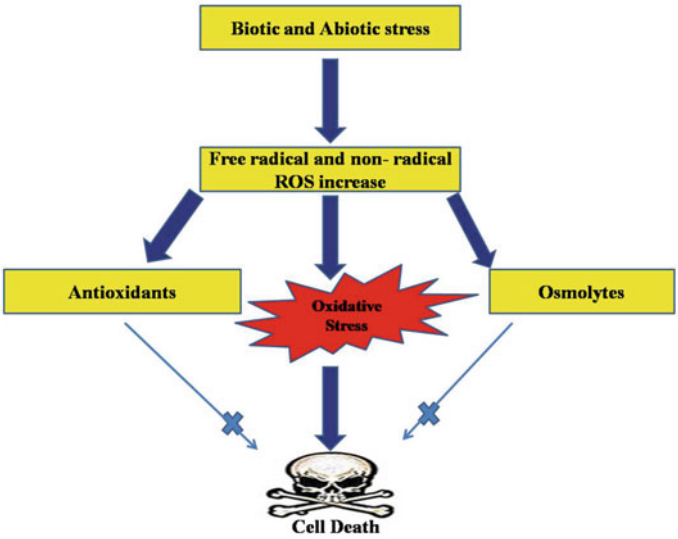


Fig. 7.2 A schematic representation depicting the production and detoxification of reactive oxygen species in plants through the antioxidant defense system

(1) **Hydroxyl radical ($OH^{\cdot}$)**

$$H_2O_2 + O_2^{\cdot -} \xrightarrow{Fe2+,3+} OH^- + O_2 + OH^{\cdot}$$

(2) **Superoxide radical ($O_2^{\cdot -}$)**

$$O_2^{\cdot -} + Fe^{3+} \rightarrow {}^1O_2 + Fe^{2+}$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2Fe^{3+}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^- \text{ (Fenton reaction)}$$

(3) **Singlet oxygen ($^1O_2$)**

$$Chl \rightarrow {}^3Chl$$

$$3Chl + {}^3O_2 \rightarrow Chl + {}^1O_2$$

(4) **Hydrogen peroxide (H_2O_2)**

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

These ROS are generated continuously at chloroplast, mitochondria, endoplasmic reticulum, peroxisomes, apoplast, cell membrane, and cell wall. The generation of ROS in different cell components has been depicted in Fig. 7.3.

A brief description of their generation in cell compartments is as follows:

Hydroxyl radical ($OH^{\cdot}$) is generated by Fenton reaction and is most reactive ROS known. In cell systems, ($OH^{\cdot}$) radicals are largely responsible for oxidation of DNA, lipids, proteins (Sharma et al. 2012; Sewelam et al. 2016). They have the distinction in cells in the manner that ($OH^{\cdot}$) radicals do not have any enzymatic antioxidants for their elimination (Vranová et al. 2002, Pinto et al. 2003; Gill and Tuteja 2010).

Operation of photosynthesis under stress conditions results in absorption of light energy in excess more than the capacity of photosynthetic machinery to utilize it through photosynthetic electron transport chain (Végh et al. 2018). This results in the formation of singlet oxygen ($^1O_2$) at photosystem PS (II) and superoxide radical ($O_2^{\cdot-}$) at PSI and PSII, respectively (Schmitt et al. 2014; Foyer 2018). As represented in Fig. 7.3, singlet oxygen ($^1O_2$) is formed by the reaction of triplet state of chlorophyll (^{3}Chl) with molecular O_2 (Das and Roychoudhury 2014). At PSII, the excess energy absorbed by the ^{3}Chl is transferred to molecular O_2 to give rise to the $^1O_2$. $^1O_2$ is thus a strong oxidant molecule that causes oxidation of macromolecules leading to cellular "damage" (Watabe et al. 2007). Singlet oxygen ($^1O_2$) is thus responsible for much of the oxidative inactivation during over-excitation of the photosynthetic electron transport chain (Telfer 2014). Superoxide radical ($O_2^{\cdot-}$) is the first ROS to be formed in plant tissues as only 1–2% of total O_2 consumption in cell tissues leads to their formation (Puntarulo et al. 1988). $O_2^{\cdot-}$ radicals may further lead to the generation of more toxic ROS like ($OH^{\cdot}$) and ($^1O_2$) as depicted above (Halliwell 2006; Gill and Tuteja 2010). H_2O_2 among all ROS is moderate ROS species and plays a dual role in plant signaling at low concentrations, it acts as a signaling molecule to mitigate biotic and abiotic stresses, at high levels, and it triggers cell death (Hossain et al. 2015; Cuypers et al. 2016; Khan et al. 2018). Production of H_2O_2 involves two-step electron reduction of $O_2^{\cdot-}$ (reaction 4). As represented in reaction 4, superoxide dismutase (SOD) catalyzes the second reduction step of $O_2^{\cdot-}$ which is finally converted into H_2O_2 (Sharma et al. 2012). As compared to other ROS, H_2O_2 has got a long half-life of 1 ms (Møller et al. 2007).

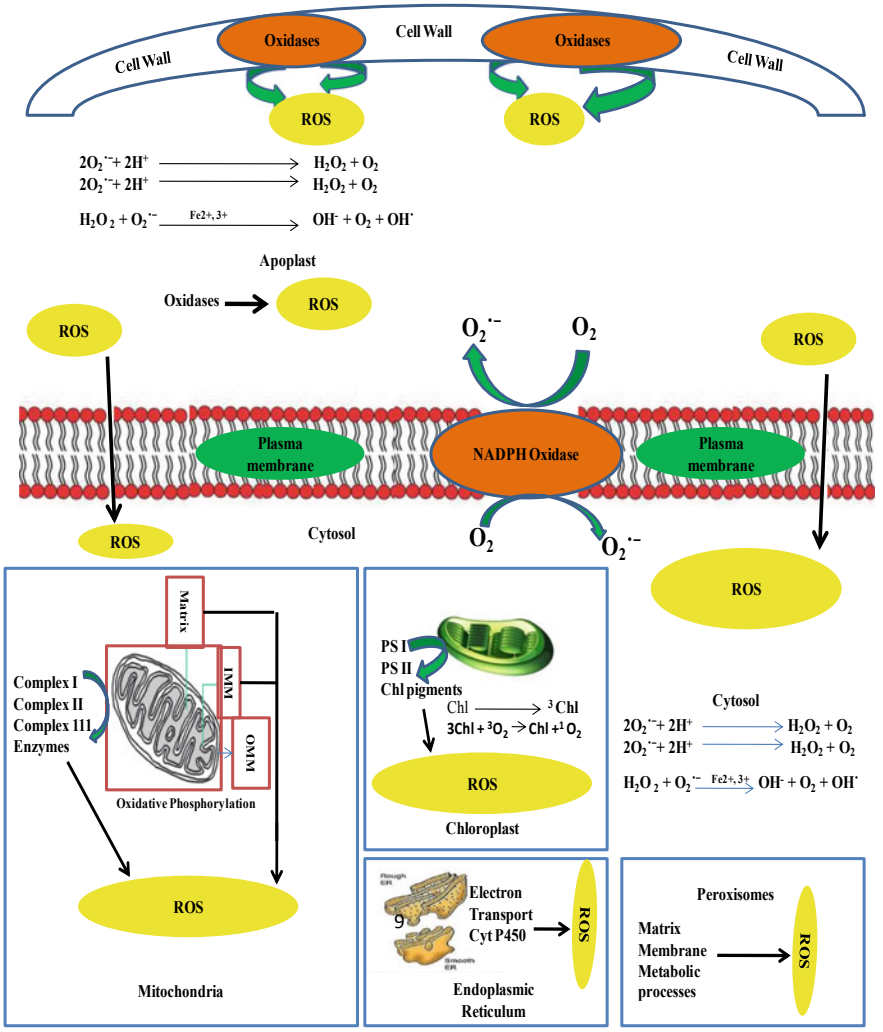


Fig. 7.3 Production sites of different reactive oxygen species (ROS) in plants. ROS are biosynthesized at various locations in the cells like chloroplast, mitochondria, plasma membrane, peroxisomes, apoplast, endoplasmic reticulum, and cell wall. The figure also shows the components of cell structures where ROS are produced

7.3 Antioxidant Batteries in Plants for Excess ROS Detoxification

As mentioned in the introductory part that plant stress tolerance mechanisms involve activation of the antioxidant defense system. The antioxidant defense system among others consists of antioxidants which are present in almost all cellular compartments that demonstrate their ability to detoxify ROS for plant survival. As ROS trigger the gene expression and signal transduction pathways in response to various stress-response programs, thus the antioxidant proteins are activated as and when ROS concentration exceeds the threshold. Here in the present section, we have covered the components of an antioxidant defense system that include enzymatic (SOD, CAT, APX, MDHAR, DHAR, and GR) and non-enzymatic (GSH, ASA, carotenoids, and tocopherols) antioxidants which are directly or indirectly engaged in the detoxification of ROS.

7.3.1 Enzymatic Antioxidants

7.3.1.1 Superoxide Dismutase (SOD, EC 1.15.1.1)

SOD is an intracellular ubiquitous enzymatic antioxidant which belongs to the metalloenzyme family present universally in all aerobic organisms. SOD is known to provide first the line of defense against excess ($O_2^{\cdot -}$) in the chloroplast, mitochondria, peroxisomes, and cytosol (Gill and Tuteja 2010).

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The reaction shows the dismutation of $O_2^{\cdot -}$ radical into molecular oxygen and hydrogen peroxide, and the reaction rate is 10,000 times faster than the spontaneous dismutation (Das and Roychoudhury 2014). SOD contains isoenzyme cofactors, viz. Mn-SOD, Fe-SOD, and Cu/Zn-SOD (Alscher et al. 2002), all of which function in the dismutation of $O_2^{\cdot -}$, thus overcoming $O_2^{\cdot -}$ radical-induced oxidative stress. An increase in SOD activity has been reported in diverse plants subjected to various environmental pressures such as salt stress (Ahmad et al. 2018), heavy metal toxicity (Zaid and Mohammad 2018; Zaid et al. 2019), pesticide stress (Fatma et al. 2018), ozone (Chen et al. 2018), wounding (Si et al. 2017, 2018), cold stress (Wani et al. 2018c; Sheteiwy et al. 2018), and drought (Ahmad et al. 2017; Moazzam-Jazi et al. 2018).

7.3.1.2 Catalase (CAT, EC 1.11.1.6)

Catalases are heme-possessing antioxidant enzymes having ability to directly scavenge H_2O_2 into H_2O and O_2.

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

As reviewed by Gill and Tuteja (2010), CAT has highest reaction turnover rates and one molecule of CAT can dismute approximately 6 million molecules of H_2O_2 to H_2O and O_2 in the 60 s time span. Animal cells contain only one CAT isoform, while plant cells are characterized by a couple of CATs (Iwamoto et al. 1998). As peroxisomes are the main sites of H_2O_2 production, however, CATs in plants also exist in chloroplasts, mitochondria, and cytosol. The expression and activity of CATs are triggered when plants are exposed to various kinds of abiotic stresses such as nematode (Vicente et al. 2015), arbuscular mycorrhizal fungi (Hashem et al. 2018), heavy metal (Zaid and Mohammad 2018), drought (Wang et al. 2018a), salinity (Fariduddin et al. 2018), cold (Jan et al. 2018a), heat (Rai et al. 2018), and UV radiation (Mariz-Ponte et al. 2018).

7.3.1.3 Enzymes of Ascorbate–Glutathione (AsA-GSH) Cycle

The AsA-GSH pathway consists of four enzymes, namely ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Fig. 7.4), and two non-enzymatic antioxidant proteins, viz. ascorbate (AsA) and glutathione (GSH). In the accompanying section, we are discussing them one by one that how ASA-GSH pathway operates to eliminate excess ROS in plants.

7.3.1.4 Ascorbate Peroxidase (APX, E.C.1.1.11.1)

APX is the first enzyme of the AsA-GSH cycle, which detoxifies H_2O_2 by causing peroxidation of AsA and yielding water and monodehydroascorbate (MDHA) radical (Asada 1999; Pandey et al. 2015) (Fig. 7.4). MDHA is then either converted to dehydroascorbate (DHA) non-enzymatically or reduced back to AsA by an enzyme (MDHAR). The reaction involved is represented below:

$$H_2O_2 + 2AsA \overset{APX}{\rightarrow} 2H_2O + 2MDHA\ (\rightarrow 2DHA)$$

Five APX isoforms have been discovered in plants, namely cytosolic (cAPX), mitochondrial (mitAPX), stromal (sAPX), membrane-bound APXs present in chloroplasts (tAPX), and peroxisomes/glyoxysomes (Asada 1999; Caverzan et al. 2012; Anjum et al. 2014). Over-expression of genes related to APX has been shown to mediate stress tolerance to various abiotic stresses in various crop plants.

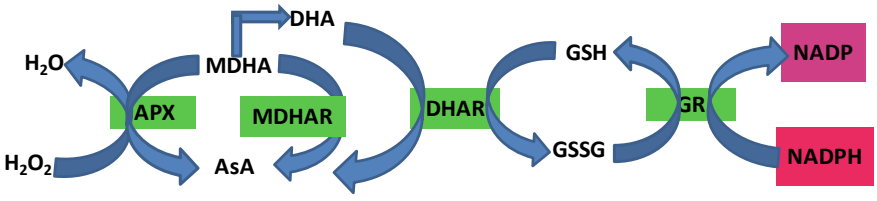
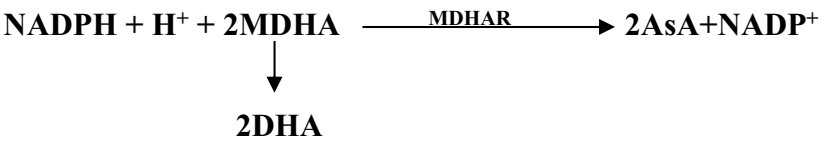


Fig. 7.4 Schematic representation of ascorbate–glutathione cycle showing enzymes and formation of reducing equivalents. Details are described in the text

For example in *Jatropha curcas* plants, quantitative polymerase chain reaction (qPCR) analysis showed over-expressing a thylakoid APX was responsible for conferring salt stress tolerance (Liu et al. 2013). In another study, ectopic over-expression of the peroxisomal APX gene (*SbpAPX*) conferred salt stress tolerance in *Arachis hypogea* plants (Singh et al. 2014). In a recent study, Balfagón et al. (2018) found that APX is the chief protein to be involved in citrus tolerance to combined conditions of drought and high temperatures. In sorghum, Akbudak et al. (2018) reported the genome-wide identification and expression profiling of APX gene families under drought stress conditions and found that APX families in leaves and roots showed significant changes in their expression levels, which, therefore, regulate drought stress tolerance. Employing bioinformatics approaches, Ozyigit et al. (2016) presented a comparative evaluation of APX gene/protein families in 18 different plant species. They analyzed the exon/intron organization of APX, studied and identified conserved motif signatures of APX, constructed the phylogenetic trees and 3D models of APX families, and analyzed the APX gene expression profiles. They conclude that APX is major H_2O_2-scavenging enzymes in plants.

7.3.1.5 Monodehydroascorbate Reductase (MDHAR, E.C.1.6.5.4)

MDHAR is a flavin adenine dinucleotide (FAD) enzyme which is responsible for rejuvenating AsA from the short-lived MDHA radical by using NADPH as an electron donor agent, thus maintaining reduced AsA pool in cellular tissues (Sharma et al. 2012). MDHA can react non-enzymatically to form DHA (Fig. 7.4). The reaction catalyzed by MDHAR is represented below:



MDHAR contains several isozymes present in the chloroplast, mitochondria, peroxisomes, cytosol, and glyoxysomes (Foyer and Halliwell 1976; Reumann and Corpas 2010). Modulated MDHAR activity has been observed in diverse crop plants in response to various abiotic stresses such as salinity (Ahanger et al. 2018), metal toxicity (Jan et al. 2018b; Hasanuzzaman et al. 2017), drought stress (Sharma and Dubey 2005), ultraviolet-B stress (Shiu and Lee 2005), and high-temperature stress (Nahar et al. 2015). Nevertheless, over-expression of MDHAR gene (*AtMDAR1*) in the cytosol has been shown to minimize the deleterious effects of ozone, salt, and polyethylene glycol-induced stress in transgenic tobacco plants (Eltayeb et al. 2007). These transgenic plants were found to exhibit up to 2.1-fold higher MDHAR activity as compared to wild-type plants. In yet another experiment, Li et al. (2010) observed that over-expression of chloroplastic MDHAR increased tolerance to temperature and methyl viologen-induced oxidative stresses by alleviating photoinhibition of PSI and PSII and elevating AsA pool. These results

suggest that an over-expressed MDHAR level confers enhanced tolerance against multiple abiotic stresses in plants.

7.3.1.6 Dehydroascorbate Reductase (DHAR, EC.1.8.5.1)

DHAR brings the reduction of DHA to AsA using reduced glutathione (GSH) as the reducing substrate (Ushimaru et al. 1997; Das and Roychoudhury 2014) (Fig. 7.4). It is thus the second enzyme apart from MDHAR which maintains the redox pool of AsA in plant cells (Qin et al. 2011). The reaction catalyzed by DHAR is given below:

$$\mathrm{DHA} + \mathrm{GSH} \overset{\mathrm{DHAR}}{\rightarrow} \mathrm{AsA} + \mathrm{GSSG}$$

DHAR also showed tolerance to abiotic stress. In an experiment, Eltayeb et al. (2011) demonstrated that transgenic potato plants over-expressing Arabidopsis *AtDHAR1* gene showed tolerance against herbicide, drought, and salt stresses.

7.3.1.7 Glutathione Reductase (GR, E.C.1.6.4.2)

GR is a flavoprotein having a disulfide bond which catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduce glutathione (GSH), is thus important for maintaining the reduced redox pool of GSH, and thus maintains homeostatic redox balance in cellular environment (Ghisla and Massey 1989; Gill and Tuteja 2010; Achary et al. 2015) (Fig. 7.4). As depicted in Fig. 7.4, GSH is used to regenerate AsA from DHA by DHAR enzyme and is itself converted to GSSG. GR is thus a crucial enzyme in AsA-GSH cycle to maintain GSH/GSSG ratio. The reaction involved is:

$$\mathrm{GSSG} + \mathrm{NADPH} \overset{\mathrm{GR}}{\rightarrow} \mathrm{GSH} + \mathrm{NADP}$$

7.3.2 Non-enzymatic Antioxidants

7.3.2.1 Reduced Glutathione (GSH)

GSH is a tripeptide (γ-glutamyl-cysteinyl-glycine) molecule, having a low molecular weight, and is one of the crucial nonprotein sulfur-containing thiols in plants to scavenge ROS and ROS-induced oxidative damage. It has been detected abundantly in reduced form (GSH) and is present in all cellular compartments like apoplast, endoplasmic reticulum, cytosol, vacuole, mitochondria, chloroplasts, peroxisomes (Foyer and Noctor 2003). GSH provides a reducing environment by functioning as an antioxidant molecule in several ways. In plants, GSH production imparts chilling stress tolerance (Lukatkin and Anjum 2014), metal/metalloid tolerance (Per et al. 2017; Kim et al. 2017), high-temperature stress tolerance (Nahar et al. 2015), and salt stress tolerance (Zhou et al. 2018). GSH is involved in redox signaling, regulation and modulation of enzymatic activities, and expression of defense gene during biotic and abiotic stresses (Zechmann 2014; Anjum et al. 2012). In the AsA-GSH

cycle, as represented in Fig. 7.4, GSH acts as a reductant to reduce DHA to AsA enzymatically and is itself oxidized to GSSG which indicates that GSH plays a crucial role in maintaining AsA pool in the cellular environment (Noctor et al. 1998). On the other hand, GSSG is reduced back to GSH by GR in the presence of reducing equivalents. This process replenishes and maintains a cellular redox of GSH which provides a reducing environment during stress conditions.

7.3.2.2 Ascorbate

AsA (vitamin C) is the most abundant water-soluble antioxidant molecule and is regarded as a key electron donating substrate to detoxify excess ROS (Khan et al. 2011; Qian et al. 2014; Akram et al. 2017). In plant cells, biosynthesis of AsA takes place in mitochondria as a result of the Smirnoff-Wheeler pathway (Wheeler et al. 1998). AsA protects cells and their organelles from toxic ROS produced as a result of biotic and abiotic stresses (Khan et al. 2012; Naz et al. 2016), controls division of cells, and acts as a cofactor of many enzymes (Lisko et al. 2014). Exogenous application of AsA increases resistance in plants against various abiotic stresses. In a study involving young peach trees, Penella et al. (2017) applied foliar AsA to improve their performance after rewatering the plants. Their results suggested that AsA improved water stress tolerance under suboptimal water regimes. In yet another study, Xu et al. (2015) found that AsA mitigated the water stress-induced root growth in tall fescue by increasing the antioxidative defense system. In wheat plants, Alamri et al. (2018) applied AsA to improve their tolerance against lead toxicity. They concluded that AsA-induced lead stress tolerance was associated with improved plants' defense systems, content of essential nutrients, reduced chlorophyll degradation, increased cysteine accumulation, maintained relative water content, and the enhancement in the activities of enzymes like ATP sulfurylase, ribulose-1,5-bisphosphate carboxylase/oxygenase, nitrate reductase, and O-acetylserine(thiol)lyase. In *Arabidopsis*, AsA has been shown to trigger the release of the cytosolic-free calcium, which is essential in plant signaling phenomenon (Makavitskaya et al. 2018). Thus, it is evident from the above discussion that AsA imparts stress tolerance by modulating various plant mechanisms.

7.3.2.3 Carotenoids

Carotenoids are a class of lipophilic antioxidant molecule present in plants, algae, and microorganisms (Young 1991; Ahmad et al. 2010; Abdelrahman et al. 2016; Patias et al. 2017). Carotenoids play an essential role in different plant processes and are characterized with antioxidant potential during plant stress signaling by acting as light harvesters by dissipating excess light as heat, light quenchers, and their ability to scavenge the triplet chlorophyll (3Chl*) state and ROS (Uarrota et al. 2018). They are also precursors of abscisic acid and strigolactones (Ruiz-Sola and Rodríguez-Concepción 2012). As ROS scavenger, carotenoids prevent oxidative stress and confer abiotic stress in plants. Carotenoids protect the photosynthetic machinery from ROS-induced oxidative stress (Srichandan et al. 1989). In a study carried out by Wang et al. (2018c), over-expression of alfalfa gene (*MsOr*) in tobacco increased tolerance to multiple abiotic stresses along with enhanced carotenoid content

showing possible cross talk between stress tolerance and carotenoid accumulation. In a classical study involving cyanobacterial species, Patias et al. (2017) found that the carotenoid extracts were shown to be a potent scavenger of peroxyl radical, having peroxyl radical scavenger ability. In mango plants during ROS stress signaling, Rosalie et al. (2018) proposed a link made between antioxidant system defense and carotenoid metabolism. In response to drought stress, over-expression of a carotenoid ε-hydroxylase gene (*SlLUT1*) in transgenic tobacco plants improved drought stress tolerance by maintaining photosynthesis as well as scavenging of ROS (Wang et al. 2018b). In *Arabidopsis thaliana*, Caliandro et al. (2013) highlighted the proper regulation of altered α- and β-branch carotenoid biosynthesis in maintaining leaf photoprotection and whole-plant acclimation in response to photooxidative stress.

7.3.2.4 Tocopherols

Tocopherols are considered as lipid-soluble antioxidant molecules which contribute to plant resistance to biotic and abiotic stresses (Munne-Bosch 2005; Cela et al. 2018). Tocopherols are exclusively present in thylakoid membranes or in plastids and have four isomers (α-, β-, γ-, and δ-) with α-tocopherol (vitamin E) possessing highest biological activity and antioxidant capability. Along with other antioxidants, tocopherols play a principal role in reducing ROS level in photosynthesizing apparatus and protect photosynthetic membranes from lipid peroxidation to maintain the stability of membranes under various stress environments (Munné-Bosch and Alegre 2002; Munné-Bosch et al. 2013). Supply of tocopherols increases resistance in plants against various abiotic stress conditions. In water-stressed *Vigna radiata* cultivars, Sadiq et al. (2017) applied tocopherol which considerably improved antioxidant enzyme activities (SOD, POD, and CAT), chlorophyll content, and also the composition of fresh pods in both the cultivars. In *Arabidopsis thaliana*, vitamin E played an essential role in enhancing tolerance to metal-induced oxidative stress (Collin et al. 2008). In response to 75 μM Cu and Cd treatment, transcript levels of genes encoding enzymes of the vitamin E biosynthetic pathway were found to be increased, while tocopherol-deficient (*vte1*) mutant showed an enhanced sensitivity toward 75 μM Cu and Cd treatment as compared to the relative wild-type (WT) control. As tocopherols protect PSII from photoinhibition, lack of tocopherol modulates the PSII antenna and thus the functioning of PSI and II under light conditions (Niewiadomska et al. 2018); however, in tocopherol mutants (*vte1*) action of ROS ($^1O_2$) on PSII resulted in permanent damage at light-harvesting complex II and at PSII core. In response to biotic stress, an alteration in the composition of tocopherol in chloroplasts negatively influences the *Arabidopsis thaliana* response to stress condition by causing marked changes in fatty acid membrane composition, highest peroxidation of lipids, and altered activation of the defense system (Cela et al. 2018).

7.3.2.5 Phenolic Compounds

Phenolics are diverse secondary metabolites found widely in plant tissues. These possess antioxidant capacity. Commonly studied plant phenolics in relation to

abiotic stress are flavonoids, tannins, hydroxycinnamate esters, and lignins. Antioxidative properties of polyphenols are due to

(1) ability to chelate ions of heavy metals,
(2) high reactivity as a donor of electrons or hydrogen,
(3) radical derived from polyphenols which stabilize unpaired and unstable electrons which have chain-breaking functions (Rice-Evans et al. 1997).

Phenolic compounds engineer plants' stress tolerance. In a study involving *Scrophularia striata* plants, Falahi et al. (2018) showed that water stress alleviation by phenolic compounds is mediated by cross talk between nitric oxide and H_2O_2. Key enzymes of phenolic pathways like phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) were increased and were deployed in response to the stress mitigation. In a recent study, Siddiqui et al. (2018) found that PAL in beetroot increased when plants were infected with pathogenic microbes. Thus, these pieces of evidence clearly indicate that phenolic compounds are directly or indirectly involved in imparting resistance to a range of stresses in plants.

7.4 Conclusion and Future Directives

During the last few decades, a rich development in our knowledge of ROS chemistry, biosynthesis, and regulation in the context of abiotic stress tolerance has emerged. However, the exact underlying ROS-signaling pathways largely remain a mystery. In the present chapter, we attempt to address the regulatory role of ROS in plant abiotic defense responses and discuss at length how batteries of the antioxidant defense machinery, the antioxidant enzymes, and the non-antioxidant metabolites work in coordination to alleviate the oxidative damage induced by various ROS to engineer tolerance against various environmental stress conditions. By collecting the literature, we have tried here to unravel the basic chemistry of various ROS and the ameliorative role of various enzymatic and non-enzymatic antioxidants in imparting plant abiotic stress tolerance. Undoubtedly, the current concept may increase our understanding of the field of ROS biosynthesis and signaling in plant defense responses. Further research is needed to accurately explain the complex regulatory mechanisms that integrate ROS-signaling pathways for regulating growth and development of plants under abiotic stresses. Functional genomic techniques, along with metabolomics and proteomics, will give detailed insights into the regulation of ROS-signaling networks and the crucial role played by the antioxidant defense system during plants' responses to various environmental pressures. These concepts might pave the way for the development of transgenic approaches to engineer tolerance for optimization of crop performance, under multiple stresses in the future.

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Lipoxygenases and Their Function in Plant Innate Mechanism

8

Meenakshi Thakur and A. C. Udayashankar

Abstract

Lipoxygenases are universally distributed non-heme iron-containing dioxygenases, widely found in plants and animals. Lipoxygenases catalyzes the addition of an oxygen molecule to polyunsaturated fatty acids such as linoleic acid and linolenic acid. The syntheses of a group of acyclic or cyclic compounds collectively called oxylipins are initiated by LOX, which are products of fatty acid oxidation, with diverse functions in the cell. These oxylipins constitute a group of cyclic and acyclic compounds that coordinately amplify defense responses. Hydroperoxides transport calcium ions from outside to the inside of cell leading to activation of phospholipase A and release of polyunsaturated fatty acids from phospholipids of chloroplast membranes. These hydroperoxides are converted to oxylipins present in the chloroplast envelope by the allene oxide synthase or the hydroperoxide lyase. These phyto-oxylipins activate the gene expression resulting in a defense response against stress. Lipoxygenase pathway results in the production of traumatin, jasmonic acid, oxylipins, and volatile aldehydes that play an important role in wound healing, synthesis of antimicrobial substances in host–pathogen interactions, and membrane damage during the hypersensitive response.

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8.1 Introduction

Lipoxygenases (LOXs; EC1.13.11.12) are non-heme iron-containing oxido-reductase enzymes. These are dioxygenase enzymes that catalyze the reaction involving the addition of oxygen molecule to polyunsaturated fatty acids such as linolenic acid and linoleic acid lead to the production of unsaturated fatty acid hydroperoxides. In plant cells, depending upon developmental and environmental conditions, various isoforms of LOX are expressed in a different time in different tissues. Young tissues contain a higher amount of LOX enzyme. However, higher activity of LOX enzyme in senescing plant tissues are reported (Siedow 1991). LOXs initiate the synthesis of oxylipins as a product of fatty acid oxidation. Oxylipins are a group of acyclic as well as cyclic signaling compounds such as jasmonic acid, 12-oxo-10, 15-phytodienoic acid (12-OPDA), traumatin, etc. Besides regulating their growth and development, oxylipins play a significant role in providing immunity against abiotic and biotic stress conditions in plants (Porta and Rocha-Sosa 2002; Eckardt 2008). LOX pathway involves fatty acid oxidation to form fatty acid hydroperoxides. These hydroperoxides act as substrates for the hydroperoxide lyase (HPL) and allene oxide synthase (AOS) pathways.

In allene oxide synthase pathway, jasmonic acid is produced by the action of different enzymes such as plastid-localized 13-LOX, AOS, 12-oxo-phytodienoic acid reductase (OPR), and allene oxide cyclase (AOC) followed by three repeated β-oxidation steps (Wasternack and Hause 2013). In HPL pathway, C_6-aldehydes and traumatin are synthesized. C_6-aldehydes are volatile in nature and are further catalyzed by hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), and alcohol acyl transferase (AAT) (Liavonchanka and Feussner 2006). The products of allene oxide synthase and hydroperoxide lyase pathways, i.e., C_6-aldehydes and jasmonic acid perform a significant function in the regulation of plant defense mechanisms against various biotic and abiotic stresses (Pauwels et al. 2009).

Plant lipoxygenases are classified into two categories, based on positional specificity for linoleic acid oxygenation, i.e., 9-LOX and 13-LOX. 9-LOX lead to the oxygenation of linoleic acid at carbon atom 9 and 13-LOX at carbon atom 13 of the hydrocarbon backbone of the fatty acid and lead to the production of two groups of compounds, the (9S)-hydroperoxy- and the (13S)-hydroperoxy derivatives of linoleic acid (Liavonchanka and Feussner 2006). These hydroperoxy fatty acid derivatives derived from oxygenation of polyunsaturated fatty acids can be additionally converted to various products by enzymes involved in lipoxygenases pathway (Fig. 8.1).

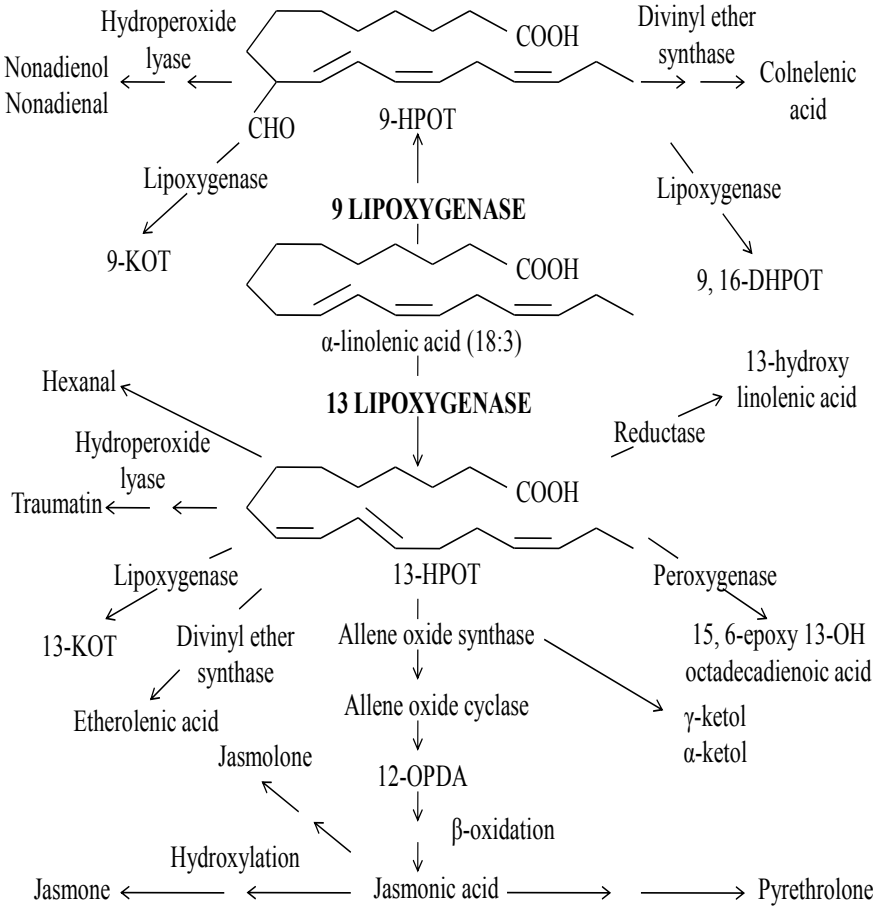

Fig. 8.1 The LOX pathway (9-KOT: (10*E*, 12*Z*)-9-keto 10,12,15-octadecatrienoic acid; 9-HPOT: (10*E*, 12*Z*)-9-hydroperoxy-10,12,15-octadecatrienoic acid; 13-KOT: (10*E*, 12*Z*)-13-keto-10,12,15-octadecatrienoic acid; 13-HPOT: (10*E*, 12*Z*)-13-hydroperoxy-10,12,15-octadecatrienoic acid; and 12-OPDA: 12-oxo-10,15-phytodienoic acid). Adapted from Porta and Rocha-Sosa (2002)

Lipoxygenases pathway results in the production of various products such as jasmonic acid, volatile aldehydes, and traumatin that play an important function in initiating signaling mechanism in plants during wound response. Besides regulating the growth and development, these compounds also act as antimicrobial compounds in host–pathogen interactions (Table 8.1). Thus, the LOX pathway leads to plant defense mechanism against pests by synthesizing different compounds with signaling functions (Creelman and Mullet 1997), antimicrobial activity (Weber et al. 1999), or by the development of hypersensitive response (Rusterucci et al. 1999).

Table 8.1 Products of LOX metabolism and their functions

Compound	Branch	Activity
13-HPOT	–	Act as mycotoxin synthesis inhibitor
9-HPOT	–	Development of hypersensitive response
Jasmonic acid	AOS	Signal transduction in stress
12-OPDA	AOS	Signal transduction in wounding and pathogen attack
C_6-volatiles (aldehydes and alcohols)	HPL	Signal transduction in wounding; attract enemies of herbivores; possess antimicrobial activities
Dinor-oxo-phytoclienoic acid	AOS	Signal transduction in wounding
9- and 13-ketodienes	LOX	Signal transduction in wounding
Traumatin	HPL	Signal transduction in wounding
(Z)-jasmone	AOS	Repel herbivores and attract enemies of herbivores; signal transduction in plant defense
Colneleic and colnelenic acids	DES	Possess antifungal activities

13 HPOT: (10*E*, 12*Z*)-13-keto-10,12,15-octadecatrienoic acid; 9-HPOT: (10*E*, 12*Z*)-9-Hydroperoxy-10,12,15-octadecatrienoic acid; 12-OPDA: 12-oxo-10,15-phytodienoic acid; AOS: Allene oxide synthase; HPL: Hydroperoxide lyase; DES: Divinyl ether synthase. Adapted from Porta and Rocha-Sosa (2002)

8.2 Proposed Model for Compartmentalization of LOXs Leading to Phyto-Oxylipin Cascades and Defense Response

Various isoforms of lipoxygenase occur in various components of cells such as cytosol, stroma, vacuole or some are associated with different membranes (Macri et al. 1994). In *Arabidopsis*, plastid envelope of leaf chloroplast and plastid stroma shows LOX activity (Blee and Joyard 1996). Lipoxygenase pathway of membrane lipids is initiated by the signal interaction with a plasmolemma receptor that leads to the activation of a membrane-bound protein and initiation of the signal circuit. Lipoxygenase circuit signals are further enhanced by autocatalytic cycles involving calcium and calmodulin ions. Lipoxygenase pathway leads to the production of hydroperoxides in the plasmolemma from linoleate and linolenate followed by the transport of calcium ions from outside to the inside of the cell (Porta and Rocha-Sosa 2002). This results in an increased calcium ion concentration within the cytoplasm, which further leads to the activation of phospholipase A. Phospholipase A, in turn, releases polyunsaturated fatty acids from phospholipids of chloroplast membrane (Liavonchanka and Feussner 2006). These fatty acids are oxygenated either by 13-LOX or 9-LOX. 13-LOX causes oxygenation of these fatty acids and results in the formation of hydroperoxides. These hydroperoxides are further acted upon by the allene oxide synthase or the hydroperoxide lyase present in the chloroplast envelope. On the other hand, phospholipase A or D releases polyunsaturated fatty acids from plasma membranes and oxygenated by cytosolic

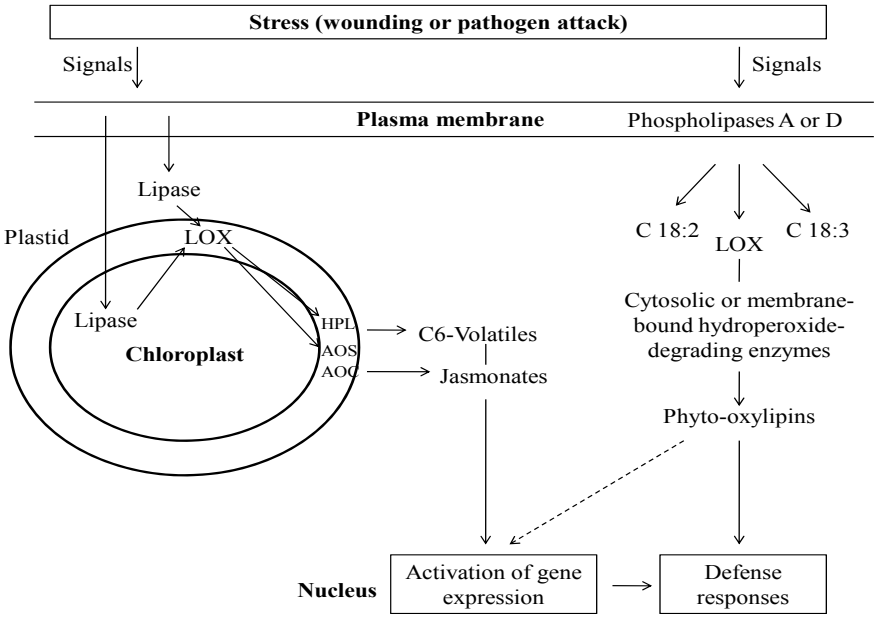

Fig. 8.2 Compartmentalization of LOXs leading to phyto-oxylipin cascades and defense response (AOC: Allene oxide cyclase; AOS: Allene oxide synthase; and HPL: Hydroperoxide lyase)

9-LOX or membrane-bound LOXs to form phyto-oxylipins. These phyto-oxylipins activate the gene expression resulting in a defense response against stress (Fig. 8.2).

The function of LOXs in plant defense mechanism has been discussed below.

8.3 Role of LOXs in Wounding Healing

In plants, wounding may occur due to herbivorous insects, animals, fungal or bacterial pathogens as well as mechanical stress. Wound healing in plants involves various defense mechanisms including strengthening of cell walls, induction of genes related to plant defense, and production of antimicrobial compounds. Products of the LOX pathway, i.e., jasmonic acid and its derivatives such as methyl jasmonates are involved in signal transduction in wounding. The function of jasmonates in defense induction in response to wounding was first demonstrated in potato and tomato by the gene expression of proteinase inhibitor I and II (Farmer and Ryan 1990; Farmer et al. 1992).

Jasmonic acid and its precursors are involved in the composite multi-component signaling system of plant innate immunity. Initial phases of hormonal signaling by exogenous jasmonates are thought to be related to the regulation of H^+ and Ca^{2+} ions transport through the cytoplasmic membrane (Ozeretskovskaya et al. 2009).

Mechanical wounding in plants caused by herbivorous animals results in quick accumulation of jasmonic acid and methyl jasmonate (Campos-Vargas and Saltveit 2002) followed by activation of main enzymes concerned in the biosynthesis of these compounds (Stenzel et al. 2003). Campos-Vargas and Saltveit (2002) observed that exogenous application of jasmonic acid on mechanically wounded plants induces the expression of protective genes including the genes of protease inhibitors and antioxidative enzyme phenylalanine ammonia lyase (PAL) gene. In another work performed by Park et al. (2002), it was observed that the inactivation of jasmonic acid synthesis genes causes the suppression of a plant protective response. In mechanically wounded tomato plants, systemin activated the expression of protease inhibitors through a general signaling pathway involving jasmonic acid (Wasternack et al. 2013).

Farmer and Ryan (1992) developed a model that involves diverse signal transduction actions including perception of the signal in response to wound followed by induction of wound-responsive genes. According to this model, as the plant cells perceive a specific stimulus such as wounding, it results in activation of a cell membrane-bound phospholipase that results in the release of linolenic acid from the membrane. Linolenic acid is oxygenated by lipoxygenases resulting in the formation of hydroperoxide that functions as a precursor for the synthesis of jasmonic acid. Jasmonic acid leads to the induction of expression of genes that synthesize various products involved in defense reactions.

Zhang et al. (2008) reported that wounding in plants leads to buildup of jasmonic acid as a result of LOX pathway. As the insects feed upon plant parts, dioxygenation of linolenic acid (18:3) and linoleic acid (18:2) occur at C_9 or C_{13} by specific LOXs resulting in the formation of (9S)- or (13S)-hydroperoxy-octadecadi(tri)enoic acids. These hydroperoxides are further transformed into 12-oxo-phytodienoic acid (12-OPDA) by allene oxide cyclase and allene oxide synthase. 12-OPDA is translocated to peroxisome and is reduced by OPDA reductase 3 (OPR3) to form jasmonic acid. Ulloa et al. (2002) observed that jasmonic acid affects the activity of calcium-dependent protein kinases (CDPK) in potato. CDPKs are serine/threonine kinases, and in plants, these play an important role in providing defense against various biotic and abiotic stresses through signal transduction (Ludwig et al. 2004). Jasmonates induce a broad spectrum of defense response including antioxidative enzymes (peroxidase and polyphenol oxidase), proteinase inhibitors, volatile organic compounds, production of alkaloid, formation of trichome and secretion of extrafloral nectar (EFN) (Kost and Heil 2005; Dickens 2006; Mao et al. 2007; Barbehenn et al. 2009; Pauwels et al. 2009; Zhao et al. 2009; Shivaji et al. 2010).

Jasmonic acid and methyl jasmonate stimulate the production of proteinase inhibitors and are involved in alkaloid release, and accumulation, provide a selective suppression of polypeptide synthesis (Santino et al. 2013). Jasmonic acid generates the active forms of oxygen and stimulates the synthesis of some protective compounds related with pathogenesis-induced plant disease resistance (Cohen et al. 1993). Jasmonic acid was found to be involved in signaling from the surface of an infected cell to the nucleus as well as in intercellular signaling (Ozeretskovskaya et al. 2009; Savchenko et al. 2014).

The defensive role of 9-lipoxygenase derived oxylipins was suggested by Vellosillo et al. (2007). It was observed that *Arabidopsis* mutant, *noxy2* that was faulty in reaction to the 9-lipoxygenase product, 9-hydroxylinolenic acid showed enhanced susceptibility to incompatible and compatible strains of *Pseudomonas syringae*. Woldemariam et al. (2018) found that *Zea mays* infested by beet armyworm (*Spodoptera exigua*) larvae resulted in elevated expression of 9-lipoxygenases as compared to 13-lipoxygenases.

Besides jasmonic acid, hydroperoxide lyase (HPL) is a dominant enzyme in hydroperoxide metabolism in leaves of many plants and due to this, volatile aldehydes that are products of HPL activity execute signaling functions. The synthesis of phenylalanine ammonia lyase, an enzyme that catalyzes lignin predecessor production and promotes thickening of cell walls, was induced by *trans*-2-hexenal (Koshio et al. 1995). PAL can catalyze salicylic acid, which provides the accumulation of hydrogen peroxide toxic for pathogens and the production of plant antibiotics—phenylpropan phytoalexins. Bate and Rothstein (1998) found that in *Arabidopsis* C_6-aldehydes, i.e., hexanal, *cis*-3-hexenal, and *trans*-2-hexenal, as well as their equivalent alcohols induce defense-related genes. These volatile compounds and alcohols are produced due to the catalytic action of the enzyme hydroperoxide lyase in damaged or wounded plant tissues. It was also shown that 4-hydroxy-2-nonenal induced the synthesis of glutathione-S-transferase that is implicated in the elimination of substances toxic for plants (Fukuda et al. 1997).

8.4 Role of LOX in the Synthesis of Antimicrobial Substances

Lipoxygenase pathway leads to the synthesis of various products that exhibit antimicrobial activity thus induce defense mechanism against pathogens (Weber et al. 1999). These molecules either exert direct antimicrobial action against plant pathogens or indirectly confer resistance in plants against microorganisms by playing an important role in fatty acid signaling between the host and the pathogen (Rosahl 1996). Rusterucci et al. (1999) proposed the role of lipoxygenases in inducing plant defense mechanism against plant pathogens either by synthesizing fatty acid hydroperoxides or by C_6-aldehydes that exhibit signal transduction mechanism. Jasmonic acid, one of the products of LOX pathway, accumulates in the leaves of tobacco during bacterial infection before cell death (Kenton et al. 1999). Rance et al. (1998) reported the increased activity of 9-LOX as well as increased *Lox1* mRNA gene expression in tobacco plants infected with *Phytophthora parasitica* var *nicotianae*. This supported the function of 9-LOX in plant defense mechanism against fungal infection as both 9-LOX activity and *Lox1* mRNA expression appeared in an incompatible plant–pathogen interaction as compared to a compatible interaction.

The primary products of the LOX pathway, i.e., hydroperoxy derivatives of linolenic acid and linoleic acid are antifungal in nature. In rice, 9-hydroperoxylinolenic acid prevents the development of blast fungus, *Magnaporthe grisea*, by inhibiting spore germination, germ tube development, and configuration of appressori. It was observed that mono- and tri-hydroxy derivatives of linolenic acid synthesized by LOX activities are highly active against plant pathogens even at minor concentrations as compared to the hydroperoxy fatty acids (Ohta et al. 1990). It was observed that the cystospore germination of *Phytophthora capsici* was inhibited by 9- and 13-hydroperoxylinoleic acids (Ricker and Bostock 1994). Other metabolites derived from the LOX pathway such as *trans*-2-hexenal at low concentration and *cis*-3-hexenol at higher concentration exhibit antibacterial activities (Croft et al. 1993). Jasmonic acid exhibiting antifungal activity in rice has been reported by Neto et al. (1991).

Lipoxygenases are accountable for lipid peroxidation during pathogen-induced plant defense mechanisms. Hwang and Hwang (2010) isolated *Xanthomonas campestris* pv. *vesicatoria* induced *CaLOX1* (encode a 9-specific lipoxygenase), 9-LOX gene, from *Capsicum annuum* (pepper) foliage and expressed in *Escherichia coli*. The enzyme activity of recombinant CaLOX1 protein was determined by incubating it with linoleic acid resulting in its hydroperoxidation with a 113.9 μm K_m value. The pepper plants with transient expression of *CaLOX1* resulted in the induction of hypersensitive response, whereas *CaLOX1*-silenced *C. annuum* plants were susceptible to *Xanthomonas campestris* pv. *vesicatoria* infection along with the low expression of genes related to plant defense and also low lipid peroxidation.

8.5 Involvement in Membrane Damage During the Hypersensitive Response

The hypersensitive response is one of the plant defense mechanisms against plant pathogens that involve rapid cell death at the infection site. Hypersensitive death leads to early physiological changes involving irreversible membrane damage owing to changes in membrane lipids. During the hypersensitive response, the peroxidation of polyunsaturated fatty acids of membranes occurs that leads to the formation of fatty acids hydroperoxy derivatives. These derivatives can experience autocatalytic degradation resulting in the production of radicals, thus initiating a series reaction of lipid peroxidation, leading to membrane damage and ultimately electrolyte leakage and rapid death of tissue (Rosahl 1996). Rusterucci et al. (1999) observed that in tobacco, cryptogein (purified protein from *Phytophthora cryptogea*) induced 9-LOX pathway that resulted in the production of free fatty acid hydroperoxides, thus playing an important role in hypersensitive response. In tobacco, both 9-LOXs and oxidative processes are crucial for the induction of hypersensitive response by the avirulent pathogen *Pseudomonas syringae* pv. *syringae* (Montillet et al. 2005).

8.6 Interaction of Abscisic Acid and Oxylipins

Abscisic acid (ABA) positively affects the LOX activity in the mechanically wounded plant (Pena-Cortes et al. 1995). ABA activates 9-LOX more than 13-LOX. When mechanically wounded, a plant forms a jasmonate-dependent response, its tissues desiccate and wounded sites accumulate ABA, expresses the cell cycle regulator ICK 1 which interacts with cycline D3, and suppresses the CDK-complexes activity (Birkenmeier and Ryan 1998). Exogenous ABA was found to stimulate lipoxygenase activity, promote jasmonic acid production, activate peroxide oxidation of membrane lipids, and contribute to the formation of tolerance when rice leaves are wounded (Roychoudhury et al. 2009). Mechanical wounds lead to an increase of LOX activity, ABA, and jasmonic acid content (Zhang et al. 2005).

8.7 Conclusion

Lipoxygenases are non-heme iron-containing fatty acid dioxygenases that cause the oxygenation of polyunsaturated fatty acids such as linolenic acid and linoleic acid present in plant cell membranes and result in the synthesis of their hydroperoxy derivatives. These derivatives are additionally converted into a variety of compounds such as C_6-aldehydes and jasmonic acid that perform a crucial part in plant defense response by synthesizing signaling as well as antimicrobial compounds and by developing hypersensitive response.

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Alkaloid Role in Plant Defense Response to Growth and Stress

9

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Abstract

In the natural habitats, plants are surrounded by a different number of enemies, including a wide variety of viruses, bacteria, fungi, nematodes, insects, and other herbivorous, which are responsible for the deleterious reduction in plant growth and production. Plants protect themselves by producing a diverse array of compounds called secondary metabolites, including terpenes, phenolics, sulfur-containing compounds, saponins, and alkaloids. Alkaloids are a diverse group of nitrogen-containing basic natural products consisting of more than 20 different classes including pyrrolidines, pyrrolizidine, quinolizidine, tropanes, piperidines, pyridines, and others. Most alkaloids are believed to function as (1) storage reservoirs of nitrogen, (2) defensive elements against predators, especially animals, vertebrates, insects as well as arthropods due to their general toxicity and deterrence capability, and (3) growth regulators, since the structures of some alkaloids are similar to known plant growth regulators. In addition, a number of alkaloids are being used as a source of medicinal drugs for thousands of years due to their structure–activity relationship, and this line of interest is still a major one for organic chemistry and pharmaceutical industries. For example, quinine, which is derived from the bark of tropical cinchona tree, has been used by Indians of South America for fever treatment and later proved to be an

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essential remedy needed for malaria disease. Considerable efforts have been carried out in the biosynthesis pathways of alkaloids and their intermediate compounds. This chapter presents an overview of the recent studies on the role of alkaloids as specific plant protectants to pathogen attack and other damaging creatures. In addition, we critically evaluate the availability and significant of alkaloid's genetic resources with desirable biotic stress resistance traits.

Keywords
Alkaloids · Plant defense · Plant–pathogen interaction

9.1 Introduction

9.1.1 What are Alkaloids

Alkaloids are naturally occurring chemical compounds containing nitrogenous organic molecules. Alkaloid name refers to the word alkaline that was used to describe any nitrogen-containing base. Most organisms produced alkaloids, including bacteria, fungi, plants, and animals and considered an essential part of secondary metabolites (Matsuura and Fett-Neto 2015). Plant alkaloids are one of the largest groups of natural products represented by approximately 12,000 natural products. Alkaloid classification depends on the presence of a basic nitrogen atom at any position in the molecule, in which nitrogen does not include in peptide bond (Robinson 1974). Plant containing alkaloids have been used by different folks for approximately 300 decades in medicines and teas, but the active compounds were not isolated and identified until the nineteenth century, and its chemical nature and structure discovered have been reported recently. Since the isolation of alkaloids in nineteenth century, they are used in the medicine of alkaloid drugs. Through the history of alkaloids, opium was the first crude drug to be chemically investigated, that had been used as an analgesic and narcotic drug for many centuries. Opium alkaloids isolated for the first time in 1803 by Derosne, three years later Sertürner (1806) recognized the alkaline nature of the somniferous principle of opium, and after ten years he titled it as morphine. From 1817 to 1820, Pelletier and Caventou discovered an exciting series of active compounds, including caffeine from coffee, strychnine from nux-vomica, emetine from ipecac, quinine and cinchonine from cinchona bark, shortly after that followed by coniine. Currently, the advanced NMR techniques and X-ray diffraction spectrometry permit the explanation of most chemically complex structures. Most alkaloids that react with acids to form salts are characterized by the crystalline shape. In the plant, they may be formed as salts or as N-oxides. As alkaloids are essential active compounds, many attempts have been made recently to produce alkaloids in plant's tissue culture. Nowadays, about 30 alkaloid compounds are commercially interested especially in medicines,

flavorings, or poisons (Bribi 2018). In ancient time, plant alkaloids had been observed and used in ancient times but without any explanation. They have been used by man more than 3000 years ago for many purposes, for example, in Mesopotamia since 2000 BCE, medicinal plants Papaver somniferum and Atropa belladonna used in therapeutic purposes, Amanita muscaria used in ancient India (Aniszewski 2007).

9.2 Alkaloids Role in Plant Defense

9.2.1 Alkaloids as Anti-Pathogens

Plants accumulate antimicrobial secondary metabolites to protect themselves. Some of these metabolites are constitutive chemical barriers to microbial attack (phytoanticipins) and (phytoalexins) as inducible antimicrobials (González-Lamothe et al. 2009; Abdelrahman et al. 2014, 2017a, b). Plant alkaloids are one of the important secondary metabolites that play a crucial role in plant defense. Plant alkaloids have both blessing and curse of nature and have the ability to produce beneficial and toxic bioactive natural compounds (Cushnie et al. 2014). Alkaloids are one of the strategies that plants use in order to defend themselves against the great variety of potential environmental threats. One of the important danger causes of plant diseases is the biotic agents (including fungi and bacteria). Figure 9.1 shows the bioactivity of some plant alkaloids used in defense against certain pathogens. Pathogens establish a close connection with their hosts to suppress and prevent plant defenses and promote the nutrient release. Plants protect themselves from pathogens by a variety of incredible strategies among them the production of toxic compounds (Freeman and Beattie 2008). Any part of the plant could produce alkaloids, and specific compounds may be limited to a certain plant's part as quinine in *cinchona* tree bark (Robbers et al. 1996). Beside microorganisms as the common source of antibiotics, higher plants have also been a source of antibiotics. Examples from higher plants as antibiotic contacting plants, *Allium sativum* (garlic) has allinine alkaloid, an effective antibiotic, and berberine alkaloid extracted from goldenseal (*Hydrastis Canadensis*) has antimicrobial effects (Trease and Evans 1972). In the following part, we discuss the role of some alkaloids as antimicrobial agents from higher plants containing alkaloids. In Table 9.1, a list of the potential role of some plant alkaloid groups and compounds as anti-pathogens. Emetine alkaloid found in the underground part of *Cephaelis ipecacuanha* and related species used as an amoebicidal drug and used for the treatment of abscesses due to the spread of *Entamoeba histolytica* infections (Iwu et al. 1999). As demonstrated in plenty of studies, secondary metabolites play a crucial role in the ecology of plants and their survival and fitness; hence in the sweet and wild-type species of *Lupinus*, alkaloids play an important role in plant defense. De la Vega et al. (1996) investigated the role of lupanine, lupinine, and gramine alkaloidal antimicrobial effect against four bacterial types: *Pseudomonas syringae P.V. phaseolicola;*

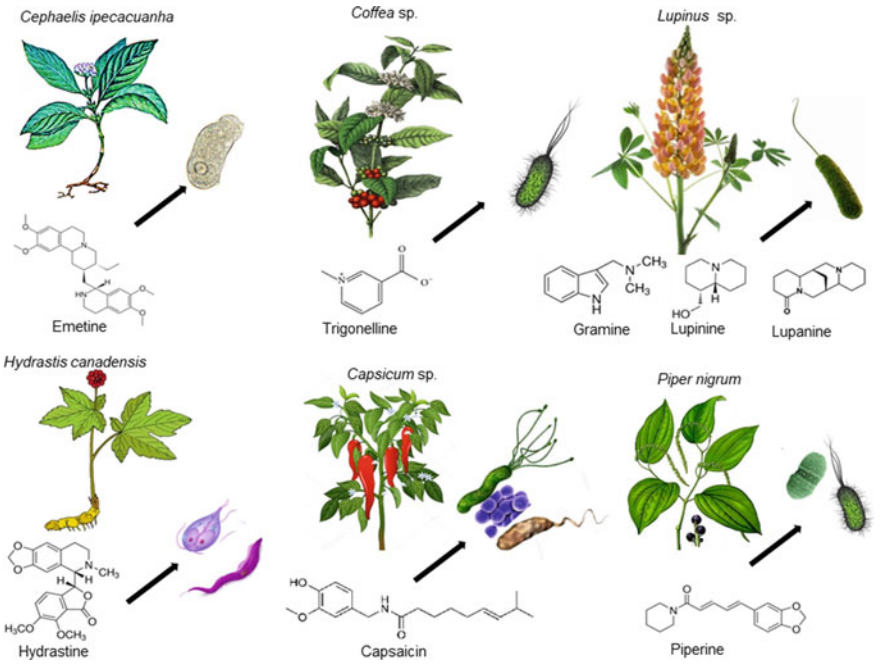

Fig. 9.1 Diagrammatic graph shows some plants and their related enemies and the role of alkaloids in defensive process

Table 9.1 Some alkaloid groups and compounds naturally occurring in plants and their defensive role in protection against pathogens

Alkaloids type	Plant species name	Pathogenic organisms	References
Lupanine, lupinine, and Gramine	*Lupinus albus (L.) and Lupinus luteus (L.)*	*Pseudomonas syringae P.V. phaseolicola; Pseudomonas syringae P.V. tomato; Pseudomonas putida; Erwinia carotovora var. carotovora*	de la Vega et al. (1996)
Quinolizidine	*Dictamnus dasycarpus*	*Erysiphe graminis*	Zhao et al. (1998)
Emetine	*Cephaelis ipecacuanha*	*Entamoeba histolytica*	Iwu et al. (1999)
α-tomatine	*Solanum lycopersicum*	*Fungi*	Eltayeb and Roddick (1984), Kozukue et al. (2004)

(continued)

Table 9.1 (continued)

Alkaloids type	Plant species name	Pathogenic organisms	References
7-demethoxytylophorine	*Cynanchum komarovii*	*Tobacco mosaic virus*	An et al. (2001)
Pyrrolizidine	*Heliotropium subulatum*	*Five bacteria, four fungi*	Singh et al. (2002)
7-deoxytransdihydronarciclasin	*Hosta plantaginea*	*Tobacco mosaic virus*	Wang et al. (2007)
Trigonelline	*Coffea*	*Escherichia coli, and Staphylococcus enterica*	López-Gresa et al. (2009), Almeida et al. (2006)
Naphthylisoquinoline	*Ancistrocladus abbreviatus, Triphyophyllum peltatum*	*Botrytis cinerea*	Aniszewski (2007)
β-carboline	*Picrasma quassioides*	*Tobacco mosaic virus*	Chen et al. (2009)
Berberines	*Hydrastis canadensis*	*Bacteria*	Ettefagh et al. (2011)
Protoberberine	*Radix Berberidis, Rhizoma coptidis and Cortex Phellodendri*	*Escherichia coli, Staphylococcus aureus, Shigella dysenteriae, Streptococcus pneumoniae and Candida albicans*	Qi et al. (2013)
Capsaicin	*Capsicum*	*Helicobacter pylori, Pseudomonas aeruginosa, Vibrio cholerae, Staphylococcus aureus, and Porphyromonas gingivalis*	Marini et al. (2015)
Ricinine and its derivatives	*Ricinus communis*	*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans*	El-Naggar et al. (2017)
β-carbolines	*Peganum harmala*	*Staphylococcus aureus, Staphylococcus aureus, Escherichia coli, Candida albicans, Candida*	Suzuki et al. (2018)

(continued)

Table 9.1 (continued)

Alkaloids type	Plant species name	Pathogenic organisms	References
		intermedia, Candida krusei	
Cocaine	*Erythroxylum coca*	*Gram-negative and gram-positive cocci*	Tiku (2018)
Piperine	*Piper nigrum*	*Lactobacillus, Micrococcus, Escherichia coli, Enterococcus faecalis*	Tiku (2018)
Colchicine	*Gloriosa superba*	*Bacteria and fungi*	Tiku (2018)
Hydrastine	*Hydrastis canadensis*	*Bacteria, Giardia duodenalis, trypanosomes*	Tiku (2018)
Berberine	*Mahonia aquifolium*	*Plasmodium Trypanosomes, general*	Tiku (2018)
Reserpine	*Vinca minor*	*General*	Tiku (2018)
Mescaline	*Lophophora williamsii*	*General*	Tiku (2018)
Quinine	*Cinchona sp.*	*Plasmodium spp.*	Tiku (2018)
Reserpine	*Rauwolfia serpentina*	*General*	Tiku (2018)
Quinolizidine	*Vicia faba*	*Bacteria*	Tiku (2018)
Oleuropein glucoside	*Solanum nigrum*	*Fungi*	Tiku (2018)
Veremivirine	*Solanum nigrum*	*Fungi*	Tiku (2018)
Myristic acid	*Solanum nigrum*	*Fungi*	Tiku (2018)
Nicotine	*Tobacco*	*Bacteria and fungi*	Tiku (2018)
Berberine	*Berberis vulgaris*	*Bacteria and protozoa*	González-Lamothe et al. (2009), Tiku (2018)
6 indole alkaloids	*Kopsia genus*	*Bacteria and fungi*	Long et al. (2018)

Pseudomonas syringae P.V. tomato; Pseudomonas putida; Erwinia carotovora var. carotovora. Lupinine was the most effective bactericidal agent against the four studied bacteria while gramine was effective controller agent of *P: phaseolicola and P: tomato* (de la Vega et al. 1996). An α-tomatine alkaloid extracted from most organs of *Solanum lycopersicum* have antifungal activity as reported by Eltayeb and Roddick (1984), Kozukue et al. (2004). An et al. (2001) studied two alkaloids 7-demethoxytylophorine and 7-demethoxytylophorine from *Cynanchum komarovii* have antiviral activity against TMV ranging from 60% to 65% at a concentration of 500 and 10 μg/mL. Several angiosperm species produce nitrogen-based secondary metabolites PAs (pyrrolizidine) alkaloids. Pyrrolizidine alkaloids (PAs) are quite

toxic and help in defense against infection caused by microbes. *Heliotropium subulatum* contains five pyrrolizidine alkaloids that have antimicrobial activity against many bacteria and fungi (Singh et al. 2002). Tobacco mosaic virus (TMV) is one of the most ancient and threatening viruses for tobacco, pepper, cucumber, and ornamental crops resulting in gigantic economic losses. Many studies attempted to solve the TMV virus crisis by eco-friendly approaches using naturally occurring secondary metabolites. 7-deoxytransdihydronarciclasin alkaloid was extracted and separated from *Hosta plantaginea* exhibited anti-TMV activity (Wang et al. 2007). Trigonelline alkaloid was firstly isolated by Jahns from the seeds of *Trigonella foenum-graecum* species belong to Leguminosae, widely cultivated in India and Egypt. Trigonelline is an alkaloid extracted from coffee that has an antibacterial effect on *Escherichia coli* and *Salmonella enterica* (López-Gresa et al. 2009; Almeida et al. 2006). Naphthylisoquinoline alkaloids extracted from tropical lianas *Ancistrocladus abbreviatus* and *Triphyophyllum peltatum* inhibit the growth of *Botrytis cinerea* fungus (Aniszewski 2007). Chen et al. (2009) used β-carboline alkaloids and a quassinoid from the *Picrasma quassioides* wood as antiviral against TMV exhibited positive results. Ettefagh et al. (2011) extracted alkaloids from roots and shoots of *H. canadensis,* but they found that the higher concentration was in roots especially berberine followed by hydrastine and canadine was the lowest. Protoberberine alkaloids extracted from three medicinal plants *Radix Berberidis, Rhizoma coptidis,* and *Cortex Phellodendri* have antimicrobial activity against *E. coli, Staphylococcus aureus, Shigella dysenteriae, Streptococcus pneumoniae,* and *Candida albicans,* where *Rhizoma coptidis* has the strongest antimicrobial activities (Qi et al. 2013). The antimicrobial and anti-virulence activity of capsaicin recently had considerable attention. Capsaicin has an antibacterial effect against food-borne pathogens, *Helicobacter pylori*, and *Pseudomonas aeruginosa* and has anti-virulence activity against *Vibrio cholerae*, *S. aureus*, and *Porphyromonas gingivalis* (Marini et al. 2015). El-Naggar et al. (2017) studied and proved the antimicrobial activity of ricinine on bacterial and fungal species as follows: *S. aureus, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and C. albicans.* β-carbolines constitute a large group of indole alkaloids and are distributed widely in different types of organisms among them plants. Plants contain simple β-carboline called harmala alkaloids and were firstly discovered form *Peganum harmala* L. (Zygophyllaceae), which is being used as a traditional medicine in the Middle East and North Africa. The effect of quaternary ammonium groups in antibacterial agents causes the forthwith death of the bacterial cell by disrupting negatively charged bacterial cell membrane followed by the release of K^+ ions and other cytoplasmic constituents (Suzuki et al. 2018). Suzuki et al. (2018) were reported the antimicrobial effects of β-carboline derivatives against bacteria and fungi: *S. aureus, S. aureus, E. coli, C. albicans, Candida intermedia, Candida krusei*; they concluded the possibility of the synthesis of naturally occurring β-carboline derivatives and their N2—alkylated analogs. Tiku (2018) reported many plants as a source for many antimicrobial alkaloids. For example, cocaine alkaloid found in *Erythroxylum coca* is effective against gram-negative and gram-positive cocci. Piperine, an antibacterial and antifungal

alkaloid formed by *Piper nigrum,* has antimicrobial effects against *Lactobacillus* sp., *Micrococcus* sp., *Escherichia coli, E. faecali.* Long et al. (2018) studied the antimicrobial effect of six alkaloids extracted from *Kopsia fruticosa* areal parts against two gram-positive bacteria and five gram-negative bacteria, and antifungal activities against five pathogens.

9.2.2 Alkaloids Toxicity to Insects

Plants have designed strong strategies to detect and defend themselves against invading organisms before causing extensive damage. As plants are the main vital food source for human, so we need to protect our food supply and develop highly disease-resistant plant species, and we should understand how plants defend themselves from pathogens and herbivores. Most alkaloids are toxic to some degree and play a primary role in plant defense against microbial infection and herbivore attack. Plant alkaloids have major role described in many scientific literatures as protecting agents against herbivores because alkaloids have special characteristics such as bitter flavor, disruption of protein activity after ingestion and metabolization, and central nervous system alteration (Matsuura and Fett-Neto 2015). Table 9.2 shows the effective role of some plant alkaloids defense against selected

Table 9.2 Plant alkaloid groups and compounds and their protective relation against insects

Alkaloids type	Plant species name	Insect	References
Quinolizidine	*Lupinus sp.*	*Insects*	Keeler (1989)
Ricinine	*Ricinus communis*	*Atta sexdens rubropilosa*	Bigi et al. (2004)
Naphthylisoquinoline	*Ancistrocladus abbreviatus* and *Triphyophyllum peltatum*	*Spodoptera littoralis*	Aniszewski (2007)
Harmine derivatives	*Peganum harmala*	*Mosquitos, mustard aphid*	Zeng et al. (2010)
Colchicine	*Colchicum autumnale*	*Apis mellifera*	Mithöfer and Boland (2012)
Alkaloidal extract	*Pergularia tomentosa*	*Locusta migratoria cinerascens*	Acheuk and Doumandji-Mitiche (2013)
Demissine	*Solanum demissum*	*Leptinotarsa decemlineata and Empoasca fabae*	Fürstenberg-Hägg et al. (2013)
Caffeine	*Coffea arabica*	*Coffea feeding insects*	Matsuura and Fett-Neto (2015)
Antofine N-oxide	*Cynanchum mongolicum*	*Spodoptera litura*	Ge et al. (2015)

(continued)

Table 9.2 (continued)

Alkaloids type	Plant species name	Insect	References
Antofine	*C. mongolicum*	*Lipaphis*	Ge et al. (2015)
Chili pepper extract	*Capsicum frutescens*	*Coptotermes gestroi*	Colon et al. (2016)
Pellitorine	*Zanthoxylum piperitum*	*Culex pipiens pallens and Aedes aegypti*	Kim and Ahn (2017)
Ricinine	*Ricinus communis*	*Atta sexdens*	Santos et al. (2018)
Amabiline	*Lycoris radiate*	*Aphis citricola*	Yan et al. (2018)
Deoxydihydrotazettine	*L. radiate*	*A. citricola*	Yan et al. (2018)
Deoxytazettine	*L. radiate*	*A. citricola*	Yan et al. (2018)
3-epimacronine	*L. radiate*	*A. citricola*	Yan et al. (2018)
Galanthamine	*L. radiate*	*A. citricola*	Yan et al. (2018)
11-hydroxygalanthamine	*L. radiate*	*A. citricola*	Yan et al. (2018)
N-allylnorgalanthamine	*L. radiate*	*A. citricola*	Yan et al. (2018)
11β-hydroxygalanthamine	*L. radiate*	*A. citricola*	Yan et al. (2018)
Lycorine	*L. radiate*	*A. citricola*	Yan et al. (2018)
Colchicine	*L. radiate*	*A. citricola*	Yan et al. (2018)
Alkaloidal extract	*Catalpa ovata*	*Mythimna separata and Plutella xylostella*	Shao et al. (2018)

insects. Quinolizidine alkaloids constitute 5% of lupin seeds, which are toxic to insects as reported by Keeler (1989). Aphids are one of the pests most economically destructive of plants that transmit plant viruses and in turn leading to fungal infection causing a negative effect on photosynthesis by ingesting plant juices with their stylets and secreting honeydew, hence severe economic financial losses worldwide appear (Yan et al. 2018). Aphids are small sap-sucking insects which infest several economical important vegetable and fruit crops in different countries, including apple (*Malus domestica*), crab apple (*Malus sylvestris*), and papaya (*Carica papaya*). Yan et al. (2018) investigated the insecticidal effect of ten alkaloids including amabiline, deoxytazettine, deoxydihydrotazettine, 3-epimacronine, galanthamine, 11-hydroxygalanthamine, N-allylnorgalanthamine, 11β-hydroxygalanthamine, lycorine, and colchicine which are extracted from *Lycoris radiate* against *Aphis citricola.* Among all tested alkaloids, the first nine compounds exhibited a potential aphicidal activity against *A. citricola.* N-allylnorgalanthamine showed the best inhibitory effect as aphicidal in both *in vivo* and *in vitro* against *A. citricola.* Ricinine alkaloid has been found in all parts of *Ricinus communis* plant and has insecticidal activity against *Atta sexdens* (Santos et al. 2018). *A. abbreviatus* and *T. peltatum* plant species have naphthylisoquinoline alkaloid with insecticidal effects on *Spodoptera littoralis* (Aniszewski 2007). *Colchicum*

autumnal, family Colchicaceae produced Colchicine alkaloid which have many harmful effects to honey bee (Apis mellifera). Colchicine alkaloid is toxic and inhibits microtubule polymerization by binding to tubulin and inhibiting mitosis (Mithöfer and Boland 2012). Caffeine, present in *Coffea arabica* and various other plant species is toxic and paralyzes insects feeding on the plant as it inhibits phosphodiesterase activity and promotes an increase in intracellular cyclic AMP level (Matsuura and Fett-Neto 2015). *Cynanchum mongolicum* contains three insecticidal alkaloids: antofine N-oxide, antofine, and tylophorine as identified by Ge et al. (2015). Antofine has the highest toxicity, and the three alkaloids have significant toxicity against aphid *Lipaphis erysimi*. Most of the alkaloids secreted by plants have potent effects on insect pests; hence, these botanical nature organic molecules provide a safe source of pesticides compounds that are eco-friendly. *Pergularia tomentosa* alkaloids extracted from shoot parts had a considerable larvicidal effect on *Locusta migratoria cinerascens* (Acheuk and Doumandji-Mitiche 2013). Chili pepper (sizzling taste) has many nutritional benefits like a source for vitamin A and C, and it also has the capability to kill household insects. Capsaicin is the dominant compound found in the many varieties of chilies with other compounds. Fruit extract of *Capsicum frutescens* prepared by Colon et al. (2016) is an insecticide against household termites. Harmine derivative compounds found in medicinal plants such as *P. harmala* are active insecticide as demonstrated by (Zeng et al. 2010) against *Culex pipiens quinquefasciatus* and *L. erysimi,* compounds 1-phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, and methyl 1-phenyl-β-carboline-3-carboxylate had an insecticidal effect in both *in vitro* and *in vivo*. Three different concentrations of the total alkaloids extracted from the root bark of *Catalpa ovata* were used to investigate the insecticide activity against *Plutella xylostella,* and *Oriental armyworm* and the findings exhibited positive result as a potential insecticide (Shao et al. 2018). *Zanthoxylum piperitum* bark contains pellitorine alkaloid which is very toxic to third-instar larvae of *Mythimna separata* and *Plutella xylostella* as demonstrated by (Kim and Ahn 2017). Ricinine alkaloid extracted from *R. communis* causes the death of leaf-cutting ant (*Atta sexdens rubropilosa*) (Bigi et al. 2004). Nightshade potato (*Solanum demissum*) contains demissine alkaloid characterized by its resistant to Colorado beetle (*Leptinotarsa decemlineata*) and potato leafhopper (*Empoasca fabae*) (Fürstenberg-Hägg et al. 2013).

9.2.3 Alkaloids as Deterrents

Deterrent alkaloids play an important role as mediators in insect–plant interactions, as they are unpalatable by many herbivorous insects (Shields et al. 2008). Recently, most alkaloids are believed to play a crucial role as defensive agents against predators, especially mammals because of their toxicity and deterrence capability (Mazid et al. 2011). Most of the alkaloids occur in higher plants with 20–30% and are mostly found in dicotyledonous angiosperms with concentrations of about more than 0.01% of the dry weight (Shields et al. 2008). Livestock death disaster is

caused by the ingestion of alkaloid-containing plants. For example, in the USA, huge percentage of all grazing livestock are poisoned and lost yearly by feeding on large quantities of alkaloid-containing plants such as lupines (*Lupinus* sp.) and larkspur (*Delphinium* sp.). Alkaloid's effects in animal cells are varied as follow, may interfere with nervous system components especially the chemical transmitters, affect membrane transport, protein synthesis and activities of the miscellaneous enzymes (Mazid et al. 2011). Nine alkaloids (acridine, aristolochic acid, atropine, berberine, caffeine, nicotine, scopolamine, sparteine, and strychnine) were investigated for their feeding deterrent behavior on gypsy moth larvae, and the result was those feeding deterrent responses for all the alkaloids tested depend on alkaloid concentration. Berberine and aristolochic acid were the most potent antifeedants comparing with other tested alkaloids (Shields et al. 2008). Nicotine and capsaicin alkaloids have decreased feeding effects when applying *Henosepilachna viginti octomaculata* to Motsch (Chowanski et al. 2016). *P. tomentosa* alkaloids extracted by Acheuk and Doumandji-Mitiche (2013) mentioned above as insecticidal against larvae of *Locusta migratoria cinerascens* and also have anti-feeding effect causing weight loss of these larvae (Acheuk and Doumandji-Mitiche 2013).

9.2.4 Allelopathic Activity of Alkaloids

Although there are great contribution of alkaloids as allelopathy but little reports dealt with alkaloids involved in allelopathy: for example: alkaloids of *Datura stramonium* inhibited the germination of many crop species, and the role of lupin alkaloids in inhibition of seed germination. Of other alkaloids reported to have allelopathic activity, cocaine from *Erythroxylon* coca Lamk (coca) (Roberts and Wink 1998). Berberine, sanguinarine, and gramine alkaloids inhibited the seedling growth of *Lactuca sativa* and *Lepidium sativum* as recorded by (Matsuura and Fett-Neto 2015). Alkaloids such as quinine, cinchonidine, nicotine, boldine, lobeline, coniine, and harmaline manifested harmful phytotoxic effects to *Lemna gibba* causing cell chlorosis or death (Matsuura and Fett-Neto 2015).

9.3 Conclusion

Plants have developed multiple defense strategies against microbial infections and various types of environmental stress. Natural alkaloids obtained from plants play an important role in plant disease prevention and promoting healthcare world-wide. Alkaloids offer a diverse range of structurally unique bioactive molecules, which have been used as a significant source of useful and innovative therapeutic agents. An in-depth study on metabolic efficacy, transformation, and safety of alkaloids will accelerate their plant natural resource utilization and development. Furthermore, studies in the field of the regulation of the biosynthesis of terpenoid indole alkaloids on the level of genes and enzymes, and the feasible to clone these genes in

various plants might eventually lead to generate gene cassettes for complete pathways, which could then be used for production of valuable defensive secondary metabolites in transgenic plants or plant cell cultures with improved productivity of the desired compounds. In addition, the recent emergence of liquid chromatography–mass spectrometry will facilitate the isolation, identification, and quantification processes of different alkaloids. This revolution in mass spectrometry has significantly enabled us to generate several metabolic databases (e.g., https://www.genome.jp/kegg/pathway.html, https://metacyc.org/ and others) which can provide an in-depth insight regarding plant–pathogen interaction. Thus, the biological screening of new active alkaloids, using a wide variety of robust tools and the interactive collaboration of experts in diverse scientific disciplines in connection with studies on the role of secondary metabolism for plants, may contribute to a better understanding of resistance of plants to diseases and various herbivores.

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Endogenous Peptides: Key Modulators of Plant Immunity

10

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Abstract

Plants have sophisticated innate immune systems based on the ability of individual cells to sense danger. The system is activated upon the perception of elicitor molecules derived from invading organisms or from the host itself. Recently, a growing number of plant endogenous peptides have been identified as regulators of immune response to herbivory and/or microbial infection. Significant progress has been done to elucidate signaling mechanisms and responses triggered by elicitors. These peptides may initiate, amplify, or fine tune defense responses against attackers. Here, we present state-of-the-art findings regarding plant endogenous peptides acting as regulator of plant immunity, providing their basic features and properties. We followed the categorization based on the structure of their precursor proteins. A special focus is placed on the *Arabidopsis thaliana* plant elicitor peptide1 (*At*Pep1) and its receptors PEPR1 and PEPR2, due to the large amount of available information of these ligand–receptor pair. Finally, we present a general discussion highlighting future perspectives.

Keywords

DAMPs · Plant elicitors · Pattern-triggered immunity PTI · AtPep1 · PEPR1 · Pattern-recognition receptors PRR

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10.1 Introduction

The sessile lifestyle of plants does not allow defense against pathogens by simply moving away. Moreover, unlike metazoans, plants lack a somatic adaptive immune system and motile defender cells. Thus, to avoid pathogens, plants have developed defense strategies such as pre-formed barriers and a sophisticated two-tiered innate immune system. The latter, used to sense danger signals by individual cells, allowed plants to colonize diverse ecosystems (Jones and Dangl 2006; Yu et al. 2017). Pre-formed obstacles are considered the first line of defense. When these barriers are overcome, an immunity system known as pattern-triggered immunity (PTI) is switched on. PTI is activated upon perception by plasma membrane pattern recognition receptors (PRRs) of elicitor molecules derived from invading organisms (Yu et al. 2017; Macho and Zipfel 2014). Elicitor molecules are commonly referred as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) (Yu et al. 2017; Macho and Zipfel 2014). Exogenous elicitors are conserved and widely distributed within pathogens and non-pathogens from invading organisms (Medzhitov and Janeway 1997), thus we chose to use the term MAMPs to refer to elicitors from invading organisms throughout this work. To date, several MAMPs —PRR interactions have been well characterized in *Arabidopsis.* Among those, we found the bacterially derived peptides flg22/elf18 recognized by FLS2/EFR receptors, respectively (Robatzek et al. 2006; Zipfel et al. 2006), and chitin from fungal cell walls that binds LYK5/CERK1 receptors (Miya et al. 2007; Petutschnig et al. 2010). Likewise, bacterial cell wall peptidoglycans are recognized by LYM1/LYM3 (Willmann et al. 2011), and lipopolysaccharides by LORE receptors (Ranf et al. 2015). Based on these studies of known ligand–PRRs pairs, a series of synchronized responses with particular spatiotemporal dynamics has been proposed as a hallmark of PTI elicitation. These mechanisms collectively contribute to plant defense against a diversity of pathogens (Yu et al. 2017) (Fig. 10.1).

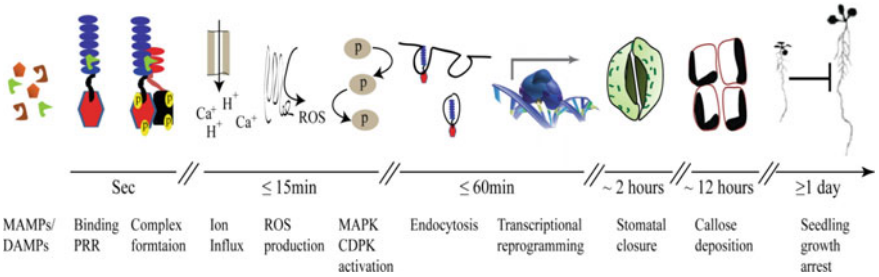

Fig. 10.1 Proposed model of pattern triggers immunity responses based on studies of known ligand–PRRs pairs. MAMPs/DAMPs are recognized by specific PRR which interact immediately with a co-regulatory receptor kinase, followed by phosphorylation and activation of intracellular kinase domains in both receptors. Early responses include increase of intracellular calcium, extracellular alkalinization, ROS production, and MAPK and CDPK activation by phosphorylation. Activated receptors are removed from the plasma membrane by endocytosis and massive transcriptional reprogramming occurs. Late responses (hours or days) after MAMP/DAMP stimuli include stomata closure, callose deposition, and seedling growth inhibition

As a response to PTI, plant pathogens have evolved a suite of diverse effector molecules secreted into host cells to interfere with pathogen detection or subsequent signaling responses (Jones and Dangl 2006; Saijo et al. 2018). In most cases, these effectors are virulence factors that promote microbial growth and disease (Boller and Felix 2009; Dodds and Rathjen 2010). However, as a counter defense to virulence effectors, plants have developed a second layer of immunity, termed effector-triggered immunity (ETI). ETI is based mostly on a large intracellular receptor's family of the nucleotide-binding leucine-rich repeat (NLR) domain class (R proteins). These proteins recognize virulence effectors directly or effector-triggered perturbation in host structures (Jones and Dangl 2006; Yu et al. 2017). ETI shows stronger responses than PTI, leading in many cases, to hypersensitive responses characterized by rapid apoptotic cell death and local necrosis (Jones and Dangl 2006; Yu et al. 2017; Dodds and Rathjen 2010).

Interestingly, PTI can also be activated by endogenous host-derived elicitor molecules released upon wounding or infection. These elicitors are recognized as danger/alarm signals by structurally similar PRRs trough which MAMPs are recognized (Yu et al. 2017; Gust et al. 2017; Zipfel 2014). Endogenous danger signals are usually referred to as damage-associated molecular patterns (DAMPs) and are thought to act with MAMPs to orchestrate an appropriate immune response (Yu et al. 2017; Albert 2013). Recently, it was proposed by Gust et al. (2017) that host-derived elicitors can be divided into two categories: (i) primary endogenous danger signals such as wall-associated or intracellular molecules passively released from plant cells upon host damage (cell debris) and (ii) secondary endogenous danger signals, which are actively processed small peptides (5–100 aa in length) with defined structures release upon tissue damage or other stimuli.

Over the last decade, numerous plant endogenous peptides have been identified as regulators of immune response to herbivory and/or microbial infection. Significant progress has been achieved elucidating their signaling mechanisms and roles in plant immunity (Gust et al. 2017; Huffaker et al. 2006; Yamaguchi and Huffaker 2011; Hou et al. 2014; Mott et al. 2014). One of the best well-characterized peptide–receptor pair in *Arabidopsis* is the plant elicitor peptide1 (*At*Pep1) and its receptors PEPR1 and PEPR2 (Huffaker et al. 2006; Krol et al. 2010; Tang et al. 2015; Ortiz-Morea et al. 2016). Elicitor peptides are typically released from large precursor proteins (Yamaguchi and Huffaker 2011). Yamaguchi and Huffaker (2011) categorized for plants these peptides in three major groups based on their precursor protein structure: (i) peptides derived from precursor proteins without an N-terminal secretion signal, (ii) peptides derived from precursor with an N-terminal secretion signal, and (iii) peptides derived from proteins with distinct primary functions (Albert 2013; Yamaguchi and Huffaker 2011). In this chapter, we present state-of-the-art findings regarding plant endogenous peptides acting as regulators of plant immunity. We followed the categorization based on the structure of their precursor proteins (Yamaguchi and Huffaker 2011) discussing their role as modulators of immunity. A special focus is placed on *At*Pep1 and its receptors PEPR1 and PEPR2, due to the large amount of available information of these ligand–receptor pair.

10.2 Peptides Derived from Precursor Proteins Without an N-Terminal Secretion Signal

This category comprises peptides expected to be exported to the extracellular space via an unconventional system (lacking a N-terminal secretion signal), including peptides derived from long precursor proteins and peptides directly translated as the active form.

10.2.1 Plant Elicitor Peptides (Peps)

Peps are the most widely studied family of defense-inducible peptides and thus have provided vast information regarding plant immunity responses and components of key signaling pathways. The first member (*At*Pep1) was isolated in *Arabidopsis* from an extract of wounded leaves using an elicitor-induced alkalinization assay on cultured cells (Huffaker et al. 2006). *At*Pep1 is a 23-aa-long peptide that matures from the carboxyl terminus of a 92 aa precursor protein known as PROPEP1 which lacks a N-terminal secretion signal (Huffaker et al. 2006; Huffaker and Ryan 2007). Originally, seven PROPEP members were described for *Arabidopsis,* and an additional PROPEP was identified by bioinformatic tools later (Huffaker et al. 2006; Huffaker and Ryan 2007; Bartels et al. 2013). Based on sequence homology on the SSGR/KxGxxN motif, all eight PROPEPs are predicted to contain a putative *At*Pep of 23–29 aa at the C-terminus (*At*Pep1 to *At*Pep8) (Bartels et al. 2013). Although only *At*Pep1 and *At*Pep5 have been biochemically isolated from *Arabidopsis* leaves, the other peptides have been synthesized and their activity confirmed (Bartels et al. 2013).

*At*Peps signaling is mediated by binding to the extracellular LRR domain of two RKsPRR, designated PEPRs. Whereas PEPR1 is able to detect all eight *At*Peps, PEPR2 can only detect *At*Pep1 and *At*Pep2 (Bartels et al. 2013). PEPR1 was identified by photoaffinity labeling and further purification from *Arabidopsis* extracts (Yamaguchi et al. 2006). Finding that T-DNA *pepr1* mutants were only partially compromised in *At*Pep1-induced responses (Yamaguchi et al. 2006), triggered the search for additional PEP receptors. PEPR2 was subsequently identified by phylogenetic analysis, and its role as *At*Pep1 receptor was experimentally demonstrated (Krol et al. 2010; Yamaguchi et al. 2010). Interestingly, the double *pepr1 pepr2* mutant completely abolished *At*Peps immune responses, indicating that *Arabidopsis* contains only two PEPRs (Krol et al. 2010; Yamaguchi et al. 2010). PEPRs belong to the XI subgroup of LRRRKs and are thus classified as kinases. PEPRs have an arginine-aspartic acid (RD) motif in the catalytic site, differing from FLS2 and EFR proteins, which are non-RD kinases (Yamaguchi et al. 2010). Non-RD kinases generally show weak autophosphorylation activity, and there is a significant correlation between the absence of this motif and a role in the early events of innate immune signaling (Dardick et al. 2012). The crystal structure of the extracellular PEPR1LRR complex with *At*Pep1 has been

determined, demonstrating that the conserved C-terminal portion of *At*Pep1 coordinates the *At*Pep1 binding to PEPR1LRR. Moreover, the non-conserved N-terminal sides of *At*Peps might possibly contribute to the preferential recognition of *At*Pep1 and *At*Pep2 by PEPR2 over other *At*Peps (Tang et al. 2015).

After recognition of *At*Peps by PEPRs, PTI responses resembling those triggered by FLS2/EFR upon bacterial MAMPs sensing, are activated (Bartels and Boller 2015). Upon ligand binding, PEPRs interact with the co-receptor BAK1 followed by phosphorylation of both BAK1 and PEPRs (Tang et al. 2015; Schulze et al. 2010). Additionally, the RLCK BIK1 that constitutively interacts with PEPR1 and probably with PEPR2 also gets phosphorylated (Liu et al. 2013). Subsequently, induction of ion fluxes across the plasma membrane, ROS and ethylene production as well as MPK3 and MPK4 activation are quickly triggered (Ortiz-Morea et al. 2016; Krol et al. 2010; Ranf et al. 2011). Then, the PEPR-*At*Pep1 complex is internalized via clathrin-mediated endocytosis (CME) and transported to the lytic vacuole, passing through early and late endosomal compartments (Ortiz-Morea et al. 2016).

In addition, *At*Peps regulate transcriptional reprogramming, inducing expression of pathogen defense genes, such as *PDF1.2*, *MPK3*, *PR-1* and *WRKY* (Huffaker and Ryan 2007; Yamaguchi et al. 2010). The fact that exogenous *At*Peps induce the expression of their own precursor genes (except *At*Pep6) and receptors, potentially indicates a positive feedback loop in *At*Peps-PEPRs signaling (Huffaker and Ryan 2007; Yamaguchi et al. 2010). Callose deposition and seedling growth inhibition occur when seedlings are maintained in the presence of *At*Peps (Krol et al. 2010; Liu et al. 2013; Ranf et al. 2011). Curiously, in contrast to flg22 that affects the whole seedling process, the inhibitory effect of *At*Pep signaling impairs mainly root growth (Krol et al. 2010; Ranf et al. 2011). Moreover, results showing that exogenous applications of *At*Peps induce a set of similar responses hint at functional redundancy (Bartels et al. 2013; Yamaguchi et al. 2010).

Bioinformatics analysis and expression localization experiments revealed that PROPEPs expression patterns differ temporally and spatially under normal and stress conditions (Bartels et al. 2013). This implies differential physiological roles among *At*Pep members (Bartels et al. 2013; Bartels and Boller 2015). Expression of *PEPR1* and *PEPR2* in *Arabidopsis* showed overlapping patterns in leaves and roots. In roots, *PEPR2* expression is restricted to the central cylinder, whereas *PEPR1* is present on most root tissue (Ortiz-Morea et al. 2016; Bartels et al. 2013; Ma et al. 2014). Curiously, when the expression of *PEPRs* was assessed by fusing their promoter sequence to the reporter β-glucuronidase gene (GUS), expression in the root tip was not found. However, when genomic *PEPR1* and *PEPR2* sequences fused to GFP were expressed under their native promoters, the presence of PEPR1-GFP and PEPR2-GFP was detected at the root tip (Ortiz-Morea et al. 2016).

Although PROPEPs have been predicted for numerous species, including important crops (Huffaker et al. 2006; Ruiz et al. 2018), they have been mainly studied in *Arabidopsis* and more recently in *Zea mays*. Yet, PROPEPs orthologs have been functionally validated in pepper (*Capsicum annuum*), potato (*S. tuberosum*), tomato (*S. lycopersicum*), soybean (Glycine max), peanut (*Arachis hypogea*),

rice (Oriza sativa), and *Prunus* spp (Bartels and Boller 2015; Huffaker et al. 2013; Huffaker et al. 2011; Ruiz et al. 2018; Trivilin et al. 2014; Lori et al. 2015). Identification of PROPEPs and PEPRs in recently sequenced plant genomes (Lori et al. 2015) suggests that the PROPEP family is largely conserved in angiosperms and that Peps might probably play roles as general defense mediators (Huffaker et al. 2013; Lori et al. 2015). Interestingly, despite the presence of PEPRs orthologs across angiosperms, interfamily incompatibility has been reported. Peps are not recognized by taxa-specific PEPRs outside family boundaries (Huffaker et al. 2013; Lori et al. 2015). This could be explained by taxon-specific co-divergence of both Peps and the extracellular ligand-binding (LRR) domain of PEPRs (Lori et al. 2015). In contrast to the PEPR LRR domain, the intracellular part of the PEPR is highly conserved, allowing activation by compatible downstream signaling molecules across plant families (Lori et al. 2015).

Overexpression and external application approaches have shown in various plant species that Peps activate PTI, induce systemic immunity, improve resistance to bacterial/fungal pathogens, and protect against herbivore attacks (Huffaker et al. 2006; Huffaker et al. 2013; Huffaker et al. 2011; Ruiz et al. 2018; Klauser et al. 2015). Most of the research has been done in the model plant *Arabidopsis*, reporting that application of Peps confers resistance against fungal (*Botrytis cinereal*) and bacterial (Pto DC3000) pathogens (Yamaguchi et al. 2010; Liu et al. 2013). Likewise, *Arabidopsis* plants overexpressing *PROPEP1* or *PROPEP2* displayed an enhanced resistance toward the root pathogen *Pythium irregulare* (Huffaker et al. 2006). Since MAMPs sensing induces the expression of some PROPEPS and its receptors, it has been theorized that Peps carry out functional interactions with MAMP signaling pathways (Huffaker and Ryan 2007). For instance, *Arabidopsis* plants pre-treated with bacterial and fungal MAMPs exhibited enhanced ROS production following Peps application (Flury et al. 2013; Klauser et al. 2013). Moreover, Peps receptors were found to be required for the maximal activation of EFR-and FLS2-triggered immunity (Tintor et al. 2013; Ma et al. 2012).

Interestingly, there is an evidence showing that PROPEP3 and PEPRs receptors are strongly induced upon herbivore feeding in *Arabidopsis.* It was found that *pepr1/pepr2* double mutant plants (insensitive to Peps) exhibit a reduced resistance to feeding by *Spodoptera littoralis* larvae (Klauser et al. 2015). It was also reported that exogenous applications of a member of the maize Peps family, *Zm*Pep3, elicits anti-herbivore responses that contribute to resistance against *Spodoptera exigua*, causing reduction of larval growth and attraction of *Cotesia marginiventris* parasitoids (Huffaker et al. 2013). In the same study, it was shown that Peps derived from PROPEP orthologs identified in plants from Fabaceous and Solanaceous families also induce herbivory responses. All these data point out the Pep-PEPR system as an important player of higher plant defense against herbivores.

Recent studies have indicated roles for Peps during plant development and abiotic stress responses. For instance, a biotic stress-related microarray experiment suggested PROPEPs involvement in plant development and reproduction besides their roles in biotic stress resistance. This study also indicated that PROPEP transcription is regulated individually and does not follow a general pattern for all

PROPEPs coding genes (Bartels et al. 2013). Likewise, it was also reported that Pep-perception in *Arabidopsis* accelerates dark/starvation-induced senescence via an early induction of chlorophyll degradation and autophagy. By contrast, the MAMPs flg22 or elf18 was not able to induce the same effect, indicating that this is a distinctive characteristic of PEPR signaling (Gully et al. 2015).

Finally, transcriptional data from *Arabidopsis* showed higher expression of PROPEPs under salinity stress relative to control conditions. PROPEP3 exhibited the highest level of expression at high salinity and knockdown PROPEP3 transgenic plants displayed a hypersensitive phenotype under osmotic stress that was suppressed by exogenous application of *At*Pep3 (Nakaminami et al. 2018). Moreover, salt-induced bleaching of chlorophyll in *Arabidopsis* seedlings was inhibited by *At*Pep3 treatment, demonstrating the role of Peps in salinity stress tolerance (Nakaminami et al. 2018). Although genetic and biochemistry approaches have indicated that Peps modulate diverse biological process besides plant immunity, the molecular mechanisms explaining how plant cells regulate and integrate Peps signaling is poorly understood.

10.2.2 Systemins

Systemin is an 18 aa peptide derived from the C-terminus of a 200 aa precursor protein named prosystemin (McGurl et al. 1992). Prosystemin was isolated from tomato (*Solanum lycipersicum*) and was the first reported plant peptide with hormone characteristic (Pearce et al. 1991). Systemin is able to promote immune responses to wounding and herbivore attacks, mainly accumulating in the cytosol of vascular phloem parenchyma cells (Pearce et al. 1991). Even though the mechanism responsible for systemin release from its precursor protein was poorly understood (Pearce et al. 1991), it has been shown recently that this process is performed by phytaspases, which are aspartate-specific proteases of the subtilase family (Beloshistov et al. 2018). The destruction of cleavage sites in prosystemin precluded its processing in vitro and abrogated systemin signaling in vivo. These findings indicate that prosystemin requires processing for signal biogenesis and biological activity (Beloshistov et al. 2018). Prosystemin homologs displaying a well-conserved systemin primary structure have only been found in the Solanoideae subfamily, which includes tomato, bell pepper, tomato, and nightshade (Constabel et al. 1998).

Systemin triggers early signaling components of plant defense as well as induction of proteinase inhibitors and other anti-nutritive proteins (Yamaguchi and Huffaker 2011; Orozco-Cardenas et al. 1993). In neighboring cells, systemin induces biosynthesis of jasmonic acid (JA) or its derivatives, which in turn propagates a systemic response throughout the plant, producing volatiles that contribute to deter plant herbivores (McGurl et al. 1992; Degenhardt et al. 2010; Li et al. 2002; Sun et al. 2011). Although systemin has been amply associated to wounding or herbivore responses, this peptide has been found to activate genes of multiple signaling pathways that enhance resistance to different biotic stresses such as

phytophagous larvae, fungi/aphids infections (Coppola et al. 2015; El Oirdi et al. 2011), and osmotic stress (Orsini et al. 2010). Tomato plants overexpressing prosytemin released higher amount of bioactive volatile organic compounds, which attract natural enemies for parasitoids (Degenhardt et al. 2010; Corrado et al. 2007).

Moreover, besides activating a systemic defense response, systemin generates metabolic changes that activate priming responses in neighboring unchallenged plants (Coppola et al. 2017). Priming is an adaptive strategy that improves the sensitivity and responsiveness to biotic stress from a prior stimulus, thus increasing the defensive capacity of plants (Mauch-Mani et al. 2017). The specific components that modulate the priming systemin effect are most probably volatile organic compounds, however, this is still unknown (Coppola et al. 2017). After the discovery of systemin, several laboratories focused on the identification of the systemin receptor. Initially, a leucine-rich repeat receptor kinase (LRR-RK) named systemin receptor 160 (SR160) was suggested as the PRR that recognizes systemin (Scheer and Ryan 2002). This receptor is a tomato ortholog of the *Arabidopsis* Brassinosteoird Insensitive 1 receptor (BRI1), however, the proposed role for SR160 could not be verified by other researchers (Malinowski et al. 2009; Holton et al. 2007). Recently, it was demonstrated that systemin signaling depends on the presence of two closely LRR-RKs, termed systemin receptor 1 and 2 (SYR1 and SYR2) (Wang et al. 2018). Nonetheless, only SYR1 was found to bind systemin with high affinity and specificity and thus acts as a bona fide systemin receptor with important roles in defense against herbivorous. Whether SYR2 is a low-affinity receptor or has a paralogous function as a receptor for a different ligand remains to be clarified (Wang et al. 2018). The identification of the systemin receptor will allow to carry out further research to elucidate evolutionary, physiological, and molecular aspects of this DAMP-PRR pair.

10.2.3 Kiss of Death (KOD)

In *Arabidopsis,* the kiss of death (KOD) is 25 aa peptide proposed as a positive regulator of the initial stages of programmed cell death (PCD) during embryogenesis and root hair development (Blanvillain et al. 2011). While two mutant alleles of *KOD* reduced PCD of the suspensor, a single cell type that supports embryo development, overexpression caused ectopic cell death in seedlings (Blanvillain et al. 2011). Although no direct link with plant immunity has been demonstrated yet, the *KOD* gene is transcribed upon biotic and abiotic stresses, suggesting roles as a potential DAMP candidate, further clarifying studies are needed (Albert 2013; Blanvillain et al. 2011). Interestingly, the *KOD* transcript contains a short ORF of 75 bp that directly encodes an active form of the peptide, avoiding the cleaving-off from a precursor protein, as observed in systemin and Peps (Yamaguchi and Huffaker 2011; Blanvillain et al. 2011). Endogenous peptides encoded by short ORFs are less described in plants, probably because they have been overlooked due to the small size of their ORFs and polypeptide chain. Transgenic plants expressing KOD fused to fluorescent proteins showed that KOD localizes to the cytosol and

nucleus (Blanvillain et al. 2011). However, considering the size of fluorescent proteins, the authors cannot disregard that the observed localization pattern is due to a dysfunctionality of KOD-GFP or KOD-RFP chimeric proteins (Blanvillain et al. 2011). Although KOD was reported in 2011, no further studies aimed to understand its role in plant immunity have been performed.

10.3 Peptides Derived from Precursor with an N-terminal Secretion Signal

This category includes peptides derived from a precursor protein with an N-terminal secretion/subcellular localization signal. Peptides are secreted to the extracellular space and processed either along the secretion pathway or in the apoplast, releasing the mature C-terminal peptide.

10.3.1 PAMP-Induced Peptides (PIPs)

A clear example in *Arabidopsis* for this category are the PAMP-induced peptides (PIPs) shown to activate immune responses and enhance resistance against *Pseudomonas syringae* and *Fusarium oxysporum* (Hou et al. 2014). *These* peptides are derived from the C-terminus of precursor proteins (prePIPs) with a N-terminal signal peptide that undergoes the secretion for subsequently extracellular processing by unknown proteases. The mature C-terminal peptide exhibits a conserved core SGPS motif (Hou et al. 2014; Vie et al. 2015). These features are hallmarks of post-translationally modified secreted peptide precursors (Matsubayashi 2011). In *Arabidopsis*, the prePIP family harbor at least 11 members (72–108 aa-length). Orthologs have been predicted in dicots and monocots species, including maize, rice, soybean, grape, and *Medicago truncatula* (Hou et al. 2014; de Bang et al. 2017).

Transgenic plants expressing the GFP gene under control of the *prePIP1* promoter exhibited strong fluorescence signal in vascular tissue, guard cells, and hydathodes, which represent either potential entry points or proliferation routes for invading organisms (Hou et al. 2014). Abundance of *AtPrePIPs* transcripts increased following MAMPs treatments and infection with bacterial or fungal pathogens. *Arabidopsis* seedlings treated with *At*PIP1 and *At*PIP2 activate typical PTI responses (Hou et al. 2014). All these together indicated that PIPs are important modulators of plant immune responses. Moreover, the fact that expression of some *PIP* genes is induced by abiotic stress, suggests distinct or additional biological roles for PIP peptides (Vie et al. 2015). Interestingly, contrary to what was found in *Arabidopsis,* there is an evidence showing that some member of PIPs in *Medicago truncatula* are *involved in macronutrient responses and nodulation, increasing lateral and root lengths*, which *suggest that PIPs could have species-specific*

functions (Hou et al. 2014; de Bang et al. 2017; Ghorbani et al. 2015). Genetic and biochemical evidence suggest that an LRR-RK, referred as receptor kinase 7 (RLK7) functions as *At*PIPs receptor. However, so far, this has been proved just for *At*PIP1 and *At*PIP2. The same study showed that *At*PIP1-RLK7 and *At*Pep1-PEPR1 cooperatively amplify MAMPs signaling (Hou et al. 2014).

10.3.2 Hydroxyproline-Rich Systemin (HypSys)

Hydroxyproline-rich systemins (HypSys) are 18–20 aa peptides derived from large precursor proteins (proHypSys) with N-terminal secretion signal for cell wall matrix localization (Pearce et al. 2001a). HypSys peptides have been described as important modulators of plant local and systemic defense, especially during herbivore attack, but also during interaction with other plant pathogens (Bhattacharya et al. 2013). Two distinct 18 aa HypSys derived from one single precursor protein were first isolated from tobacco leaves (*Nt*HypSysI and *Nt*HypSysII). The single gene product encodes a precursor protein of 165 aa, which transcription is induced by methyl jasmonate, wounding and certain elicitors (Pearce et al. 2001a; Rocha-Granados et al. 2005). Tobacco plants overexpressing preproHypSys are more resistant to herbivory by *Helicoverpa armigera* larvae (Ren and Lu 2006). The amino acid sequence of HypSys resembles that of canonical systemin orthologs, but, due to their passage through the secretory system, the polyprolines are hydroxylated and then glycosylated with pentose sugar chains (Pearce et al. 2001a). Orthologs of *Nt*HypSys have only been isolated in Solanaceae and Convolvulaceae (Narváez-Vásquez et al. 2007). Expression of *proHypSys* has been localized to phloem parenchymal cells of the midveins of leaves and petioles in tomato (Narváez-Vásquez et al. 2005). In sweet potato, expression of *proHypSyst* was induced in leaves and petioles after wounding, and suppressed *Spodoptera litura* growth by enhancing lignin biosynthesis (Li et al. 2016). The HypSys receptor still remains to be identified.

10.3.3 Rapid Alkalinization Factor (RALF)

Rapid alkalinization factors (RALF) are members of a cysteine-rich peptide family that usually present four Cys residues able to form two disulfide bridges (Pearce et al. 2001b; Haruta et al. 2014). The first RALF was isolated from tobacco leaves and described as a factor causing rapid pH increase in tobacco cell suspensions (Pearce et al. 2001b). RALFs have been identified in several species, being considering ubiquitous in the plant kingdom. RALFs are found either as single-copy genes, as is the case in *Nicotoiana attenuata* or as multigene families in rice, *Arabidopsis*, maize, and poplar (Wu et al. 2007; Cao and Shi 2012; Sharma et al. 2016). The *Arabidopsis* genome contains 39 RALF encoding genes (*At*RALFs)

(Sharma et al. 2016). RALF is derived from the C-terminal of a larger precursor protein (proRALFs) of up to 134 aa, which carries an N-terminal signal peptide for conventional endoplasmic reticulum dependent secretion (Sharma et al. 2016). ProRALFs are processed by a subtilase family of proteases that release the mature active peptide located at the C-terminus (Matos et al. 2008; Srivastava et al. 2009; Stegmann et al. 2017). Recently, the *Arabidopsis* malectin-like receptor kinase FERONIA was identified as a receptor for *At*RALFs (Haruta et al. 2014). Additionally, it was found that the receptor kinase BAK1 interacts physically with RALF1 in a specific manner, suggesting that BAK1 may play a role in *At*RALF signaling as a co-receptor (Dressano et al. 2017).

Typically, RALF peptides are associated to cell expansion in root cells, mobilization of calcium, MAP kinase activation, alkalinization of the extracellular medium, and pollen tube growth (Pearce et al. 2001b; Haruta et al. 2014; Morato do Canto et al. 2014; Campos et al. 2018; Ge et al. 2017). Nevertheless, recent data suggest a strong link between RALF and plant immunity. It was reported that the *Arabidopsis* SITE-1 PROTEASE (S1P) cleaves proRALF23, releasing its active form. RALF23 subsequently interacts with FER, inhibiting the formation of stable plant PRR complexes. FER enhances MAMP-induced stability of PRR complexes during PTI responses (Stegmann et al. 2017). Therefore, it is suggested that *At*RALF23 is a negative feedback regulator of plant immune activation (Stegmann et al. 2017).

Interestingly, this negative regulation is exploited by some fungal phytopathogens that encode functional RALF23 homologs to suppress plant immunity (Masachis et al. 2016). On the other hand, *Arabidopsis* seedlings treated with RALF17—a RALF member that lacks a predicted S1P cleavage site, induced ROS production, acted additively to MAMPs and was able to induce resistance to Pto DC300. These observations indicate that some RALFs can also act as elicitors of PTI responses (Stegmann et al. 2017). Newly, it has been shown that RALF4 and RALF19 bind to ANXUR1 (ANX1) receptor regulating pollen tube integrity. ANX1 is the closest homolog of FER and has been identified as negative regulator of PTI and ETI (Ge et al. 2017). Whether RALF4/RALF19 are also ligands of ANX1/ANX2 in plant immunity, remain to be clarified.

10.4 Cryptic Peptides Derived from Proteins with Distinct Primary Functions

This category groups peptides derived from ubiquitous plant proteins with primary functions different than plant immunity. These molecules experience proteolysis under herbivore attack, generating elicitor peptides. Low concentrations of these peptides are able to trigger plant defense responses.

10.4.1 Inceptin Peptides

Inceptin are the first cryptic peptides discovered to regulate immunity. These acidic 11–13 aa peptides originate from the disintegration of the ATP synthase γ-subunit in the gut of fall armyworm (*Spodoptera frugiperda*) larvae (Schmelz et al. 2006, 2007). These peptides are present in the oral secretions of fall armyworm and can trigger plant defense responses. Inceptin treatment of cowpea (*Vigna unguiculata*) and common bean (*Phaseolus vulgaris*) leaves enhanced the production of jasmonic/salicylic acid and other metabolites with defensive roles that together reduced fall armyworm growth (Schmelz et al. 2006, 2007). The elicitor activity of inceptins seems to be specific for *Phaseolus* and *Vigna* genera (Schmelz et al. 2007). Still, receptors for inceptin peptides have not been reported to date. Interestingly, the velvet bean caterpillar (*Anticarsia gemmatalis*), a harmful herbivores of legumes, preferentially process inceptin-related proteins into a biologically inactive form that work as a natural antagonist of plant defense mechanism triggered by inceptins (Schmelz et al. 2012). This adaptation allows velvet bean caterpillar to evade plant recognition and activation of defense responses.

10.4.2 Glycine Max Subtilase Peptide (GmSubPep)

The *Glycine max* (soybean) subtilase peptide (*Gm*SubPep) is another member of this peptide category. *Gm*SubPep is a 12 aa long peptide that was discovered embedded in the protein-associated domain of a putative extracellular subtilase. Similar to other DAMPs, *Gm*SubPep is able to trigger extracellular alkalinization and to induce expression of defense- and stress-related genes (Pearce et al. 2010a). The *Gm*SubPep release mechanism and its receptor have not yet been identified. Structure-activity studies of GmSubPep reported that the C-terminal extreme has important signal transduction properties and probably is essential for receptor interaction (Pearce et al. 2010b).

10.5 Final Discussion and Future Perspectives

Plants have developed a sophisticated immune system that relays on specialized receptors to switch on plant defense after the perception of danger signals (Yu et al. 2017). The integration between exogenous and endogenous danger signal is expected to collectively contribute to plant defense against a broad spectrum of invading organism. Plant-derived molecules are suggested to be divided into two categories; primary endogenous danger signals passively released from plant cells upon host damage (cell debris), and secondary endogenous danger signals that are actively processed small peptides, release upon herbivory and/or microbial infection (Gust et al. 2017). Tremendous progress has been achieved over the last years in the identification of endogenous elicitor peptides and their receptors, as well as,

characterization of signaling networks that triggered plant immune responses. Here, we presented eight different endogenous peptide elicitor families that are thought to amplify, modulate, or fine-tune plant immunity. Typically, endogenous peptides are released from large precursor proteins that can either have or not an N-terminal secretion signal, or derive from proteins with distinct primary functions (cryptic peptides) (Yamaguchi and Huffaker 2011).

Probably, the Peps-PEPRs system is the most widely peptide–receptor pair studied in plants. There is a vast amount of evidence showing that this system acts mainly as an amplifier of innate immunity (Fig. 10.2.). This role has also been proposed for most of the endogenous elicitor peptides. As amplifiers, elicitor peptides are released from precursor proteins into extracellular spaces, where they subsequently bind specific plasma membrane receptors in neighboring cells, thus triggering defense responses (Yamaguchi and Huffaker 2011; Hou et al. 2014). This assumption is based mostly on data generated from genetic and biochemistry experiments. The subcellular dynamics of the peptide–receptor complexes remains largely unknown. Recently, Ortiz-Morea et al. (2016), elucidated the internalization pathway of the peptide *At*Pep1, which is probably similar to other plant peptides

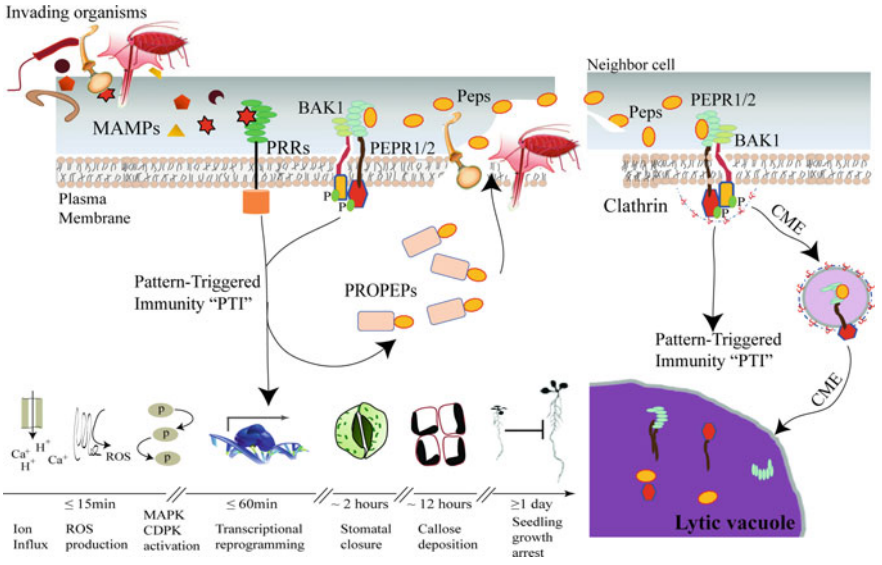

Fig. 10.2 Proposed model of Peps as amplifiers of innate immunity. After the detection of an external danger/alarm signal molecule through pattern recognition receptors (PRR), the cell triggers pattern triggered immunity (PTI). At the same time, PROPEPs are produced and Peps released by unknown mechanism to the extracellular medium. Neighboring cells perceive Peps by PEPR1 and PEPR2 receptors, interacting immediately with the co-receptor BAK1 followed by phosphorylation and activation of intracellular kinase domains in both receptors. Subsequently, PTI is triggered. Finally, the Pep-receptor complex undergoes clathrin-mediated endocytosis and is transported to the lytic vacuole, thus allowing desensitization of the signal

that trigger a comparable set of downstream signaling responses. However, the internalization mechanism of active *At*Peps after release from their precursor proteins (PROPEPs) is poorly understood.

Precursors of *At*RALF23 are processed within minutes after MAMPs treatment, releasing the active peptide, which in turn affects negatively MAMPs initiated immune responses (Stegmann et al. 2017). This observation indicated antagonistic effects for endogenous peptides, besides the agonistic effect amplifying immune responses triggered by exogenous elicitors. Recently, it was shown that systemin triggers metabolic changes capable of inducing a primed state that alerts neighboring unchallenged plants of possible incoming attacks (Coppola et al. 2017). These plants become well prepared to fight invading organisms and likely to prevent further spread of the pest. Although this effect has been shown only for systemin, the capacity of other endogenous peptides to induce plant-to-plant communication processes leading to a prime state remains unknown. Further studies are needed to completely understand the role of endogenous peptides in plant immunity. Moreover, some endogenous elicitor peptide families have also been described to modulate developmental and abiotic stress responses. It is intriguing to understand how plant cells translate and integrate diverse responses to distinct signals.

An outstanding question that remains to be clarified is if endogenous elicitor peptides are released without loss of cellular integrity and what are the modes of secretion, especially for peptides derived from precursors without an N-terminal secretion signal. It can be argued that peptides with an N-terminal secretion signal are mainly secreted through a classical secretion pathway under microbe attack without loss of cellular integrity, and that leaderless peptides are secreted mainly under herbivory or when cell integrity is compromised. However, because Peps and systemins (leaderless peptides) have been associated with both microbe and herbivore responses, and because evidence for peptide releases via non-classical secretion pathways are emerging (Ding et al. 2012), active secretion of leaderless elicitor peptides may not be ruled out. Likewise, proteins with functions other than plant immunity served as precursors of cryptic peptides thought to be release just after plant damage under herbivore attack.

Among the hundreds of plasma membrane receptor kinases (RKs), encoded in plant genomes, there are several of them (likely PRRs) with the ability to modulate plant immunity (Ghorbani et al. 2014; Shiu et al. 2004). Approximately 1000 genes encoding small secreted endogenous ligands able to bind RKs have been found in *Arabidopsis thaliana* (Lease and Walker 2006). Nonetheless, in spite of the large possible peptide–receptor pairing, only a few peptide–receptor combinations have been identified and their ability to activate immune responses experimentally demonstrated. Although the characterization of regulatory pairs is essential to understand communication networks in plants, the task is challenging because PRRs' encoding genes are often redundant and their expression restricted to few cells and/or particular conditions (Butenko et al. 2014). The study of matching peptide–receptor pairs could take advantage of labeling peptide techniques combined with ectopic expression of receptor genes in suitable plant cells and by

assessing endocytosis as read-out of their interaction. Ligand-induced endocytosis is emerging as a hallmark of RKs (Ortiz-Morea et al. 2016; Di Rubbo and Russinova 2012; Mbengue et al. 2016).

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Phytoanticipins: The Constitutive Defense Compounds as Potential Botanical Fungicides

11

Gyula Oros and Zoltán Kállai

Abstract

Present control technologies of plant pathogenic fungi decouple the pathogen's life cycle mainly in two points of ontogeny, either by destroying spores prevent the infection or inhibit the biotrophic thallus, thus anticipating the formation of new infective propagules. Although, nowadays, the only tool for credible control of cultivated plants is the use of synthetic chemicals, the calculability of yield sureness has been worldwide threatened by the emergence of acquired tolerance to this group of pesticides as well as anxious feelings for their undesirable side effects. This situation urges the development of efficient alternative control agents, as threatening the net return even 10% disease incidence can cause economic loss. One approach to discover newer antimicrobial compounds is to search for their presence in natural sources exploiting the defense strategies of plants against their pathogens. Contrary to phytoalexins that are synthesized *de novo* after the plant is exposed to microbial attack, i.e., being produced in response of elicitors or stressors, the phytoanticipins are not formed in the tissue or released from preexisting plant constituents. These substances are plant antibiotics presented in tissue prior to infection, serving as the basis of pest tolerance. Several thousands of such molecules of different structure have been identified; however, few of them met practical application. In this chapter, we focus on constitutive mechanisms that might be used for controlling phytopathogenic fungi with special regard to organic substances, which might serve either as botanical fungicides or as lead compounds for molecular design.

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Consequently, the introduction of alien phytoanticipins and precursors of phytoalexins into the proper host/parasite system can represent a prospective tool for disease management. We summarized the results and experiences of past three decades searching for candidates for biofungicides useful in pest management practices. The efficacy of over 100 plant species used as either spices or preparations in traditional medicine or culinary was demonstrated in vitro against 25 phytopathogenic fungi, and possible use of promising candidates was discussed.

Keywords
Phytoanticipin · Fungicide · Phytopathogen · Defense · Yeast · Herb · Spice

11.1 Introduction

Plants have evolved finely regulated complex of metabolic processes to sustain homeostatic balance as well as constitutive and inducible defense mechanisms to help in both wound healing and defense against attack by microbes and herbivores. The constitutive defenses include static structures, such as lignified cell walls, mineral or organic crystals that create physical barriers as well as wide variety of organic compounds. The inducible defenses are also multitudinous, involving gene activation-linked *de novo* enzyme syntheses and various metabolites called phytoalexins. The role of phytoalexins in defense mechanisms was intensively studied (Van Etten et al. 2001), while to constitutive compounds has been paid less attention. In this chapter, we focus on constitutive mechanisms that might be used for controlling phytopathogenic fungi with special regard to organic substances, which might serve either as botanical fungicides or as lead compounds for molecular design.

Contrary to phytoalexins that are synthesized *de novo* after the plant is exposed to microbial attack, i.e., produced in response of elicitors or stressors, the phytoanticipins are not performed in the tissue or released from preexisting plant constituents, but are plant antibiotics presented in tissue prior to infection, serving as the basis of pest tolerance. Several thousands of such molecules of different structure have been identified; however, few of them met practical application. These compounds represent heterogeneous chemical structures, and significant part of them is synthesized via polyketide, isoprenoid, shikimate, and phenylpropanoid pathways (Pedras and Yaya 2015). The progress in separation and analytical techniques has allowed the rapid identification of plant secondary metabolites. The screening of their biological activities combined with molecular genetic techniques elucidated various roles in defense mechanisms (Mazim et al. 2011; Carere et al. 2016).

Present control technologies of plant pathogenic fungi decouple the pathogen's life cycle mainly in two points of ontogeny. The applied chemicals either destroy spores, preventing the infection or inhibit the biotrophic thallus, anticipating the formation of new infective propagules. Although the tolerance of cultivated plants can be enhanced by diverse methods, the possibilities of biocontrol, as well as the enhancement of plant resistance with chemical treatment, are limited; none of these approaches resulted in the economically acceptable level of control for long term of application in recent plant cultivation technologies, contrary to modern synthetic pesticides. Nowadays, the only tool for creditable control of cultivated plants is the use of synthetic chemicals. However, the calculability of yield sureness has been worldwide threatened by the emergence of acquired tolerance to this group of chemicals as well as by anxious feelings for undesirable side effects. All these are major causes of concerns as even 10% disease incidence can cause economic loss threatening the net return. This situation urges the development of efficient alternative control agents. One approach to discover newer antimicrobial compounds is to search for their presence in natural sources exploiting the defense strategies of plants against their pathogens. Microbial species or strains that do not invade the plant are usually more sensitive to the components of performed barriers than a viable pathogen of this plant. Consequently, the introduction of alien phytoanticipins and precursors of phytoalexins into the proper host/parasite system can represent a prospective tool for disease management (Piasecka et al. 2015).

The possible use of botanicals in pest control technologies intrigued big expectations hitched up by social movements. Indeed, in some special cases, these preparations performed well.

However, in comparative studies, the new generation of synthetics surpassed the botanicals at some orders of magnitude (Table 11.1). The use of natural compounds as lead molecules is seemingly more prospective, and the new techniques of

Table 11.1 Antisporulant activity of commercial fungicides and reference substances

Treatment		Concentration (%) of substances				
Substances	Form[a]	0.0005	0.005	0.05	0.5	5
Dimethomorph	A	−	+	+	++	++
Metalaxyl	A	−	+	+	++	++
Mikal	B	−	−	+	+	++
Digitonin	A	−	−	+	+	++
Podophyllotoxin	A	−	−	−	+	++
Veratrin	A	−	−	−	+	+
Nutri-Neem	B	−	−	−	+	+
Milsana	B	−	−	−	+	++

Test organism: *Sclerospora graminicola* (Sacc.) J. Schröt

The antisporulant activity was evaluated by the following scale; full inhibition (++), partial inhibition (+), and no inhibition (−)

[a]A = 25% methanolic stock solution of active ingredients containing 1% of Tween 20 was used for preparing dilution series. The methanol and Tween 20 did not exhibit any inhibitory effect alone when applied at maximum doses (5 and 0.2%, respectively). B = Commercial preparations were used (Deepak et al. 2005)

molecular design help to map the parts of the active molecule that respond for the desired biological effect. In past decades, the losses caused by peronosporaceous pathogens are increasing, and only a few synthetics are available to control them at an economically acceptable level. Unfortunately, the populations of pathogens rapidly adopt to these highly active monosite inhibitors. Some natural compounds in model experiments exhibited notable antiperonospora effect, especially in their abiotrophic stages of ontogeny (Deepak et al. 2007).

Some natural compounds in model experiments exhibited notable antiperonospora effect, especially in their abiotrophic stages of ontogeny, among them the known Na^+ ion channel activator ceveratrum alkaloids effectively inhibited the systemic invasion of the parasitizing thallus as well (Oros 2010). These amphiphilic steroid alkaloids are thought to act by direct incorporation into the microbial membrane disrupting its structural and functional integrity. Examination of the effect of veratridine on the alkali metal salt tolerance of *Plasmopara halstedii* showed that this steroid alkaloid dramatically impaired the tolerance of microbes to Li^+, Na^+, Cs^+, and, especially, to K^+. Modifying its structure synthetically, the sporicidal activity was successfully increased about thousand times (Oros and Ujváry 1999). The non-steroidal analogues of ceveratrum alkaloids designed by molecular modeling have an anti-oomycetes activity that depends significantly on the chemical structure and is confined to certain biotrophic and abiotrophic developmental forms of *P. halstedii* (Table 11.2).

Interestingly, the main structural features of these non-steroidal compounds presented here bear a certain resemblance to known commercial fungicides such as fenpropimorph and fenpropidin as well as to the experimental diaryltetrahydropyridines (Takayama et al. 1995). Thus, the new compounds, on the one hand, refine the structure–fungicidal activity relationship for substituted piperidines and, on the other hand, define an extended structural scaffold for new fungicide development (Ujváry and Oros 2002). The ecological role of the botanical steroid alkaloids is not fully known. Nevertheless, it can be assumed that these substances have multiple functions in the wild plants among them to protective against herbivores and diseases (Wink 1993). In this context, it is interesting that digitonin, α-solanine, and their aglycones showed activity against the asexual spores of *P. halstedii* and *S. sclerospora* even though it is generally believed that cleavage of the glycoside bond of plant glycoalkaloids represents a deactivation process utilized by glycoalkaloid-resistant fungi. It should be emphasized, however, that *P. halstedii* and *S. sclerospora* are specific and obligate pathogens, and their host plants have not been shown to contain glycoalkaloids; thus, these pathogens are unlikely to have evolved such deactivation mechanism.

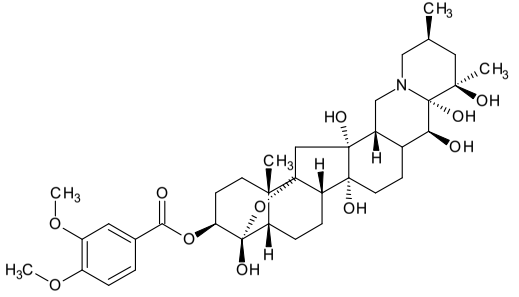
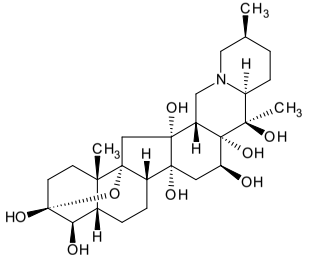
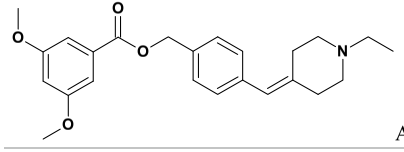
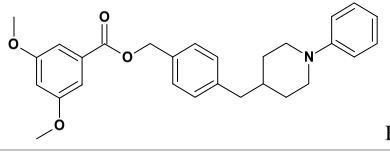
From now on, we summarize results of the past two decades searching for promising candidate botanicals useful in pest management practices. The selected for screening plants are attractive for humans, because of their characteristic organoleptic properties (smell and taste). Most of them are cultivated plants being part of the human diet. Their features are well known, and the marketed samples refer to traditionally accepted standards, that is important, as these plants exceptionally rich in secondary compounds of divergent structure (Table 11.3).

Table 11.2 Effect of steroid alkaloids and non-steroid analogues on sunflower downy mildew and on the asexual spores of *Plasmopara halstedii*

Compounds	Inhibition of plant disease symptoms[a]		Response of asexual spores			
			Zoosporangium		Zoospore	Cystospore
	Damping off [%]	Leaf chlorosis [%]	Survival	Release	Motility	Germination
			MIC [μM]			
	Alkaloids with cevane skeleton					
Veratridine	60 de	73 c	>1000	100	100	10
Veracevine	93 ab	73 c	>1000	100	10	100
	Alkaloids with pregnane skeleton					
α-Solanine	95 a	30 g	>1000	1000	10	1
Solanidine	85 b	48 e	1000	100	10	1
	Non-steroidal analogues					
A	−28 i	84 b	100	10	1	0.1
B	100 a	0 h	>1000	>1000	10	100
C	60 de	80 b	100	10	1	0.01
D	0 h	100 a	>1000	>1000	10	10
	Reference fungicides[b]					
Tridemorph	70 cd	30 g	>1000	1000	10	10
Metalaxyl	100 a	73 c	>1000	>1000	>1000	1000

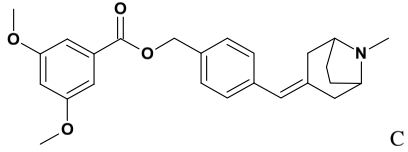
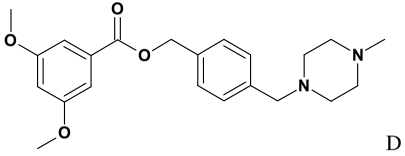
(continued)

Table 11.2 (continued)

Compounds	Inhibition of plant disease symptoms[a]		Response of asexual spores			
			Zoosporangium		Zoospore	Cystospore
	Damping off [%]	Leaf chlorosis [%]	Survival	Release	Motility	Germination
			MIC [μM]			
 Veratridine			 Veracevine			
Veratridine			Veracevine			
 A			 B			

(continued)

Table 11.2 (continued)

Compounds	Inhibition of plant disease symptoms[a]		Response of asexual spores			
			Zoosporangium		Zoospore	Cystospore
	Damping off [%]	Leaf chlorosis [%]	Survival	Release	Motility	Germination
			MIC [μM]			
 C			 D			

[a]One μM test compound per plant germling was applied. The activities marked with the same letter did not differ at $P = 5\%$ level (Fisher's test)
[b]Data from Ujváry and Oros (2002)

The composition can largely vary within samples. Chemotypes—chemically distinct entities within plant species on genetic variation—are exceptionally frequent for secondary compounds and can influence the quality of plant materials, which property has been largely used in chemotaxonomy.

From the agroindustrial point of view, the herbs have special advantages as their effects on mammalians are well known. Thus, the risk of elaboration of botanical preparation for pest control is significantly lower and less risky than the introduction of the plant with unknown biological effects in details. The use of pure compounds is more favorable; however, their production in industrial scale frequently meets difficulties and unprofitable. Thus, the herbal preparations may have a place in pest control technologies. Moreover, the protective effect can be resulted by synergic joint action of several secondary metabolites that phenomenon needs further studies.

11.2 Standard Operating Protocols

The growth response of 25 filamentous fungi pathogenic to 100 herbal preparations and seven culinary mushrooms was compared in model experiment applying poisoned agar technique following, in general, the route of Walker et al. (1937).

The herbal preparations of plant species listed in Table 11.3 were either home prepared of the plants collected in Protected Landscape Area of Buda Mountains (N 47°33′00″, E 18°52′60″) following traditional manners or purchased in drug store (Herbaria Co., Budapest). The desiccated plants were stored protected of light at ambient conditions over silica gel. The dry material was micronized before use.

The test fungi listed in Fig. 11.1 were maintained on potato dextrose agar slants at 22–25 °C (CM0139B, OXOID, Basingstoke) amended with two gL^{-1} casein digest (Difco, Detroit, USA), vitamins, and mineral salts (Oros and Naár 2018). All strains were isolated from various sources in Hungary and deposited in the Mycology Collection (WDCM824) of PPI.

Toxicity test: The conidia of fungi for inoculation of agar plates were washed up with sterile distilled water containing 0.05% Tween 20 of 8-day-old colonies grown up on PDA slants.

The herbal preparation was mixed with the agarized medium (2500 mg in 100 mL) and poured into Petri dishes (20 mL into a 90-mm-diameter dish). Then these plates were overlayered with 5 mL sterile agar (1.5 gL^{-1} in distilled water) and after solidification were inoculated with conidial suspensions (10^5 cell per mL) using a multipoint inoculator, and subsequently incubated at 20–22 °C. The intensity of colony growth was evaluated after 24 and 48 h by the following four-grade scale: 0 = no growth, 1 = growth on the limit of visual apperception, 2 = apparent but retarded growth as related to the untreated control, and 3 = the colony is not visually distinguishable from the untreated control and −1 = stimulation.

Table 11.3 Inhibitory effect of plant preparations on the growth of phytopathogenic fungi

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
Fungi Ascomycota						
	Agaricales					
1	*Agaricus bisporus* L.	Button mushroom	77	74	24	E8, E12, Y1, S36, E27, S26, B5, B1, S43, O1, S17
2	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Oyster mushroom	73	79	20	E4, E12, E29, Y1, E27, Y8, S37, S32, D3
3	*Lentinula edodes* (Berk.) Pegler	Shiitake	100	*n*	0	E29, Y1, O10
4	*Marasmius quercophylus* Pouzar	White-rot fungus	86	132	5	No
Basidiomycota						
	Auriculariales					
5	*Auricularia auricula-judae* (Fr.) Quel.	Wood ear	100	160	21	E4, E29, Y1, E27
	Boletales					
6	*Boletus edulis* Bull.	Penny bun	68	71	9	S25, S26
	Cantharellales					
7	*Cantharellus cibarius* Fr.	Cantharelle	72	75	24	E4, Y1, L4, Y8, S26, B2
Moss Lecanorales						
8	*Cetraria islandica* (L.)	Iceland moss	79	76	27	Y1
Plants Equisetales						
9	*Equisetum arvense* L.	Field horsetail	61	54	19	Y1, O10, L2, L4
	Cycadales					
10	*Zamia floridana* A.D.C.	Arrowroot	76	73	15	No
	Taxales					
11	*Taxus baccata* L.	English yew	88	83	39	D15, S22, Y1

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Pinales					
12	*Araucaria heterophylla*	Norfolk pine	83	87	27	No
13	*Juniperus communis* L.	Common juniper	75	72	20	E8, E30, Y1, E26, D17, E29, E25, E8
14	*Cupressocyparis leylandii*	Leylandii	85	90	16	D15, S22, S35
15	*Thuja occidentalis*	Arbor vitae	88	90	26	D15, D17, D19, S26, B8, O12
16	*Chamaecyparachilis obtusa*	Hinoki	89	117	12	Y1, D15, O6, B10, S7, L10, D29, S36, S35
17	*Sequoiadendron giganteum* J. Buchh.	Giant redwood	90	113	14	E8, W1, E29, Y7
18	*Picea pungens* Engelm.	Colorado spruce	64	53	23	No
19	*Picea abies* (L.) H. Karst.	Norway spruce	83	95	13	E29, L4, O4, O11, B22, O5, S4, D12, E21, S31
20	*Pinus nigra* J.F. Arnold	Black pine	88	111	18	E8, Y1, S22, E18, Y6, D5, S40, B9
	Austrobayleales					
21	*Illicium verum* Hook	Chinese Star anis	86	143	2	Y1, E26, S39, E25, E18, D16, S23, D18, O2, E9, B12, S31
	Acorales					
22	*Acorus calamus* L.	Sweet flag	80	95	6	E8, E4, M3, E30, Y1, L4, D15, S30, E26, D17, B10, E27, D16, E12, B3, B4, Y8, E19, D9, S19, D14, S25, D7, E1

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
Monocotyledones Zingiberales						
23	*Elettaria cardamomum* Maton	Cardamom green	83	93	22	E8, E4, E26, D17, S39, Y6, Y8, D9, S19, Y3, Y4, E25, B14, M2
24	*Elettaria cardamomum* Maton	Cardamom black	80	79	14	
25	*Zingiber officinale* L.	Ginger	80	83	10	E8, E29, E30, Y1, D15, S39, E27, B4, Y8, E25, Y9, E5, M2, S26, S1, S5, O12
26	*Curcuma longa* L.	Turmeric	89	98	19	E8, E4, E29, E30, D17, D19, S7, E25, E27, S23, M2, S26, S11, S29, S25, S32, D23, S13, D27, D2, S5, S8, D8, E10
27	*Kaempferia galanga* L.	Galangal	100	*n*	0	E8, E4, E29, B10, E5
	Asparagales					
28	*Allium schoenoprasum* L.	Chives	68	58	12	E8, L4, L10, S26, E17, L6
Dicotyledones Ranunculales						
29	*Chelidonium majus* L.	Greater celandine	70	59	20	E29, Y1, L3, L4, D15, S22, S30, E26, Y6, S23, E25, E5, D4, L7, D30, S38
30	*Clematis vitalba* L.	Old men's beard	77	96	7	E8, E4, Y1, Y8, M2, S26, E20

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Magnoliales					
31	*Myristica fragrans* Houtt.	Nutmeg	89	140	7	E4, Y1, O10, D15, S39, B10, S7, L1, L10, M2, S26, E9, S11, S29, B27, B26
32	*Flos myristicae*	Nutmeg	85	80	23	
	Fagales					
33	*Alnus glutinosa*	Black alder	77	67	10	Y4
34	*Juglans regia* L.	Walnut	95	187	3	E8, E4, E29, E30, E26, Y7, E27, E25, E11
	Laurales					
35	*Laurus nobilis* L.	Bay laurel	87	112	15	E8, E13, Y1, D35, D15, Y7, O6, B10, L10, D16, S23, B4, Y8, D9, S26, E9, E11, E15, S25, B8, D7, L8, B17, E14, B16, B13, B21, D26, D10, B27
36	*Cinnamomum verum* J. Presl.	Cinnamon	100	*n*	0	E4, Y1, L2, L4, D15, D17, D19, E22, O6, L10, Y8, D9, S19, S16, Y4, M2, S26, E16, D23, S1, D30, E14, D26, D10, D13, D25, B20, B15

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Piperales					
37	*Piper nigrum* L.	Black pepper	86	100	25	E8, E4, Y1, D15, S22, D19, E22, S36, Y8, S19, S26, E9, D24, S13, E10, B27, O12
	Caryophillales					
38	*Rumex patientia* L.	Patience dock	76	116	−6	E29, E28
	Cucurbitales					
39	*Momordica charantia* L.	Balsam pear	83	80	32	Y1, D15, S30, E26, S26, S11, D7, S6
	Brassicales					
40	*Sinapis alba* L.	Yellow mustard	100	*n*	0	E8, S26
41	*Wasabia japonica* (Miq.) Matsum	Wasabi root	100	707	1	D27
	Ericales					
42	*Arctostaphylos uvaursi* (L.) Spreng.	Bearberry	77	70	16	Y1, E29
43	*Camellia sinensis* L.	Green tea	69	52	12	Y1, E27, Y6, B4, Y4, O01, B17, B9
44	*Camellia sinensis* L.	Black tea	77	67	27	E29, Y1, E27, S25, S42, O01, B17, B9, B18, S44
45	*Primula veris* L.	Cowslip	90	125	19	No
46	*Vaccinium myrtillus* L.	Bilberry	80	73	33	Y1, D15, L10, S16, Y8, M2, S26
	Malvales					
47	*Hibiscus sabdariffa* L.	Red sorrel	89	145	9	D17, D19, E7
48	*Tilia cordata* P. Mill.	Lime	79	78	25	No

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Apiales					
49	*Foeniculum vulgare Mill.*	Fennel	84	87	23	Y1, O10, L4, D15, B10, M2, M2, E15, D7, B6, B27
50	*Apium graveolens* L.	Celery	75	64	16	B22, B27, B20, B15
51	*Anethum graveolens* L.	Dill	73	62	29	E8, E4, Y1, E7, S19, S12, M4
52	*Petroselinum crispum*	Parsley	86	87	31	E4, S37, E16, E21, B27
53	*Levisticum officinalis* L.	Lovage	79	68	27	L4, S22, B10, B22
54	*Carum carvi* L.	Caraway	84	92	15	E4, E13, Y1, D15, Y7, S19, D2, D7
55	*Coriandrum sativum* L.	Coriander	69	66	23	E8, E13, E29, Y1, L4, Y6, S29, E15, S41, O7, D1
56	*Pimpinella anisum* L.	Anis	88	121	16	E8, E13, E29, E30, Y1, E26, E22, Y7, O6, B10, L10, E27, Y4, E15, S25
57	*Panax ginseng* C. Meyer	Ginseng	84	95	24	E29, E30, S24, S30, S39, E25, S23, S26, S18, B7, D11, D22, O9
	Malpigiales					
58	*Hypericum perforatum* L.	St. Johnswort	73	59	21	E29, E30, S24, E26, E27, E25, S41
	Fabales					
59	*Galega officinalis* L.	Goat's rue	65	60	24	E8, Y1, D30
60	*Ononis spinosa* L.	Cammock	83	87	23	Y1
	Myrtales					

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
61	*Epilobium parviflorum* Schreb.	Willow herb	64	54	19	Y1
62	*Pimenta officinalis* L.	Allspice	83	93	2	No
63	*Punica granatum* L.	Pomegranate	99	196	18	E8, E4, E29, E30, Y1, L4, D15, E11, E26, D17, S26, E15, O1, M4, L8, E14, Y2, B2, E2, S28, E28
64	*Punica flos*	Pomegranate	85	87	22	
65	*Syzygium aromaticum* L.	Clove	100	*n*	0	E8, E4, Y1, D15, S22, D19, S23, B4, Y8, S26, D23, E14, O9, E10, D13, B20, B15
	Rosales					
66	*Alchemilla alpina* L.	Lady's mantle	80	80	19	No
67	*Frangula alnus* P. Mill.	Buckthorn	80	115	−12	No
68	*Humulus lupulus* L.	Hop	82	111	2	E8, E4, E13, E12, M3, E29, E30, M1, D15, S22, E22, D16
69	*Kerria japonica* (L.) DC.	Japanese rose	87	98	27	No
70	*Rosa canina* L.	Dog briar	74	69	30	E4, Y1, D17, D19, E22, E7, B4
71	*Urtica dioica* L.	Great nettle	73	65	23	E26, E25, B16, B21
	Sapindales					
72	*Citrus lemon* L.	Lemon	89	97	28	E8, S30, D17, D19, E18, S26, D30, E14, O12
73	*Schinus terebinthifolius* Raddi	Pink peppercorn	90	107	20	E4, Y1, L4, S22, S4, B4, M2, S26, B25, E28

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Vitales					
74	*Vitis vinifera* L.	Wine grape	86	84	25	S24, O11, B22, L2
	Asterales					
75	*Achillea millefolium* L.	Common yarrow	63	56	19	D15, S22, D9, D20
76	*Arctium lappa* L.	Great burdock	76	71	20	E8, Y1, E22
77	*Artemisia dracunculus* L.	Tarragon	98	140	25	E8, E4, E30, Y1, E26, S39, L10, E27, S4, E5, M2, S26, E3
78	*Calendula officinalis*	Marigold	67	58	21	E8, E4, E29, E30, D15, S30, E26, D17, E7, Y7, B10, L10, E27, E25, S26, S41, Y5, D7, B6
79	*Carthamus tinctorius* L.	Safflower	80	80	30	E8, Y7, E11
80	*Cnicus benedictus* L.	Holy thistle	68	64	15	No
81	*Echinacea purpurea* (L.) Moench	Echinacea	69	62	24	Y1, L4, Y6, B4, Y8
82	*Matricaria chamomilla* L.	Chamomile	91	109	30	E8, E4, E13, S24, D15, E22, M2, E15, S41, Y5, B16, D13
83	*Taraxacum officinale* L.	Dandelion	67	54	25	No
	Dipsacales					
84	*Sambucus nigra* L.	European elder	68	55	18	Y8, S38
	Gentianales					
85	*Asperula odorata* L.	Sweet woodruff	66	61	18	No
86	*Centaurium erythreae* Rafn.	Centaury	85	121	2	E8, E4, O10, D15, D9, E9, E11, S37
87	*Coffea arabica* L.	Coffee	68	56	24	E8, Y1, E22, E9, D24, B23

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
	Boraginales					
88	*Myosotis sylvatica* Hoffm.	Forget-me-not	67	59	27	No
	Lamiales					
89	*Hyssopus officinalis* L.	Hyssop	64	53	14	S26, S34
90	*Lavandula officinalis* L.	Lavender	75	70	24	Y1, O10, S30, E22, D16, E9, S29, S37, B8, L5, M4, L9, B17, B16, B21, B11, O7, D1, D13, D21
91	*Majorana hortensis* Moench.	Marjoram	70	60	33	E4, E13, Y1, L4, D15, O3
92	*Marrubium vulgare* L.	White horehound	66	55	28	E8, Y1, L4
93	*Melissa officinalis* L.	Common balm	100	*n*	0	E8, S22, Y8, S26, M4, B16, E23
94	*Mentha piperita* L.	Peppermint	75	69	33	E29, Y1, S1, E7, O6, B10, L10, S36, S23, S19, S26, E9, S29, S25, S17, S32, D30, Y5, B16, B13, B21, O7, D1, O3
95	*Ocimum basilicum* L.	Basil	81	79	31	E8, E4, E29, Y1, E30, L4, S30, E26, E7, Y7, B10, E27, E25, M2, E9, B24, S10, M4, S2, B27, O12, L3
96	*Origanum vulgare* L.	Oregano	83	94	23	E8, E13, E29, Y1, O10, L4, D15, S30, E22, B10, S36, E18, O4, S16, M2, S26, E9, S29, E15, S17, S37, S32, M4, L8, O8, E14, E10, S2, O7, D1, D25, D21, O3, S38, B20, B15

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
97	*Plantago major* L.	Common plantain	82	83	29	Y1
98	*Rosmarinus officinalis* L.	Rosemary	97	130	24	E8, Y1, O10, S24, D15, D17, E22, D16, Y4, M2, S26, E9, S29, E15, S41, S37, S32, Y5, E10, S2, O7, D1, B27, D21, O3
99	*Salvia officinalis* L.	Garden sage	81	88	6	Y1, O10, L4, D15, D17, O11, D16, S23, Y8, Y4, Y9, M2, S26, E15, S17, E23, S2
100	*Satureia hortensis* L.	Summer savory	89	147	2	E8, E4, L4, E5, M2, S26, S33, O9
101	*Syringa vulgaris*	Common lilac	81	78	27	No
102	*Thymus vulgaris* L.	Thyme	88	110	25	E8, E4, E13, M3, E29, Y1, O10, L4, D15, S22, S30, E22, B10, S36, E18, S23, Y8, S16, M2, M2, S26, E9, S29, E15, S25, S34, S17, L8, D30, O8, E14, E1, O7, D1, D13, B20, B15
103	*Verbascum phlomoides* L.	Mullein	84	86	31	Y1, D17, E22, Y4

(continued)

Table 11.3 (continued)

	Species (order)	Common name	Activity[a] (*R* %)			Reported activity[b]
			PA	SA	PD	
104	*Verbena officinalis* L.	Common vervain	84	174	13	E13, L4, E18, S4, S16, O8
105	*Veronica officinalis* L.	Speedwell	83	87	20	No
	Solanales					
106	*Capsicum annuum* L.	Pepper	82	69	24	E8, E4, Y1, O10, D15, O6, S26
107	*Ipomoea tricolor* Cav.	Morning glory	98	112	14	No

The cases where the only mammalian pathogens have been mentioned are not included into the references, but those where a phytopathogenic or food rotting species were tested and pathogenic species included into the tests the latter are mentioned

[a]Potential activity values (PA) have been calculated by potency mapping technique according to Lewi (1976); the largeness of activity spectrum (SA) refers to the set of fungi tested, which is negatively proportional to the given value, i.e., the *n* means complete inhibition of all strains tested; PD = the intensity of degradation (%) of the effect during 24 h of incubation, where zero means no deterioration of the efficacy

[b]The fungal species with reported sensitivity to extracts of the given plants are as follows:

Zygomycota: M1—*Mucor rouxii* (Shigeyuki and Yuko 1985), M2—*Mucor sp.* (bin Jantan et al. 2003; Szakiel et al. 2011; Abdolahi et al. 2010), M3—*Rhizopus oryzae* (Ujváry and Oros 2002; Niknejad et al. 2015; Khan et al. 2017; Nabigol and Farzaneh 2010), M4—*Rhizopus sp.* (Ali et al. 2017; Camele et al. 2010; Tehranifar et al. 2011; Lopez et al. 2007)

Basidiomycota: B1—*Malassezia furfur* (Waithaka et al. 2017), B2—*Rhodotorula mucilaginosa* (Visnjevec et al. 2017; Dulger et al. 2004), B3—*Cryptococcus gastricus* (Devi and Ganjewala 2009), B4—*Cryptococcus neoformans* (bin Jantan et al. 2003; Phongpaichit et al. 2005; Ewais et al. 2014; Shreaz et al. 2016; Pinheiro et al. 2017; Sigei 2013; Lewi 1976; Kovatcheva et al. 2011; Mir-Rashed et al. 2010; Thirach et al. 2003), B5—*Ustilago maydis* (Waithaka et al. 2017; Cardoso et al. 2017), B6—*Athelia rolfsii* (Fonseca et al. 2015a; Turkolmez and Soylu 2014), B7—*Coprinus comatus* (Ng and Wang 2001; Lam and Ng 2001), B8—*Pleurotus ostreatus* (Lelono et al. 2018), B9—*Schizophyllum commune* (Eberhardt and Young 1994), B10—*Rhizoctonia solani* (Fonseca et al. 2015a; Turkolmez and Soylu 2014; Kwon et al. 2017; Huang et al. 2010; Prasad et al. 2016; Yoon et al. 2013; Ojala et al. 2000; Osorio et al. 2010; Thobunluepop et al. 2009; Mullerriebau et al. 1995; Lee et al. 2007a), B11—*Gloeophyllum trabeum* (Sen and Yalcin 2010), B12—*Anthrodia sp.* (Hedenstrom et al. 2016), B13—*Ceriporiopsis subvermispora* (Sen and Yalcin 2010), B14—*Coriolus versicolor* (Shreaz et al. 2016), B15—*Laetiporus sulphureus* (Xie et al. 2017), B16—*Oligoporus placenta* (Sen and Yalcin 2010), B17—*Phanerochaete chrysosporium* (Arora and Ohlan 1997), B18—*Phlebia radiata* (Arora and Ohlan 1997), B19—*Sporotrichum pulverulentum* (Arora and Ohlan 1997), B20—*Trametes hirsuta* (Xie et al. 2017), B21—*Trametes versicolor* (Sen and Yalcin 2010), B22—*Heterobasidion parviporum* (Ojala et al. 2000; Kusumoto et al. 2014), B23—*Hemileia vastatrix* (de Colmenares et al. 1998), B24—*Peridiopsora mori* (Maji et al. 2005), B25—*Phakopsora pachyrhizi* (Bigaton et al. 2013), B26—*Puccinia triticina* (Cho et al. 2007), B27—*Uromyces appendiculatus* (Arslan et al. 2009)

Ascomycota: Saccharomycetales: Y1—*Candida albicans* (bin Jantan et al. 2003; Waithaka et al. 2017; Visnjevec et al. 2017; Devi and Ganjewala 2009; Ewais et al. 2014; Thirach et al. 2003; Millot et al. 2017; Uslu et al. 2013; Khan et al. 2013; Hong et al. 2004; Glisic et al. 2007; Sarac et al. 2014; Digrak et al. 1999; Yazdani et al. 2009; Houksey et al. 2010; Al Taweel 2007; Meng et al. 2009; Constantine 1966; Girardot et al. 2014; Iyer et al. 2017; Dulger et al. 2015; Nofouzi 2015; Roco Gauch et al. 2014; Ehssan and Saadabi 2012; Hearst et al. 2009; Castillo et al. 2018; Iwalokun et al. 2000; Verma et al. 2008; Dellamura and Edmar 2013; Ika et al. 2018; Alves et al. 2013; Al-Zubairi et al. 2017; Sasidhran and Menon 2010; Hammer et al. 1999; Jagessar et al. 2008; Namdar et al. 2014; Hirasawa and Takada 2004; Kosalec et al. 2005; Seidler-Łożykowska et al. 2013; Silva et al. 2011; Ertürk 2010; Altuner et al. 2010; Urziya et al. 2016; Bouterfas et al. 2016; Tonea et al. 2016; Johann et al. 2008; Endo et al. 2010; Hayouni et al. 2011; Kochthressia et al. 2012; Binns et al. 2000; Talib and Mahasneh 2010; Lopes-Lutz et al. 2008; Gundidza et al. 2009; Schmourlo et al. 2005; Giamperi et al. 2002; Bajer et al. 2017; Mejd et al. 2015; Bouterfas et al. 2016; Chen et al. 2013), Y2—*Candida glabrata* (Kochthressia et al. 2012), Y3—*Candida orthosilosis* (Badiee et al. 2012), Y4—*Candida parapsilosis* (Visnjevec et al. 2017; Sigei 2013; Nofouzi 2015; Roco Gauch et al. 2014; Seidler-Łożykowska et al. 2013; Hayouni et al. 2011; Badiee et al. 2012; Hofling et al. 2010), Y5—*Candida sp.* (Kasiri and Heidari-Soureshjani 2018; Ratha Bai and Kanimozhi 2012), Y6—*Candida tropicalis* (Sigei 2013; Digrak et al. 1999; Meng et al. 2009; Talib and Mahasneh 2010; Badiee et al. 2012; Taha and Shakour 2016), Y7—*Geotrichum candidum* (Seidler-Łożykowska et al. 2013; Silva et al. 2011; Lovecka et al. 2017; Bouzouita et al. 2003; Bibi et al. 2016), Y8—*Saccharomyces cerevisiae* (bin Jantan et al. 2003; Dulger et al. 2004; Phongpaichit et al. 2005; Mir-Rashed et al. 2010; Iwalokun et al. 2000; Sasidhran and Menon 2010; Namdar et al. 2014; Talib and Mahasneh 2010; Badiee et al. 2012; Wen 2009; Cioch et al. 2017; Farcasanu et al. 2006; Smith et al. 2008; Araujo et al. 2003; Barla et al. 2007; Baerlocher and Oertli 1978), Y9—*Torulopsis glabrata* (bin Jantan et al. 2003; Hofling et al. 2010). Hyphomycetales: W1—Aquatic hyphomycetes (Chantawannakul et al. 2005). Eurotiomycetes: E1—*Ascosphaera apis* (Boudegga et al. 2010; Kumar et al. 2010), E2—*Exophiala dermatitidis* (Visnjevec et al. 2017), E3—*Fonsecaea pedrosoi* (Gundidza et al. 2009), E4—*Aspergillus flavus* (Niknejad et al. 2015; Devi and Ganjewala 2009; Ewais et al. 2014; Castillo et al. 2018; Verma et al. 2008; Ika et al. 2018; Al-Zubairi et al. 2017; Binns et al. 2000; Gundidza et al. 2009; Schmourlo et al. 2005; Wen 2009; Kapoor et al. 2008; Al-Sohaibani et al. 2011; Uddin et al. 2003; Vânia et al. 2014; Simonić et al. 2014; Shiva Rani et al. 2013; Tian et al. 2012; Vokk et al. 2011; Dorman 1999; Skrinjar et al. 2009; Krishnamurthy et al. 2008; Mileva et al. 2014; Sagar et al. 2011; Tajehmiri et al. 2018; Preeti and Sudhir 2014; Dhingra et al. 2007; Cai et al. 2012; Rizwana et al. 2016), E5—*Aspergillus fumigatus* (bin Jantan et al. 2003; Binns et al. 2000; Tajehmiri et al. 2018; Babu et al. 2007), E6—*Aspergillus glaucus* (Simonić et al. 2014), E7—*Aspergillus nidulans* (Glisic et al. 2007; Preeti and Sudhir 2014), E8—*Aspergillus niger* (bin Jantan et al. 2003; Niknejad et al. 2015; Nabigol and Farzaneh 2010; Ali et al. 2017; Waithaka et al. 2017; Devi and Ganjewala 2009; Ewais et al. 2014; Glisic et al. 2007; Sarac et al. 2014; Verma et al. 2008; Sasidhran and Menon 2010; Altuner et al. 2010; Rakatama et al. 2018; Kochthressia et al. 2012; Binns et al. 2000; Gundidza et al. 2009; Taha and Shakour 2016; Mehrabian et al. 2000; Lovecka et al. 2017; Wen 2009; Kapoor et al. 2008; Uddin et al. 2003; Vânia et al. 2014; Simonić et al. 2014; Shiva Rani et al. 2013; Dorman 1999; Tajehmiri et al. 2018; Preeti and Sudhir 2014; Dhingra et al. 2007; Cai et al. 2012; Rizwana et al. 2016; Rodrigez 2017; Matthews and Haas 1993; Fierascu et al. 2018; Kawachi 2010; Singh et al. 2002; Saglam et al. 2009; Kloucek et al. 2012; Atta-Ur-Rahman Choudhary et al. 2000), E9—*Aspergillus ochraceus* (Verma et al. 2008; Cioch et al. 2017; Simonić et al. 2014; Houicher et al. 2016; Santos et al. 2014; Salem et al. 2016; Basilico and Basilico 1999; Stupar et al. 2014), E10—*Aspergillus parasiticus* (Saglam et al. 2009; Suganthi et al. 2013), E11—*Aspergillus versicolor* (Verma et al. 2008; Caputo et al. 2017), E11—*Penicillium chrysogenum* (Niknejad et al. 2015; Devi and Ganjewala 2009; Kumar and Yadav 2014), E12—*Penicillium citrinum* (Niknejad et al. 2015; Caputo et al. 2017; Boyraz and Ozcan 2005; De Martino et al. 2009; Felsociova et al. 2015), E13—*Penicillium digitatum* (Nabigol and Farzaneh 2010; Kloucek et al. 2012; Kharchoufi et al. 2018; Nicosia et al. 2016; Yahyazadeh et al. 2008; Vitoratos et al. 2013), E14—*Penicillium expansum*

(Lovecka et al. 2017; Caputo et al. 2017; Houicher et al. 2016; Felsociova et al. 2015; Nicosia et al. 2016; Yilmaz et al. 2016; Matos et al. 2011), E15—*Penicillium funiculos* (Verma et al. 2008; Linde et al. 2016; Simic et al. 2004), E16—*Penicillium gladioli* (Cai et al. 2012), E17—*Penicillium italicum* (Camele et al. 2010; Tehranifar et al. 2011; Digrak et al. 1999; Vitoratos et al. 2013), E18—*Penicillium marneffei* (Phongpaichit et al. 2005), E19—*Penicillium notatum* (Wen 2009), E20—*Penicillium ochrochloron* (Wen 2009; Salem et al. 2016), E21—*Penicillium sp.* (Ali et al. 2017; Nofouzi 2015; Sasidhran and Menon 2010; Preeti and Sudhir 2014; Matthews and Haas 1993; Fierascu et al. 2018; Boyraz and Ozcan 2005; Felsociova et al. 2015; Mizhir et al. 2016; Nionelli et al. 2018), E22—*Penicillium verrucosum* (Ozcakmak et al. 2012), E23—*Penicliium pallidum* (Chen et al. 2013), E24—*Epidermophyton floccosum* (bin Jantan et al. 2003; Ewais et al. 2014; Cavaleiro et al. 2006; Yazdani et al. 2009; Wuthi-Udomlert et al. 2000; Massiha and Zolfaghar 2015; Xue et al. 2017), E25—*Microsporum canis* (Abdolahi et al. 2010; Devi and Ganjewala 2009; Ewais et al. 2014; Cavaleiro et al. 2006; Yazdani et al. 2009; Seidler-Łożykowska et al. 2013; Gundidza et al. 2009; Hemamalini et al. 2015), E26—*Microsporum gypseum* (Waithaka et al. 2017; Phongpaichit et al. 2005; Cavaleiro et al. 2006; Ika et al. 2018; Seidler-Łożykowska et al. 2013; Gundidza et al. 2009; Shiva Rani et al. 2013; Wuthi-Udomlert et al. 2000; Massiha and Zolfaghar 2015; Xue et al. 2017; Sharma and Sharma 2013; Cespedesa et al. 2006), E27—*Paracoccidioides brasiliensis* (Xu et al. 2014), E28—*Trichophyton mentagrophytes* (Shigeyuki and Yuko 1985; bin Jantan et al. 2003; Cavaleiro et al. 2006; Yazdani et al. 2009; Houksey et al. 2010; Mehrabian et al. 2000; Wuthi-Udomlert et al. 2000; Sharma and Sharma 2013; Wegiera et al. 2011; Rautio e al. 2012), E29—*Trichophyton rubrum* (Okubo et al. 1991; bin Jantan et al. 2003; Seidler-Łożykowska et al. 2013; Kochthressia et al. 2012; Gundidza et al. 2009; Massiha and Zolfaghar 2015; Xue et al. 2017; Lis-Balchin et al. 1998; Hemamalini et al. 2015; Cespedesa et al. 2006), E30—*Trichophyton verrucosum* (Ghosh 2006; Hemamalini et al. 2015), E31—*Trichosporium vesiculosum* (Cobos et al. 2015), E32—*Phaeomoniella chlamydospora* (Saha et al. 2005). Dothideomycetes: D1—*Pyrenochaeta lycopersici* (Bajer et al. 2017), D2—*Botryodiplodia theobromae* (Begum et al. 2013; Chu et al. 2005), D3—*Botryosphaeria berengeriana* (Pan et al. 2017), D4—*Botryosphaeria dothidea* (Sherwood and Bonello 2013), D5—*Diplodia pinea* (Burger et al. 2010), D6—*Diplodia seriata* (Saha et al. 2005), D7—*Macrophomina phaseolina* (Fonseca et al. 2015a; Turkolmez and Soylu 2014; Cobos et al. 2015; Ghosh 2006; Chu et al. 2005; Ghosh 2006; Lee et al. 2007b), D8—*Phyllosticta caricae* (Mungkornasawakul et al. 2002), D9—*Cladosporium cladosporioides* (Verma et al. 2008; Simic et al. 2004; Endah 2005; Bekhechi et al. 2011; Minova et al. 2015), D10—*Fulvia fulva* (Simic et al. 2004), D11—*Mycosphaerella arachidicola* (Lam and Ng 2001), D12—*Mycosphaerella fragariae* (Hoyos et al. 2012), D13—*Pseudocercospora griseola* (Krauze-Baranowskaa and Wiwa 2003), D14—*Septoria chrysanthemi* (Endah 2005), D15—*Alternaria alternata* (Kwon et al. 2017; Cho et al. 2007; Verma et al. 2008; Shiva Rani et al. 2013; Sagar et al. 2011; Rizwana et al. 2016; Saglam et al. 2009; Kloucek et al. 2012; Simic et al. 2004; Chen et al. 2013; Chu et al. 2005; Minova et al. 2015; Johann et al. 2010; Xu et al. 2014; Gupta et al. 2017; Thakur et al. 2013; Glazer et al. 2012; Badawy and Abdelgaleil 2014; Cabral et al. 2016; Fawzi et al. 2009; Bayar et al. 2018; Pane et al. 2016), D16—*Alternaria solani* (Huang et al. 2010; Baka 2010; Thobunluepop et al. 2009; Itako et al. 2008; Dellavalle et al. 2011), D17—*Alternaria sp.* (Glisic et al. 2007; Nofouzi 2015; Preeti and Sudhir 2014; Mizhir et al. 2016; Endah 2005; Babu et al. 2007; Fiori et al. 2000; Tonucci-Zanardo et al. 2015), D18—*Bipolaris maydis* (Huang et al. 2010), D19—*Curvularia sp.* (Preeti and Sudhir 2014; Singh et al. 2002; Bekhechi et al. 2011; Fiori et al. 2000), D20—*Didymella bryoniae* (Wang et al. 2018), D21—*Epicoccum nigrum* (Stupar et al. 2014), D22—*Exserohdurn turcicum* (Rizvi et al. 1980), D23—*Exserohilum rostratum* (Bekhechi et al. 2011), D24—*Helminthosporium sp.* (Bekhechi et al. 2011; Smid et al. 2013), D25—*Phoma foveata* (Pedras and Sorensen 1998), D26—*Phoma helianthi* (Simic et al. 2004), D27—*Phoma sp.* (Bekhechi et al. 2011; Nagy et al. 2014), D28—*Stemphylium botryosum* (Badawy and Abdelgaleil 2014), D29—*Stemphylium solani* (Kwon et al. 2017), D30—*Venturia inaequalis* (Cho et al. 2007), Letiomycetes: L1—*Blumeria graminis* (Cho et al. 2007), L2 —*Erysiphe necator* (Pazmiño-Miranda et al. 2017), L3—*Phyllactinia corylea* (Maji et al. 2005), L4—*Botrytis cinerea* (Abdolahi et al. 2010; Nabigol and Farzaneh 2010; Camele et al. 2010; Tehranifar et al. 2011; Dulger et al. 2004; Ojala et al.

2000; Lee et al. 2007a; Lopez-Reyes et al. 2013; Cai et al. 2012; Nicosia et al. 2016; Vitoratos et al. 2013; Yilmaz et al. 2016; Matos et al. 2011; Chen et al. 2013; Endah 2005; Hoyos et al. 2012; Pazmiño-Miranda et al. 2017; Ikeura and Fumiyuki Kobayashi 2015; Pârvu et al. 2008; Dafarera et al. 2003; Bouchra et al. 2003; Corato et al. 2010; Elshafie et al. 2016; Zarai et al. 2011; dos Santos et al. 2010; Li et al. 2011; Parvu et al. 2013), L5—*Botrytis fabae* (Itako et al. 2008; Baka 2010), L6—*Botrytis peaoniae* (Cai et al. 2012), L7—*Botrytis tulipae* (Hussein and Joo 2017), L8—*Monilia laxa* (Nicosia et al. 2016; Elshafie et al. 2016; Li et al. 2011; Lopez-Reyes et al. 2013), L9—*Sclerotinia nivalis* (Thomidis and Filotheou 2016), L10—*Sclerotinia sclerotiorum* (Fonseca et al. 2015a; Kwon et al. 2017; Yoon et al. 2013; Mullerriebau et al. 1995; Cai et al. 2012; Chen et al. 2013; Pane et al. 2016; Thomidis and Filotheou 2016). Orbiliomycetes: O01—*Monacrosporium ambrosium* (Maji and Banerji 2015). Sordariomycetes: S1—*Pestalotiopsis theae* (Begum et al. 2013; Bekhechi et al. 2011), S2—*Pilidiella granati* (Meepagala et al. 2002), S3—*Valsa mali* (Zhang et al. 2006), S4—*Colletotrichum acutatum* (Hoyos et al. 2012; Zarai et al. 2011; Johnny et al. 2011), S5—*Colletotrichum cameliae* (Begum et al. 2013; Yanar et al. 2011a), S6—*Colletotrichum capsici* (Karimi et al. 2016), S7—*Colletotrichum coccodes* (Kwon et al. 2017; Cho et al. 2007; Xu et al. 2014; Schnee et al. 2013), S8—*Colletotrichum falcatum* (Singh et al. 2002), S9—*Colletotrichum gloeosporioides* (Lee et al. 2007a; Cho et al. 2007; Cho et al. 2007; Simonić et al. 2014; Dhingra et al. 2007; Rizwana et al. 2016; Santos et al. 2014; Yilmaz et al. 2016; Bekhechi et al. 2011; Xu et al. 2014; Pârvu et al. 2008; Johnny et al. 2011), S10—*Colletotrichum lindemutianum* (Caputo et al. 2017), S11—*Colletotrichum musae* (Simonić et al. 2014; Dhingra et al. 2007; Rodrigez 2017), S12—*Colletotrichum nymphaeae* (Arslan and Dervis 2010), S13—*Colletotrichum orbiculare* (Bekhechi et al. 2011), S14—*Colletotrichum sublinenola* (Owaid et al. 2017), S15—*Colletrotrichum truncatum* (Osorio et al. 2010), S16—*Verticillium dahliae* (Owaid et al. 2017), S17—*Verticillium fungicola* (Sokovic and VanGriensven 2006; Atmaca et al. 2017), S18—*Cylindrocarpon destructans* (Rizvi et al. 1980), S19—*Fusarium sp*. (Ali et al. 2017; Chu et al. 2005; Endah 2005; Badawy and Abdelgaleil 2014; Bouchra et al. 2003), S20—*Fusarium avenaceum* (Johann et al. 2010), S21—*Fusarium clamidosporum* (Chen et al. 2013), S22—*Fusarium culmorum* (Ojala et al. 2000; Dorman 1999; Golah et al. 2013; Johann et al. 2010; Zhang et al. 2006; Kumar et al. 2016), S23—*Fusarium graminearum* (Huang et al. 2010; Houicher et al. 2016; Chen et al. 2013; Rizvi et al. 1980; Zhang et al. 2006; Tomescu et al. 2015; Santamarina et al. 2016; Sales et al. 2015), S24—*Fusarium guttiforme* (Pinto et al. 2007), S25—*Fusarium moniliforme* (Sigei 2013; Thobunluepop et al. 2009; Mullerriebau et al. 1995; Singh et al. 2002; Ghosh 2006; Houicher et al. 2016; Cobos et al. 2015; Imtiaz 2016), S26—*Fusarium oxysporum* (Bowers and Locke 2000; Szakiel et al. 2011; Waithaka et al. 2017; Dulger et al. 2004; Fonseca et al. 2015a; Ng and Wang 2001; Lam and Ng 2001; Lee et al. 2007a, b; Verma et al. 2008; Al-Zubairi et al. 2017; Simonić et al. 2014; Singh et al. 1994; Dorman 1999; Rizwana et al. 2016; Fierascu et al. 2018; Cabral et al. 2016; Bayar et al. 2018; Pane et al. 2016; Fiori et al. 2000; Zhang et al. 2006; Imtiaz 2016; Rongai et al. 2017; Merali et al. 2003; Sesan et al. 2017; Matsubara et al. 2015; Elsherbiny et al. 2010), S27—*Fusarium poae* (Chen et al. 2013), S28—*Fusarium sambucinum* (Joseph et al. 2008), S29—*Fusarium semitectum* (Simonić et al. 2014; Dhingra et al. 2007), S30—*Fusarium solani* (Fonseca et al. 2015a; Sagar et al. 2011; Baka 2010; Preeti and Sudhir 2014; Itako et al. 2008; Bouchra et al. 2003; Zhang et al. 2006; Shuzhen et al. 2016; Liu et al. 2017; Singh and Rai 2000), S31—*Fusarium udum* (Milovanović et al. 2014), S32—*Fusarium verticillioides* (da Silva Bomfim et al. 2015; Avanço et al. 2017; Lopez et al. 2004; Mehrparvar et al. 2016), S33—*Lecanicillium fungicola* (Glamoclija et al. 2005), S34—*Mycogone perniciosa* (Potocnik et al. 2010), S35—*Trichoderma atroviridae* (Balkan et al. 2017), S36—*Trichoderma harzianum* (Sokovic and VanGriensven 2006; Yeo et al. 2009; Sasidhran and Menon 2010; Oros et al. 2010; Atmaca et al. 2017; Balkan et al. 2017), S37—*Trichoderma viride* (Verma et al. 2008; Vânia et al. 2014; Shiva Rani et al. 2013; Linde et al. 2016), S38—*Trichothecium roseum* (Endo et al. 1990), S39—*Pyricularia oryzae* (Cho et al. 2007; Xu et al. 2014; Rizvi et al. 1980; Zhang et al. 2006; Engelmeier et al. 2004; Fonseca et al. 2015b), S40—*Ceratocystis coerulescens* (Eberhardt and Young 1994), S41—*Chalara paradoxa* (Xue et al. 2017; Pinto

et al. 2007), S42—*Chaetomium globosum* (Cabral et al. 2016), S43—*Humicola grisea* (Kumar and Yadav 2014), S44—*Daldinia concentrica* (Arora and Ohlan 1997)

Oomycota: Peronosporales: O1—*Pythium sp*. (Osorio et al. 2010; Oros et al. 2010), O2—*Pythium aphanidermatum* (Huang et al. 2010), O3—*Pythium insidiosum* (Lee et al. 2007a; Kozlowski and Métraux 1999), O4—*Pythium ultimum* (Shenvi et al. 2011), O5—*Phytophthora cactorum* (Hoyos et al. 2012), O6—*Phytophthora capsici* (Shreaz et al. 2016; Mullerriebau et al. 1995; Zhao et al. 2004; Garcia et al. 2018; Bohinc et al. 2015), O7—*Phytophthora cinnamomi* (Bajer et al. 2017), O8—*Phytophthora citrophthora* (Camele et al. 2010), O9—*Phytophthora megasperma* (Rizvi et al. 1980), O10—*Phytophythora infestans* (Cho et al. 2007; Itako et al. 2008; Yanar et al. 2011b; Soylu et al. 2006a, b; Baka 2010; Godeanu-Matei et al. 2016), O11—*Plasmopara viticola* (Pazmiño-Miranda et al. 2017; Gabaston et al. 2017; Dagostin et al. 2011), O13—*Sclerospora graminicola* (Deepak et al. 2005)

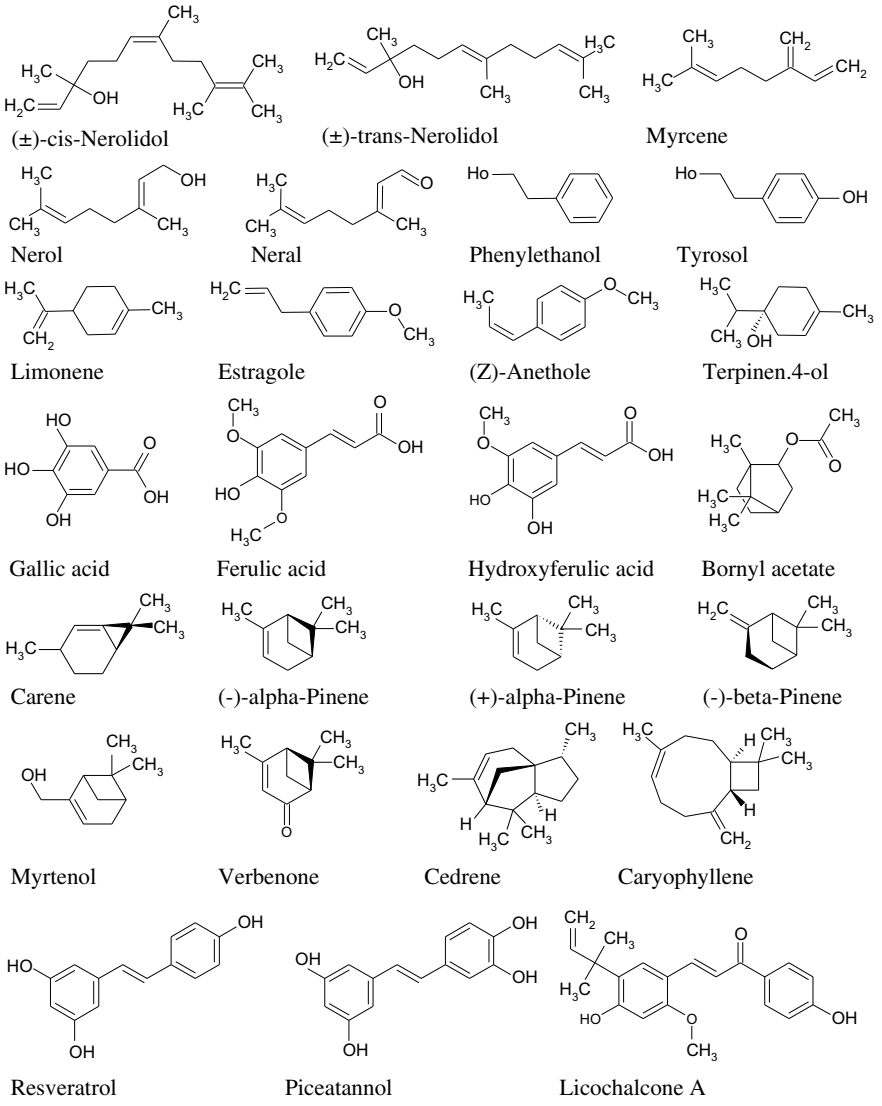
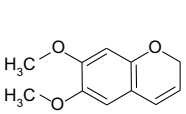
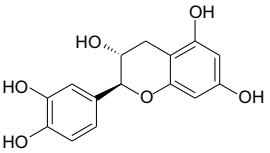
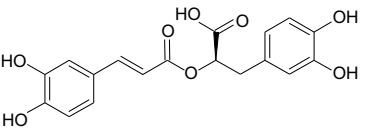
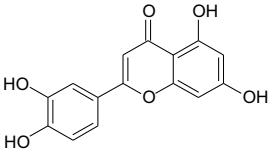


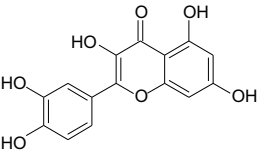
Fig. 11.1 Major secondary metabolites with approved antifungal effect of the most potent culinary and medical herbs tested. R^1—gluocose, R^2—galactose-glucose[xylose]-galactose-glucose. The presence of as minimum as one of listed compounds has been demonstrated in proper marketed spice or herb at more than 10% of active ingredients

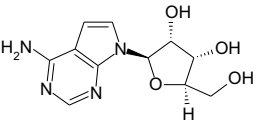

(Z)-Asarone

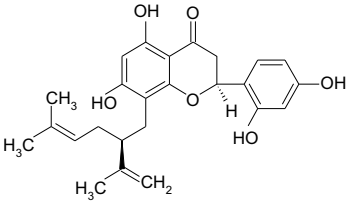

Catechin

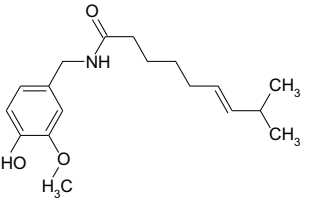

Rosmarinic acid

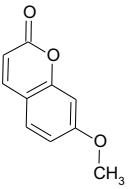

Luteolin

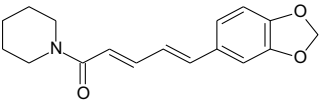

Quercetin

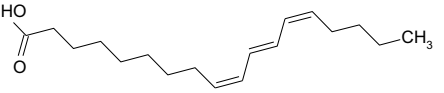

Tubericidin

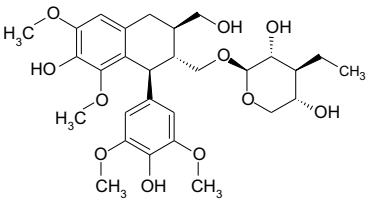

Sophoraflavanone G


Capsaicin


Herniarin


Piperine


Punicic acid


Lyoniside

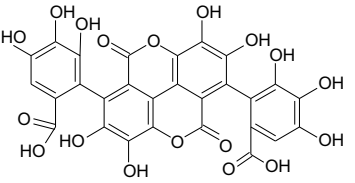

Gallagic acid

Fig. 11.1 (continued)

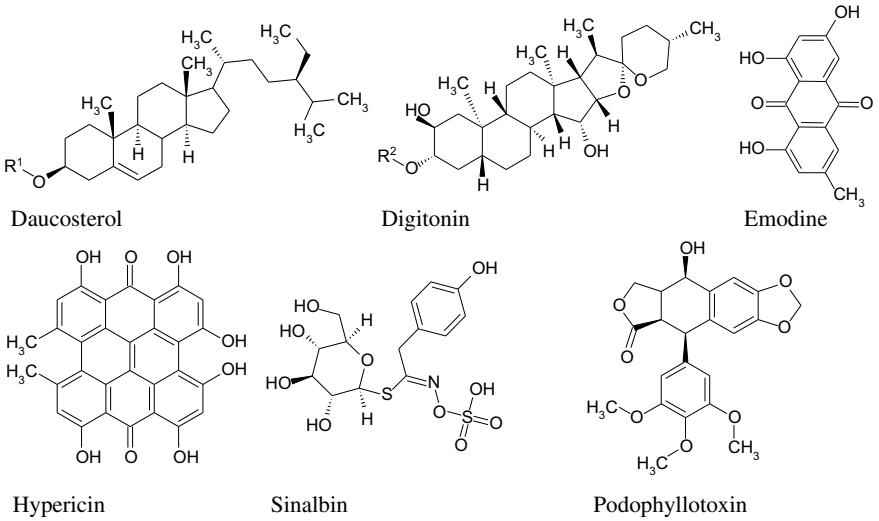


Fig. 11.1 (continued)

Data Analysis Fisher's test was applied to evaluate the significance of differences between variants at $p = 0.05$ level. The basic data matrix (107 preparations × 25 target strains × 2 evaluations) comprising response values by the scale of evaluation was subsequently analyzed with multivariate statistical methods following previously described scheme to elucidate the number of factors affecting the selective response of target fungi to toxic principles (Magyar and Oros 2012).

Potency mapping (PM) and spectral component analysis (SCA) were employed to disclose differences between both antifungal activity of preparations and sensitivity responses of test strains following Lewi (1976). The SCA separates the basic data matrix into two part; the first is a vector proportional to overall strength of responses (PM), while the second is a matrix of spectral components (Spectral Map, SPM) characterizing the spectrum of activity or sensitivity.

PCA was carried out on the correlation matrix calculated of basic data matrix, and only the components having an eigenvalue greater than one were included into the evaluation of data to demonstrate potential number of factors influencing on sensitivity responses of target fungi. Moreover, principal component regression analysis (PCRA) was employed to reveal changes in weight of influencing factors during the incubation, i.e., time dependence of the growth inhibitory effect.

Box plot analysis was applied to demonstrate time-dependent alterations in sensitivity responses. Cluster analysis (CA) combined with SCA was used to reveal relationships among the spectrum of sensitivity responses of phytopathogenic fungi to preparations.

Statistical functions of Microsoft Office Excel 2003 (Microsoft, Redmond, USA) and Statistica5 program (StatSoft 5.0., Tulsa, USA) were used for analysis of data. The graphical presentation of result of data analysis was edited uniformly in MS Office PowerPoint 2003.

11.3 Results

The conidia of all strains germinated and start to form well-distinguishable colonies within 24 h after inoculation, and the intensity of radial growth corresponded to character of species on untreated control plots. The differences between parallels did not surpass 1 mm, so their growth was near synchronous.

The germination of conidia of all strains was inhibited by various degree by herbs after 24 h of inoculation with the exception of *Alternaria* that start to form colony growing on *Clematis vitalba*: Therefore at given dose, all herbs exhibited outstanding antifungal effect being the *Hyssopus officinalis* the least active (Table 11.3). However, this situation changed dramatically after 24 h when the only ten herbs inhibited the growth of all strains (Table 11.4). The loss of activity varied within large limits, and no pattern could be recognized about the taxonomic position of plants (details of the analysis of SMP are not shown). The increase of inhibition as compared to untreated control was observed in 99 cases of 2675 pairs; the more than half of such cases were observed in Ranunculales, Caryophyllales, Myrtales, and Rosales (7, 8, 7, and 23, respectively), and no cases occurred in culinary fungi and moss. The relationship between the initial activity of herbal preparations and activation process needs further studies, although, seemingly the moderately active herb suffered the major deterioration of their antifungal effect.

The sensitivity response of strains varied in large limits; however, none of them was inhibited completely by all preparations. With exception of *Colletotrichum musae* and *Gliocladium catenulatum,* all strains activated as minimum as one of herbs, taking into the consideration the 99 of 2675 pairs, so this process seems to be highly specific and depends on target fungus. Clustering the fungi based on daily changes in their response to herbal preparation (A_{24}-A_{48}), two big clusters have been separated (Fig. 11.2). The strains of soil origin and the insect pathogen were separated of those isolated of foliage. The abilities to either deteriorate or activate the antifungal effect seemingly were not related to taxonomic position of target fungi, as, for example, *Geotrichum candidum* and *Trichotecium roseum* formed a close cluster, or two *Glomerella cingulata* anamorphs (Sour Cherry 1 and 2) have been linked into two different subclusters. The clusters A and B forming a super-cluster comprise more sensitive strains than C, D, and E; moreover, the latter are more heterogeneous in respect of the origin of strains. Thus, one can suppose that former environmental adaptation takes more influence on their sensitivity responses to herbs than traits formed during phylogeny.

Table 11.4 Similarity of hidden variables influencing the performance of growth response of target strains

First day		Principal components second day							
PCs	No.	1	2	5	8	10	11	12	13
No.	Weight	54.6	10.6	4.2	2.7	2.3	1.9	1.6	1.5
1	49.6	**−0.843**	−0.167	−0.011	0.147	0.278	0.075	0.099	0.126
2	9.6	0.094	**0.734**	−0.049	0.117	0.203	0.071	0.263	0.088
3	3.8	0.281	−0.135	−0.083	0.007	**0.527**	0.063	0.175	0.095
7	2.5	−0.091	−0.072	**−0.618**	0.053	0.012	−0.069	−0.070	0.033
8	2.1	0.114	−0.050	−0.180	**0.507**	0.177	−0.103	−0.239	−0.021
10	1.8	−0.066	0.130	−0.100	−0.226	0.058	**−0.531**	0.051	0.447
11	1.5	−0.107	0.205	0.292	−0.196	0.290	−0.019	**−0.564**	−0.244
12	1.4	0.096	0.079	0.162	0.037	0.198	0.145	0.003	**0.573**

The weight of principal components is given in percents of total variation they comprised. Significance of correlation coefficients: $r_{0.01} = 0.5256$; $r_{0.02} = 0.4815$

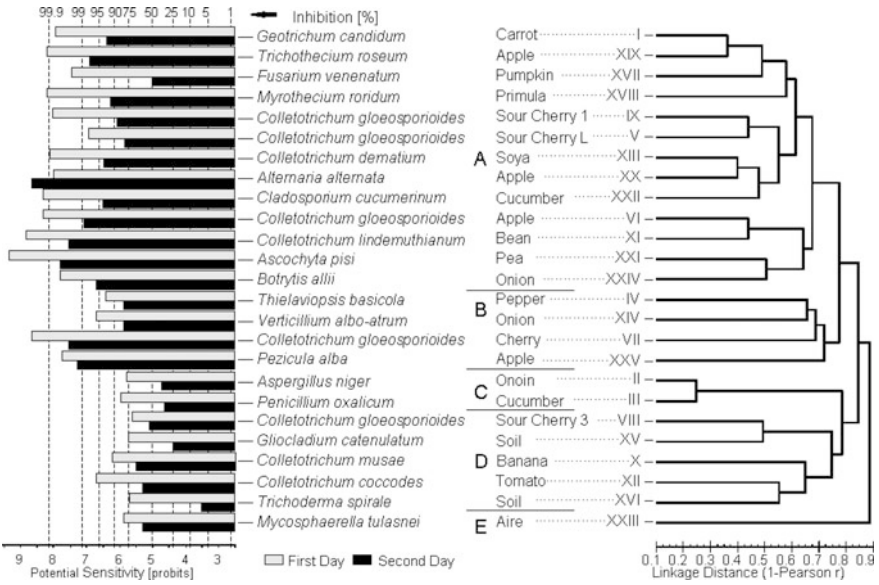
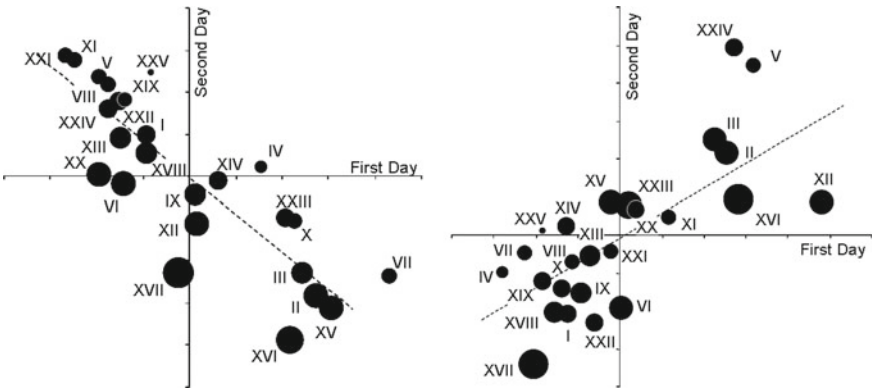


Fig. 11.2 Relationship between spectrum of deterioration of inhibitory substances and growth response of test fungi. Prior to SCA, the growth responses were converted to probit values. Potency mapping technique was used to calculate potential overall sensitivity of strains (growth response to strength of herbal action). The similarity of the sensitivity spectra of strains was analyzed applying unweighted pair group averages method based on correlation matrix of spectral variables. Subclasses were sorted at $p < 0.05$ level

The principal component analysis revealed high number of factors determining the action of herbal preparations. The response of fungi was influenced during conidial germination and germ tube elongation, i.e., start of colony formation (first day of evaluation) by sixteen principal components (PCs) having an eigenvalue greater than one, which comprised 95% of total variation, and among them four hidden factors were seemingly responsible at 70% of the inhibitory effect of herbs. After subsequent incubation (second-day evaluation), the growth response of the same set altered as it was delineated above (see Table 11.3); the PCA elucidated 13 relevant PCs comprising 93% of total variation, where three of them related to 73% of inhibition of colony formation.

This time-dependent reduction of the number of PCs (hidden variables) that influences significantly the performance of strains growing on poisoned agar plates indicates that some factors were eliminated of the medium. Indeed, comparing sets of data recorded at first and second evaluations by means of PCRA sorted out eight PCs in both sets (Table 11.4), which were correlated significantly and explaining majority of acting hidden factors (72 and 81%, respectively). In both sets were separated five PCs which did not show similarity (explaining 19 and 12% of total variation, respectively). The increase of the weight of similar hidden variables as well as decrease of their number (three PCs of 3.7% weight) as compared to the first



A - First PCs explaining 49,6 and 54,6 percents of total variation

B - Second PCs explaining 9,6 and 10,6 percents of total variation

Fig. 11.3 Similarities between hidden variables influencing the performance of sensitivity responses of target strains at various phases of their ontogeny. The Roman numerals indicate strains listed in Fig. 11.2. The size of pies is proportional to potential capacity of strain to deactivate the growth inhibitory effect of herb incorporated into the medium. The path coefficients of the fitness of regression lines in graphs A and B are 0.7099 and 0.7845, respectively

evaluation might indicate the changes in the level of active compounds in the medium resulted by metabolic activity of target fungi. As the activity of various herbs was affected by strain-dependent manner, only some general aspects of the character of major hidden variables could be postulated. Plotting strains as PC variables by intercorrelating the major PCs of two sets (Fig. 11.3) elucidated remarkable selectivity of interaction between herbs and strains. The first pair (Fig. 11.3a) negatively influenced the performance of herbs, so it can be most probably related to metabolic degradation of active principles, while the second pair (Fig. 11.3b) affected positively, which may indicate the increase of importance of permanent target sites in expression of antifungal effect (characterized by intensity of growth inhibition).

11.4 Exploitation of Findings

The anthracnose caused by *Glomerella* anamorph has caused increasing losses in Hungarian sour cherry orchards since 2006. The pathogen rapidly acquired tolerance to most effective triazole fungicides. Because of the short tolerance period (maximum 6 days), the protection of sour cherry fruit is a special problem, and use of rapidly deteriorating fungicide is requested. The botanical preparations can stand this prerequisite. The possible use of ten herbs proved to be most active among tested ones (shiitake, galangal, cinnamon, yellow mustard, clove, oregano, summer

flavory, wasabi root, wood ear, pomegranate) had been examined to control anthracnose of almond, bilberry, cherries, green pepper, grape, and tomatoes.

However, the promising results in model experiments could not be reproduced in large scale in the sour cherry orchard, where the situation was similar to those observed in the case of pathogenic *Glomerella* anamorphs (Oros et al. 2010). The only pomegranate preparation acted at acceptable way at 1 kg ha^{-1} rate in model experiments that means the preparation manufactured of aborted flowers can control the pathogen, while the others either should be applied at irrational for control doses

Table 11.5 Most important relationships to be evaluated for development and application of pest control agents

Exposed organisms			Therapeutic index	Persistence (days)
To be controlled		To be protected (P)		
Pest (C)	Traditional	*Homo sapiens*	No harm	Not
		Host plant	>5	1–30
		Vertebrates	>100	Not
		Bees	>100	Not
		Saccharomyces	>3	Not
	Future	Symbionts	>10	Not
		Antagonists	?	Not
		Predators	?	Not
		Ecosystems	?	?

Therapeutic index (T.I.) = MTD_P/MID_C, where MTD and MID are maximum tolerated and minimum inhibitory doses of control agent, respectively

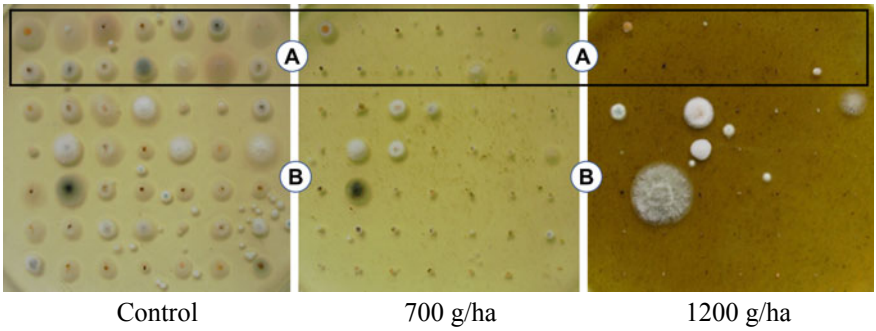


Fig. 11.4 Growth response of *Glomerella* anamorphs to various doses of pomegranate. The standard preparation made of micronized aborted pomegranate flowers was incorporated into PDA prior to pouring into Petri dishes (5-mm-thick layer) at rate mimicking the concentration of the spray to be used in field conditions. The strains in black bordered box (A) are various *Glomerella* anamorph (*Colletotrichum* species), while those of group B were isolated of sour cherry fruits collected in orchards of Fruitculture Research Institute (47°40′22.2″ N, 21°41′24.7″ E). The positions of the proper strains are identical on plates

or proved to be phytotoxic in effective dose, i.e., their therapeutic value lagged behind highly active synthetic monosite inhibitors (Table 11.5).

Unfortunately, 5 of 35 lines of anthracnose pathogen proved to be highly tolerant to prospected new biofungicide (Fig. 11.4). Most probably, in the case of other host/pathogen pairs, similar results can be expected that shows the limit of development of botanical fungicides based on crude preparations.

11.5 Discussion

The use of chemical fungicides is costly and potentially harmful to the environment. The trend toward the environmentally friendly pesticides has led to the search for new antifungal agents from various sources, including medicinal herbs, however, to plants of culinary use have paid less attention. Alternative control with herbal preparations showing the greatest antifungal potential could provide economical, safe, and non-hazardous tools for management of cultivated plants and increase food quality from sustainable production (Khaskheli et al. 2016). Most probably all plants have phytoanticipins of diverse molecular structure and size of simple myrcene or phenylethanol to steroid alkaloids, oligocarbohydrates, proteins, etc. There are increasing number of studies dealing with the isolation and chemical characterization of such molecules as well as their role in host–pathogen interactions. Several and successful efforts have been made to introduce compounds of plant origin (strychnine, rotenone, cevadine, pyrethrins) to use against pests; however, the botanicals of similar activity or formers active against phytopathogenic fungi have not been marketed yet. Here we investigated only the heat-tolerant compounds of low molecular weight.

There are increasing number of studies dealing with the isolation and chemical characterization of phytoanticipins as well as their role in host–pathogen interactions. Nevertheless, it seems to be clear that the defense molecules either predisposed or induced cannot be regarded as the agents of a single defense mechanisms. Very little detailed information is at our disposal about the multiple mechanisms for plant resistance against pests and pathogens, and these are still a matter of debate.

The Biopesticides and Pollution Prevention Division in the Office of Pesticide Programs of Environmental Protection Agency of USA encourages the development of biopesticides as well as the use of safer pesticides, including biopesticides. Since generally accepted that biopesticides tend to pose fever risks than conventional ones, EPA generally requires much less data to register them than latter. In fact, new biopesticide is often registered less than a year, compared with an average of more than three years for those based on synthetic chemicals. However, using any chemical in pest control management the same requirements have to be taken into the consideration, when these preparations aimed to be applied at large scale! Moreover, the selectivity of action also has to be evaluated by the same manner, independently of the character of active ingredient, and this requirement is more strict than those used in the case of pharmaceuticals (Table 11.5). For example, the

bees meet regularly the essential oil flavonoids that are mighty attractants for pollinating animals. However, the dose in concerted activities is very low. It is well known; the essential oils might be detrimental for humans in elevated doses when inhaling for long-term exposure. Numerous reports support that content of preformed antifungal compounds correlates with disease resistance, for example, the fall of preformed antifungal compounds in strawberry fruits was correlated with a decline in natural disease resistance against *Botrytis cinerea* (Terry et al. 2004). Analogies of medicine frequently used as some botanical preparations are traditionally applied against dermatomycoses. However, the decision on therapeutic value is different: In medicine, some iatrogenic effect might be accepted, for example, the drug applied more harmful to cancer cells than regular cells, or the use of arsenic derivatives to eliminate parasitic protozoans. In these cases usually, the ration of ED50 or LD50 values is used. Contrarily, the adverse effects in the case of host/parasite pairs are rarely accepted, and the ration of maximum tolerated dose by host plant and minimum inhibitory dose for pathogen should be taken (Table 11.5); moreover, the decisionmaker should take account of suspected knowledge of users when recommends dose for practical applications, i.e., the three- to fivefold overdose cannot harm the exposed cultivated plant.

The separation and identification of active principles of herbs important as well as the use of well-identified molecules have advantages. However, the crude extracts and herbal material per se often differ in the activity being the latter more effective (Al-Sohaibani et al. 2011). The preparation may destroy the active principle, or separate synergically interacting substances (Kapoor et al. 2008). The content of single molecules and their ratios often differ batch to batch, which shows similar affectivity due to synergic interaction of component. This fact indicates that the use of homemade crude preparations may have advantages in special regards in microscale applications.

Dramatic advancement in biology can be seen within the last fifty years. The contemporary plant biology, which led through meristem culture to the clonal propagation as well as these procedures led to tissue culture techniques, which were utilized to grow cells in suspension cultures with subsequent ability to regenerate whole plants that created a whole new era in plant biology. Some efforts have been made, and there is an increasing interest to introduce alien genes coding performed defense molecules into cultivated plants. These new properties also should be approved by selectivity criteria that are identical to those requested in the case of synthetic pesticides (Table 11.2). The experiences are contradictory: Unexpected adverse effect has manifested both in biocoenoses and in pests themselves, mainly due to acquired tolerance in populations of target organisms, like Lepidopteras to thuringiensis toxins or innumerable weed species to herbicides. No doubt about that microbes of agricultural interests will also rapidly adapt to new properties. The introduction of toxic substances alien to edible plants can also induce serious damages as it was demonstrated in the case of galanthus toxin—all this underlines that the soft practice of EPA cannot be kept when the registration of biopreparations for large-scale use takes place. Nevertheless, the intentions to improve the plant resistance by rationally designed genetic manipulations using biotech methods are

Table 11.6 Expected application of gene engineering to approve efficiency of pest control by biorational way

Type of compound	Field of application		
	Food chain	Industrial plants	Wild areas
Phytoalexin	+	+	+
Phytoanticipin	?	+	+
Signal molecules	?	+	+

promising together with to develop botanical preparations to combat losses in agriculture (Table 11.6).

Some questions need answers, first of all problems of unwanted exposures. In spite of intensive studies on defense molecules our knowledge regarding their mode of action and the flow of signal transmitters from the pathogen to the plant cells is still poor. The protective functions are highly diversified, and the variegation of defense mechanism shows multifunctional character. The exposed population, being not uniform genetically, is a mixture of strains as well as the ratio of different isomers can vary in botanical preparation depending on source and mode of manufacturing, the strain-specific action and stereometric-dependent response may limit the usefulness of herbal preparations. Nevertheless, studies on the biological activities of herbs are increasingly important in the search for natural and safe alternative pesticides in recent years. There is a lot of to be done before their use in large scale. More in-depth knowledge of potentially useful plants can provide results of economic importance for food and even pharmacological industry.

The abundant use of antimicrobial agents resulted in the emergence of drug-resistant bacteria, fungi, and viruses both in medicine and agriculture. To overcome this threat, there is necessary to find new, effective antimicrobial agents with novel modes of actions. The plant defense molecules are promising candidates for lead compounds. Some compromise among yield sureness, quality, and number of products is requested for making the biorationally designed and carefully selected new varieties.

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Plant Nutritional Deficiency and Its Impact on Crop Production

12

J. W. Park, J. C. Melgar and M. Kunta

Abstract

Nutritional imbalances in plants influence their responses and defense mechanisms against abiotic stress, pests, and diseases, ultimately impacting crop production. Normal functioning and growth of the plant are affected due to insufficient availability of an essential nutrient(s). Plants have developed highly complex and specialized nutrient sensing and signaling systems to respond to varying nutrient availability in the soil. Interaction of nutritional status and complex signaling mechanisms play a crucial role in plant's tolerance against diseases and pests. The potential role of deficiency or excess nutrients in plant's defense against pest and diseases and nutrient sensing and signaling mechanisms in plants is discussed in this chapter. These insights will lead to the development of strategies for a long-term sustainable nutrient management and improved nutrient use efficiency.

Keywords

Plant signaling · Defense mechanisms · Nutritional management · Diseases tolerance · Nutrient uptake

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12.1 Introduction

Nutritional imbalances in tree crops and their responses and defense mechanisms against pests and diseases have been much less studied than in annual crops. Understanding these interactions in trees requires knowledge of seasonal and annual patterns, as nutrient requirements are highly influenced by tree phenology, as well as by its growth and productivity, which changes throughout their life span. Unlike annual crops, nutritional management in tree crops should consider reserve organs as they play a key role in satisfying tree nutrient requirements. Thus, nutrients mobilized to new organs in spring come mostly from reserve organs, although the source organs and the percentage of nutrients mobilized from them depend on tree species and their leaf drop habit (deciduous vs. evergreen). As an example, in citrus, more than 70% of N accumulated in developing organs during spring is estimated to come from tree reserves, especially old leaves and roots (Legaz et al. 1995). In a hysteranthous deciduous tree species such as peach, reserve organs exclusively provide nutrients to growing reproductive and vegetative structures for the first month after bloom, and a combination of nutrients from reserves and the soil are used during the first two and a half months after bloom (Rufat and DeJong 2001). For these reasons, nutrient resorption and storage processes during fall and winter are essential in fulfilling tree nutritional requirements in spring. Since nutrient resorption, storage and remobilization processes are also affected by biotic and abiotic factors; these can also cause indirect effects on crop production through their impact on tree nutrition.

One of the most relevant areas where nutritional status and plant signaling interact is on resistance to plant diseases and insects. The role of nutrients in controlling plant diseases was extensively reviewed by Dordas (2008) although, once again, there are less research studies that focus on tree crops compared to annual crops. Some studies have reported interactions between nutrient uptake/transport and the plant–fungi parasitic relationship (Zeilinger et al. 2016). For instance, high concentrations of N increase the severity of infections by obligate parasites but decrease the severity of the infection by facultative parasites. In this sense, orchard management practices such as fertilization or pruning can also have a remarkable impact on reducing the incidence of a disease. It is well known that cultural practices that induce luxuriant vegetative growth or vigor (e.g., excessive N fertilization, pruning) can negatively affect fruit tree architecture and within-tree microclimate and increase the development of pests and pathogens. As a consequence, practices that aim to provide a more aerated canopy and allow greater distances between shoots reduce disease inoculum and contribute to crop protection, especially in organic or integrated pest management orchards (Simon et al. 2015). Similarly, other cultural practices that improve soil nutrient content and/or retention such as the application of organic amendments or mulching can affect the control of diseases, although the response mechanisms are not well understood (Huber and Graham 1999). The opposite can also occur: pathogen infection may drive nutrient resorption, as it happens in citrus trees infected with '*Candidatus*

Liberibacter asiaticus', the causal agent of citrus huanglongbing. Infected trees show callose deposition in phloem sieve tubes that restrict phloem transport of nutrients (Kim et al. 2009; Koh et al. 2012).

In the last decades, research studies focusing on the production of secondary metabolites with roles in plant defense from insect herbivores or diseases have described how nutrient fertilization influences the production of these metabolites (War et al. 2018). Nevertheless, at the same time, the production of certain chemicals involved in plant defense can also reduce nutrient uptake under specific orchard practices. For instance, plant phenolic compounds such as lignin increase leaf toughness and reduce insect feeding but could also reduce nutrient uptake if nutrients are applied through foliar fertilization. In the following section, we will review specific mineral nutrients (N, P, K, and Ca) and how their deficiency or excess could trigger defense signals against biotic and abiotic factors that can significantly impact tree crops.

12.2 Role of Specific Mineral Nutrients in Plant's Defense Against Biotic and Abiotic Factors

12.2.1 Nitrogen

Nitrogen is a key component of amino acids; therefore, an excessive supply of N can increase the content of amino acids and other N-containing compounds in plant tissues and, consequently, attract insects and pathogens. Some of the factors attracting insects and pathogens are the timing and intensity of flushing; young or rapidly growing trees are more likely to suffer attack by pests than older or slower-growing trees. Appropriate nutrition and pruning can be used to partly control flushing, as flushes need to be vigorous enough to provide photosynthates and support growth of reproductive structures but not so vigorous as to increase the risk of attack by pests. These mineral imbalances lower resistance to fungal diseases by reducing the physical resistance to the insect pests and creating a more favorable environment for pathogens. Adequate N levels also increase tree resistance to most bacterial diseases, whereas they can thrive in trees with low and high N conditions (Schumann et al. 2010). Low supplies of N may predispose trees to infections by facultative parasites such as *Fusarium* spp. In contrast, an excessive N supply increases disease or damage caused by some pests and pathogens. For instance, the effect of high N fertilization in pears is well known since several decades ago: freckle pit, cork spot, and blemishes on fruit from *Psylla pyricola* increased in trees treated with high rates of N fertilizers (Raese and Staiff 1989), and less fire blight was observed in inoculated peach trees that received low N and high K. While, the greatest blight was seen in trees receiving high N and low K (Keil and Shear 1972). Furthermore, N fertilizers could affect secondary metabolites with relevant plant defense properties. For instance, in a study on wild crucifers, Hugentobler and Renwick (1995) reported lower leaf concentrations of cardenolides [notably known

for its high content are milkweed (*Asclepias*) plants] in leaves of plants with high levels of N, and similar responses have also been found in other plant species (Agrawal et al. 2012). Nevertheless, there is a lack of understanding of how nutrition affects plant defense elicitors in tree crops.

Nitrogen acts as a signaling element in plants, although processes involved are not well understood. For instance, studies on the influence of N on phytohormones have shown that auxin, ethylene, and cytokinins are involved in root architectural responses to nitrates (Tian et al. 2009; Ruffel et al. 2011; and Jin et al. 2012). Similarly, the availability of N can modify leaf longevity and transition to reproductive and senescence stages through its influence on ethylene (Khan et al. 2015). However, most of these studies have been done on annual (model) plants, and there is a lack of research on the effect of N deficiency or excess in fruit trees, where ethylene plays a major role, especially during post-harvest (Khan et al. 2015). Although high, medium, or low N fertilization has not been reported to affect ethylene production in peach (Okamoto et al. 2001) or apple trees (Wargo et al. 2004), low and high N fertilization has been found to delay fruit ripening in different crops (Okamoto et al. 2001; Khan et al. 2015).

Nitrogen nutrition can also interact with other abiotic factors to modify the level of infection. Keller et al. (2003) demonstrated that the stress response of grapevines to low N and high UV conditions contributed to creating an unfavorable environment for powdery mildew growth through the enhanced synthesis of constitutive phenolic compounds such as flavonol glycosides and hydroxyl-cinnamic acid derivatives.

12.2.2 Phosphorus

Tree demands of P are much less important (quantitatively) than those of other nutrients such as N or K. This is due to the low amounts of P (compared to N or K) extracted from tree crops through different orchard management practices, for instance, when the fruit is harvested or wood is pruned. Nevertheless, P is a key element for many physiological processes and low availability of P can impair plant growth, development, and productivity. Understanding how plants sense P deficiency and trigger the responses via signaling networks has become of significant interest, and these networks have been studied in model plants (Chiou and Lin 2011) but research on tree crops is very limited, although they have been suggested to be based on a complex signaling process (Smith 2009). Even the mechanisms by which P nutrition contributes to fruit tree productivity are scarcely studied (Erel et al. 2016). As with N, the maintenance of a balanced nutrient system is critical for coping with diseases, and one example is HLB in citrus: maintaining optimum or higher P concentrations within the citrus plants have been proposed as a defensive strategy against the HLB-bacterial infection (Cao et al. 2015).

Phosphorus nutritional status of trees has been reported to influence the severity of diseases, although the role of P in resistance is variable and seemingly inconsistent (Dordas 2008; Abdelrahman et al. 2018). In some cases, the increase in

resistance seems to be a consequence of P on the development of new growth; improved root growth by P nutrition may allow the plant to "escape" attack by soil-borne fungal pathogens or nematodes (Prabhu et al. 2007). In any case, foliar sprays of phosphate salts inhibited the development of the powdery mildew on shoots and leaves of apple trees, wine grapes, mango, and nectarine (Reuveni et al. 1998). However, the mode of action of foliar-applied phosphate salts in controlling powdery mildew is not clear.

12.2.3 Potassium

Potassium is one of the nutrients required at higher concentrations by tree crops since it is removed at high levels with fruit harvest and pruning. Its cycling (resorption, storage, and remobilization) within trees need to be further studied, but it is relevant because it is a very mobile nutrient and it is involved in the regulation of many different physiological processes. Some of the functions in regard to disease and pest resistance are related to its essential role for the synthesis of proteins, starch, and cellulose in plants. Cellulose, for instance, is a primary component of cell walls, and K deficiency causes cell walls to become leaky, resulting in high sugar (starch precursor) and amino acid (protein building blocks) concentrations in the leaf apoplast, which makes cells more susceptible to fungal diseases.

Optimum levels of K have been suggested to improve the resistance of plants against biotic (Prabhu et al. 2007) and abiotic stress (Cakmak 2005). It is important to consider that K deficiency can be induced by high concentrations of other cations such as Ca or Mg, which can displace K from the cation exchange complex of the soil. Potassium deficiency has been found to be linked to diseases in some temperate crops. In this sense, K deficiencies created by overapplication of dolomite or Mg can lower this resistance although the mechanism of resistance in some disease-resistant genotypes might be related to greater efficiency in K uptake (Prabhu et al. 2007). The N/K ratio can also affect resistance: if it is too high, cells may have thinner cell walls and weaker membranes and are more prone to pathogen attack (Marschner 1995).

12.2.4 Calcium

Calcium is the most studied nutrient regarding its effects on improving acclimation against abiotic and biotic stresses, and that is due to its role in strengthening and promoting the cell wall integrity by cross-linking pectin molecules. Fungi and bacteria typically release enzymes that dissolve the middle lamella, which weakens cell walls, causes membrane leakage, and increases fungal and bacterial infections. Furthermore, Ca deficiency triggers the accumulation of sugars and amino acids in the apoplast, which also lowers disease resistance. Fruit tissue that is low in Ca is also less resistant to bacterial diseases and physiological disorders that cause rotting during storage (Schumann et al. 2010). Thus, many physiological diseases and

susceptibility to disease infections are related to the Ca content in leaves and fruits (Brunetto et al. 2015). As a consequence of this, treatments with Ca have been often explored to reduce pests and disease incidence, and an adequate supply of Ca has shown to improve resistance to pathogens such as *Rhizoctonia solani*, *Sclerotium* spp., *Botrytis* spp., and *Fusarium oxysporum* (Agrios 2005). However, Ca is immobile in the phloem and treatments have not always been successful.

Trees treated with prohexadione-calcium were found to have reduced fire blight infections in apples and pears, which opened new ways for controlling this serious bacterial disease (Rademacher et al. 2006). Moreover, reduced disease incidence was also achieved in apple scab and powdery mildew. Similarly, treated trees also had reduced incidence of several insect pests such as green and woolly apple aphids, and apple and pear psylla (Paulson et al. 2005). The mode of action of prohexadione-Ca for inducing resistance is achieved through changes in the metabolism of flavonoid, specifically by triggering the biosynthesis of luteoforol, which cannot be naturally found in pome fruits. Luteoforol has been reported to inhibit the growth of all strains of *Erwinia amylovora* tested in vitro but causes phytotoxicity (Spinelli et al. 2005). Thus, upon prohexadione-calcium application, luteoforol is usually found compartmentalized inside the cell to avoid toxic effects but when pathogens infect tissues, cells release it, and then it acts against pathogens and induces a hypersensitive reaction in the host plant.

12.3 Macronutrients Sensing and Signaling in Plants

Plants are sessile organisms facing continuous fluctuation of environmental factors that can affect their fitness in nature. To adapt to such swaying environment, plants have evolved complex interwoven molecular networks providing them with physiological plasticity safeguarding their survival and growth under various growth conditions. Plants obtain mineral nutrients from soil using their root system and distribute them through xylem vessels to other parts of a plant. Among these mineral nutrients, four-key macronutrients, nitrogen (N), phosphorous (P), potassium (K), and sulfur (S) are essential for major plant metabolic processes, and limited availability of any of these macronutrients could significantly compromise plant growth and yield (Nath and Tuteja 2016; Wang et al. 2018).

In the natural environment or in the agricultural field, the availability of mineral nutrients fluctuates due to diverse factors such as agricultural activities, location, season, and climate. Since the yield of crop species is greatly impacted by the availability of these mineral nutrients in the soil, regular fertilizer application became one of the most common agricultural practices. However, the increasing cost of fertilizer application and the escalating public awareness of its negative impact on the environment have been leading plant scientists to investigate how plant senses and orchestrates its innate genetic networks toward the fluctuating nutrient availability in the field, aiming at breeding new crop species with better nutrient use efficiency. Plants have developed highly specialized nutrient sensing

and signaling system to respond to varying nutrient availability in soil by utilizing various membranes bound proteins with transporter activities (Gutiérrez 2012; Wang et al. 2018). In this section, we summarized recent findings about how plant senses and responses to the limited availability of four-key mineral nutrients and a few other macro- and micronutrients.

12.3.1 Nitrogen Sensing and Signaling

Nitrogen is an essential macronutrient whose limited availability in the rhizosphere interferes with normal plant development and growth and eventually its productivity. Plants obtain mineral nitrogen with help from nitrogen-fixing bacteria or by direct uptake of inorganic nitrogen compounds from the soil through the root system (Nath and Tuteja 2016). Among inorganic nitrogen compounds, nitrate (NO_3^-) is the most abundant source in the soil that is taken up by nitrate transporters (e.g., *Arabidopsis thaliana* NRT1 and NRT2) present on the root tissue (Krouk et al. 2010a; O'Brien et al. 2016; Zhao et al. 2018a). Once inside the cell, the transported nitrate is subjected to a series of reduction process leading to the conversion of nitrate to nitrite (NO_2^-) by nitrate reductase, then to ammonium (NH_4^+) by nitrite reductase which is assimilated into amino acids by nitrogen assimilatory enzymes (Lam et al. 1996; Stitt 1999; Nath and Tuteja 2016; Zhao et al. 2018a). Nitrate can also be stored in the vacuole in roots and shoots as a reserve for future use under nitrate stress condition (Miller and Smith 2008; Noguero and Lacombe 2016).

In addition to functioning as an essential nutrient, inorganic nitrogen compounds and their metabolites act as signaling molecules to maintain the physiological homeostasis of plants not only by regulating the expression of nitrogen-responsive genes (Wang et al. 2003; Krouk et al. 2010a; Alvarez et al. 2012; Gutiérrez 2012; Vidal et al. 2015; Varala et al. 2018) but also by modulating plant developments (e.g., leaf and root development, seed dormancy, flowering time and circadian clock, etc.) (Alboresi et al. 2005; Rahayu et al. 2005; Gutierrez et al. 2008; Krouk et al. 2010a; Marín et al. 2011; Alvarez et al. 2012; O'Brien et al. 2016).

Nitrate sensing and uptake mechanism have been extensively studied using a model species, *A. thaliana*, which showed that nitrate uptake is operated by two major nitrate transport systems, high-affinity transport system (HATS) under low nitrate concentration (<0.1 mM) and low-affinity transport system (LATS) under high nitrate concentration (>0.1 mM) (Crawfors and Glass 1998; Krouk et al. 2006; Gutiérrez 2012; Noguero and Lacombe 2016). Under each transport system, both constitutive and inducible forms of transport systems are present, providing efficient nitrate uptake routes under both limited and sufficient nitrate availability in soil (Tsay et al. 2007; Noguero and Lacombe 2016). The chemical cue caused by low nitrogen resource in the soil triggers drastic temporal changes in gene expression profiles, starting with the rapid induction of genes related to nitrogen uptake and assimilation (5–15 min post nitrogen stress), followed by induction of genes for energy generation (20–30 min), and then genes involved in metabolic and

developmental processes (45 min $\leq$) (Varala et al. 2018), indicating that nitrate stress initiates reprogramming of plant genetic and physiological networks to adapt to new environmental condition.

NRT1.1 (also known as NPF6.3 and CHL1) is one of the most studied nitrate transporter, which is a dual-affinity nitrate transporter enabling nitrate uptake and assimilation over a wide range of nitrate concentration (Liu et al. 1999; Liu and Tsay 2003; Ho et al. 2009; Léran et al. 2014; Sun and Zheng 2015). The activities of NRT1.1 as a nitrate sensor and as a nitrate transporter were identified from the *Arabidopsis* mutant chl1-9 (Ho et al. 2009). *chl1-9* mutant has a point mutation (P492L residue, proline to leucine) in *Chl1* gene resulting in defective nitrate transport activity regardless of exogenous nitrate concentration. However, *chl1-9* mutant is able to regulate the primary nitrate responses that are activated rapidly without a need of novel protein synthesis upon exposure to nitrate as wild type (Redinbaugh and Campbell 1991; Ho et al. 2009; Vert and Chory 2009; Sun and Zheng 2015).

NRT1.1 senses nitrate in the rhizosphere whose affinity for nitrate can be switched by phosphorylation and dephosphorylation of a threonine residue at amino acid position 101 (Thr101) from high to low or vice versa depending on the nitrate concentration (Ho et al. 2009; Sun and Zheng 2015). Under nitrate stress condition, Thr101 of NRT1.1 is phosphorylated by a protein complex of calcineurin B-like protein 9 (CBL9) and CBL-interacting protein kinase 23 (CIPK23), turning NRT1.1 into a high-affinity nitrate transporter which subsequently limits the primary nitrate response to low level including the expression of NRT2.1, a high-affinity nitrate transporter gene and other genes involved in nitrate assimilation (Redinbaugh and Campbell 1991; Krouk et al. 2006; Ho et al. 2009; Vert and Chory 2009; Sun and Zheng 2015). On the other hand, under sufficient nitrate condition, NRT1.1 is dephosphorylated and functions as a low-affinity nitrate transporter that then induces the primary nitrate responses including the expression of the genes for nitrate transport (e.g., NRT2.1) and assimilation (Krouk et al. 2006; Ho et al. 2009; Gutiérrez 2012; Sun and Zheng 2015). NRT2.1 is a component of induced HATS (Tsay et al. 2007) whose expression is rapidly increased when nitrate is provided to nitrate-stressed roots and decreased upon steady nitrate supply due to the feedback repression mechanism by nitrate metabolites (Lejay et al. 1999; Zhuo et al. 1999; Tsay et al. 2007). Interestingly, low nitrate concentration in the presence of high ammonium concentration induced the expression of NRT2.1 that is dependent on NRT1.1 activity (Muños et al. 2004; Krouk et al. 2006). This indicated that plant could use the induced nitrate HATS to prevent the toxic effect of ammonium as a nitrogen source which is under the control of NRT1.1 activity (Krouk et al. 2006; Bouguyon et al. 2015). The transporter activity of NRT2.1 requires the expression of a NAR2-like gene, NRT3.1 that is also under the control of NRT1.1 activity as NRT2.1 (Krouk et al. 2006; Okamoto et al. 2006). In addition to the nitrate transporter activity of NRT1.1 that leads to the activation of primary nitrate responses, NRT1.1 is also involved in root development via its auxin transport activity which is dependent on the nitrate availability (Remans et al. 2006; Walch-Liu and Forde 2008; Krouk et al. 2010b; Bouguyon et al. 2016; Zhao et al.

2018a). These data indicated that NRT1.1 functions as a nitrate response regulator resulting in low- and high-level primary nitrate response depending on its phosphorylation status as well as the root development.

Upon exposure of a plant to nitrate stress, not only genes belonging to the primary nitrate responses are induced as an immediate response (e.g., induction of genes involved in nitrate transport and assimilation such as NRT, nitrate reductase (NIA), and nitrate reductase (NiR).) (Wang et al. 2000, 2003; Scheible et al. 2004; Gutierrez et al. 2007; Wang et al. 2007; Zhao et al. 2018a), but also as a long-term strategy, plant adjusts its growth and developmental status (e.g., root growth, seed dormancy, and flowering) using nitrate as a signaling molecule to better adapt to the fluctuating nitrate availability (Alboresi et al. 2005; Rahayu et al. 2005; Marín et al. 2011; Marchive et al. 2013; Zhao et al. 2018a). Although significant progress has been made in recent years in the area of nitrate sensing and signaling, still limited information is available which is far from the full understanding of a molecular mechanism coordinating plant genetic response to nitrate stress (Marchive et al. 2013; Vidal et al. 2015). As an attempt to identify a key regulatory factor(s) responsible for plant response to nitrate, both microarray and next-generation sequencing approaches have been widely adopted to investigate the spatial and temporal gene expression patterns which showed that the expression of a vast amount of genes is under the control of nitrate (Wang et al. 2003; Scheible et al. 2004; Xu et al. 2011; Vidal et al. 2013; Varala et al. 2018).

The first identified regulatory factor in nitrate response network in the plant was the nitrate-inducible MADS-box transcription factor, *Arabidopsis* nitrate regulated 1 (ANR1) which is involved in lateral root proliferation (Zhang and Forde 1998). ANR1-repressed transgenic plants showed an altered sensitivity to nitrate and failed to show the lateral root growth in the region exposed to nitrate-rich patch, indicating that ANR1 transcription factor has a regulatory function in nitrate signaling pathway related to the lateral root growth responding to local nitrate availability (Zhang and Forde 1998).

Another well-studied regulator in nitrate response is the transcription factor, NLP7 (NIN (nodule inception)-like protein 7) which binds to the nitrate response *cis*-element (NRE) present on the promoter region of hundreds of genes related to primary nitrate response, including genes for nitrate transport activity and assimilation, protein kinases (e.g., CIPK8), and transcription factors like LBD37/38, suggesting that NLP7 may play a major regulatory role in nitrate signaling pathway (Castaings et al. 2009; Konishi and Yanagisawa 2013; Marchive et al. 2013; Yu et al. 2016; Zhao et al. 2018a). The transcription of NLP7 gene is not under the control of nitrate, but instead, the nuclear localization of NLP7 is mediated by nitrate via the phosphorylation of serine residue at amino acid position 205 by Ca^{2+}-sensor protein kinases (CPK10/30/32) of which expression is up-regulated by nitrate (Liu et al. 2017). NLP7 binds to the promoter region of NRT1.1 in the presence of ammonium, suggesting the involvement of NLP7 in NRT1.1 gene expression and also indicate that NLP7 works upstream of NRT1.1 in nitrate signaling pathway (Marchive et al. 2013; Zhao et al. 2018b). Recently, another nitrate regulatory gene, nitrate-regulated gene 2 (NRG2), was identified by forwarding the

genetics approach using *Arabidopsis* mutant lines (Xu et al. 2016). The data indicated the involvement of NRG2 in NRT1.1 expression and that NRG2 functions upstream of NRT1.1, independent from NLP7 in nitrate signaling pathway (Xu et al. 2016). The fact that NRG2 could interact with NLP7 in vitro and in vivo indicated NRG2 might play an essential role as a major regulatory factor in nitrate signaling pathway (Xu et al. 2016; Zhao et al. 2018a).

In addition to NLP7, reverse genetics, and bioinformatics approaches identified more transcription factors with a regulatory function in primary nitrate response (Zhao et al. 2018a). Three zinc-finger transcription factors, known as lateral organ boundary domain(LBD) 37/38/39, are induced by nitrate and known to act as a negative regulator of primary nitrate responses as the overexpression of these transcription factors down-regulates the genes involved in primary nitrate response (Rubin et al. 2009). Predictive network modeling using a series of transcriptomics data obtained at different time points (Krouk et al. 2010c) identified that nitrate response in plant is interconnected with hormone-regulated response network as seen on NRT1.1 with auxin transporter activity (Krouk et al. 2010b). Also, the network modeling approach predicted another transcription factor, squamosa promoter binding protein-like 9 (SLP9) as a potential regulatory hub in nitrate response network of which expression is controlled by miR165 (Wang et al. 2009; Krouk et al. 2010c). Bioinformatics data analysis identified that two bZIP transcription factors, TGA1 and TGA4, are induced by nitrate in roots and induce the expression of NRT2.1 and NRT2.2 (Alvarez et al. 2014). The investigation using *Arabidopsis* mutant lines indicated that TGA1/TGA4-NRT2.1/NRT2.2 are likely involved in the lateral root development responding to nitrate (Zhuo et al. 1999; Wang et al. 2003; Little et al. 2005; Okamoto et al. 2006; Alvarez et al. 2014). Additionally, tga1tga4 double mutant line has shorter primary root growth than the wild type which suggested the involvement of TGA1/TGA4 for primary root growth, independent of NRT2.1 and NRT2.2 (Alvarez et al. 2014; Zhao et al. 2018a).

In addition to the genes related to primary nitrate responses in the plant, non-coding small RNAs such as microRNAs (miRNAs) play a regulatory role in nitrate-mediated root development (Wu et al. 2006; Gifford et al. 2008; Vidal et al. 2010). It has been shown that miR167, which is expressed in pericycle and lateral root cap, is down-regulated by nitrate treatment (Gifford et al. 2008). miR167 targets auxin responsive factor 8 (ARF8) that regulates lateral root development responding to nitrate (Gifford et al. 2008). On the other hand, miR393 is induced by nitrate, which down-regulates auxin receptor AFB3 resulting in the inhibition of primary root growth (Vidal et al. 2010).

Nitrate stress results in significant changes in the gene expression profiles not only to respond immediately to fluctuating nitrate availability (Varala et al. 2018) but also for long-term strategy (e.g., root architecture system adjustment) for better adoption to nitrate stress (Bouguyon et al. 2015, 2016). Although there has been a substantial amount of progress made in the research area of nitrate sensing and signaling mechanism regarding the number of protein components identified functioning as a regulatory factor in nitrate response mechanism, it is still far from

full understanding. In spite of this fact, the research efforts using a model plant species, *A. thaliana*, has provided valuable information, ultimately, that can be applied for the genetic improvement of major crop species. With the advancement of technological and computational methodologies, more information will be obtained which could be valuable for the improvement of nitrogen-use efficiency of major crop species.

12.3.2 Phosphorous Sensing and Signaling

Phosphorous is an essential mineral nutrient for all living organisms as it is a critical element in nucleotides, nucleic acids, proteins, and membrane phospholipids, which mediates diverse metabolic processes (Bowler et al. 2010; Abel 2017; Yeh et al. 2017). Plants obtain phosphorus as a form of inorganic phosphate (Pi) from soil where the availability of mobile Pi is limited due to the formation of insoluble organic Pi (Yeh et al. 2017). The low Pi availability in the soil triggers a localized signal in the plant root tissue that affects the overall root system development by limiting primary root growth and enhancing lateral root formation, which will change the architecture of the root system for more efficient soil exploration to overcome the Pi limitation (Ticconi et al., 2009; Peret et al. 2014; Abel 2017; Puga et al. 2017). Although there has been a significant progress in understanding the molecular mechanism of plant adaptation to Pi stress condition, how plants perceive the low Pi availability in the soil is remained to be further investigated (Ticconi et al. 2009; Chiou and Lin 2011; Abel 2017; Ham et al. 2018).

It has been shown that the root caps are the significant channels where the most Pi uptake takes place in undifferentiated root tip cells (Kanno et al. 2016; Abel 2017). The low Pi signal in the rhizosphere, sensed at the root tips, facilitates the interaction of phosphate deficiency response 2 (PDR2) with low phosphate root 1/2 (LPR1/2), both of which reside in the endoplasmic reticulum, that results in the arrest of the primary root growth (Svistoonoff et al. 2007; Ticconi et al. 2009; Ham et al. 2018). In this process, a transcription factor, sensitive to proton rhizotoxicity 1 (STOP1) induces the expression of aluminum-activated malate transporter 1 (ALMT1) that is involved in the transport of malate into root meristematic apoplasm where it is incorporated into Fe-redox cycling system which in turn leads to the accumulation of reactive oxygen species (ROS) (Müller et al. 2015; Abel 2017; Balzergue et al. 2017; Mora-Macías et al. 2017; Ham et al. 2018). The accumulation of ROS in the root meristem results in the callose deposition that disrupts the symplastic movement of a transcription factor, short root (SHR) that is required for normal primary root development (Müller et al. 2015; Abel 2017; Puga et al. 2017; Ham et al. 2018).

In order to maintain Pi homeostasis at the whole plant level, systemic Pi stress signal is translocated from root to other parts of a plant that will induce Pi-starvation response cascade followed by phloem-mediated source-to-sink transfer of the signal to reprogram the plant development process for Pi acquisition and its remobilization in plants (Zhang et al. 2014; Ham et al. 2018). The candidates of those systemic

signaling molecules include small non-coding RNAs (e.g., miRNAs and sRNAs) as well as mRNAs that are enriched in the phloem sap during Pi stress condition (Huang et al. 2013; Park et al. 2014; Zhang et al. 2016; Ham and Lucas 2017; Ham et al. 2018). The sensing and responding mechanism to these systemic signaling molecules in the plant is modulated by transcription factors including phosphate starvation response 1 (PHR1) and closely related MYB transcription factors that regulate the gene expression involved in Pi scavenging and transport, membrane lipid remodeling, and plant vegetative growth responding to Pi stress (Zhou et al. 2008; Pant et al. 2015; Sun et al. 2016; Abel 2017).

PHR1 is constitutively expressed and is negatively regulated by SPX (SYG1/PHO81/XPR1)-domain proteins that sense inositol polyphosphates (InsP) (Puga et al. 2014; Wang et al. 2014; Wild et al. 2016; Puga et al. 2017). Under limited availability of Pi, PHR1 induces the expression of Pi transporters (PHT1) and phosphate transporter traffic facilitator 1 (PHF1) that mediates the translocation of PHT1 to the plasma membrane from the endoplasmic reticulum to increase Pi uptake (Gonzalez et al. 2005; Puga et al. 2017). PHR1 also induces the expression of miR399 and miR827 that inhibits the expression of phosphate 2 (PHO2) and NLA, a SPX-domain protein with ubiquitinase activity, both of which under Pi-sufficient condition, are involved in PHT1 protein degradation by ubiquitination under sufficient Pi condition (Fujii et al. 2005; Aung 2006; Bari et al. 2006; Kant et al. 2011; Huang et al. 2013; Lin et al. 2013; Park et al. 2014; Puga et al. 2017; Ham et al. 2018). PHO2 also causes the degradation of PHO1, an SPX-domain protein involved in Pi xylem loading, and affects PHF1 accumulation under Pi-sufficient condition (Hamburger et al. 2002; Liu et al. 2012; Huang et al. 2013; Puga et al. 2017). In addition, it has been shown that under sufficient Pi condition, ALIX, a cytosolic protein cargo protein, and CK2α2β3 kinase down-regulate the PHT1 activity. ALIX directs PHT1 to the vacuole for degradation, and CK2α2β3 phosphorylates PHT1 resulting in ER retention of PHT1 (Cardona-López et al. 2015; Chen et al. 2015; Puga et al. 2017). Plant vacuole stores Pi as a reserve that is mediated by SPX-MFS (major facilitator superfamily) domain-containing proteins, which plays a critical role in Pi homeostasis toward fluctuating Pi level (Liu et al. 2015; Wang et al. 2015; Liu et al. 2016; Puga et al. 2017; Ham et al. 2018). The cellular components involved in Pi export from the vacuole is not identified yet (Ham et al. 2018).

Plants respond to Pi stress in two ways; (1) by modulating the root system architecture to promote root Pi foraging activity where PDR2 and LPR play a major role and (2) by regulating Pi stress-responsive gene expression where PHR1 plays a central regulatory role for Pi homeostasis in plants under Pi stress condition. Although significant progress has been made in the understanding of the molecular mechanism regulating the phosphate-starved response in plants, the Pi-sensing mechanism in the plasma membrane still needs to be further investigated (Ham et al. 2018).

12.3.3 Potassium Sensing and Signaling

Potassium (K^+), the most abundant cation in plants, constitutes up to 10% of plant dry matter (Leigh 1984; Gierth and Mäser 2007; Nieves-Cordones et al. 2018). Unlike other macronutrients, K^+ is not metabolized into other macromolecules in plants and is involved in diverse cellular processes such as the enzyme activation and cell turgor maintenance (Schachtman and Shin 2007; Anschütz et al. 2014; Nieves-Cordones et al. 2014). Plant requires K^+ in large quantity and can accumulate K^+ up to several hundred millimolar in cytoplasm while the K^+ concentration in the soil is relatively low and varies ranging from 0.1 to 6 mM (Leigh 1984; Ashley et al. 2006; Britto and Kronzucker 2008; Maathuis 2009; Nieves-Cordones et al. 2014, 2018). While the K^+ concentration in the cytoplasm is tightly controlled and maintained at ~100 mM, optimal for the activities of cytosolic enzymes to function, the amount of K^+ reserved in plant subcellular compartments (e.g., vacuole) greatly varies depending on the potassium status of the plant (Ashley et al. 2006; Gierth and Mäser 2007).

Although potassium deficiency is rare, plants encounter the temporal variation in the availability of K^+ in the soil (Ashley et al. 2006; Maathuis 2009). Plants obtain K^+ through the root system which is then transported to shoots and leaves. Distribution of K^+ within the cell in different subcellular organelles requires K^+ to cross the plasma membrane once it is retrieved from the soil (Gierth and Mäser 2007). Although very little information is available regarding how plant senses and transduces the signal for potassium deficiency in plants, it has been shown that the potassium uptake takes place in two transport modes, passive transport through ion channels with millimolar Km (low-affinity transport) and active transport through H^+-cotransporters at micromolar Km (high-affinity transport) (Epstein et al. 1963; Maathuis and Sanders 1994, 1995; Ashley et al. 2006; Maathuis 2009).

The first cloned K^+ channel in the plant is the Shaker-type K^+ channels, AKT1, and KAT1 (Anderson et al. 1992; Sentenac et al. 1992; Ashley et al. 2006). *Arabidopsis* AKT1 that is expressed in the root cortex is a potassium-selective inward rectifying channel functioning at a wide range of K^+ concentration including micromolar concentration (Hirsch 1998; Ashley et al. 2006; Maathuis 2009). KAT1 is a guard-cell-specific K^+ channel involved in the regulation of stomatal aperture (Nakamura et al. 1995; Ashley et al. 2006). The K^+ uptake activity of AKT1 in root tissue needs another Shaker family protein, AtKC1, resulting in the formation of a heterotetrameric functional channel (Reintanz et al. 2002; Pilot et al. 2003; Ashley et al. 2006; Duby et al. 2008). While the transcription of AKT1 is not induced by low potassium signal, the K^+ uptake mediated by AKT1 is increased by the phosphorylation of AKT1 by CIPK23 that is activated by the Ca^{2+} sensors, CBL1 and CBL9 under K^+ deficient condition (Xu et al. 2006; Maathuis 2009).

Arabidopsis HAK5, a K^+/H^+ symporter which is a member of KT/KUP/HAK family, is induced by K^+ deficient condition and is involved in high-affinity potassium uptake (Kim et al. 1998; Armengaud et al. 2004; Gierth 2005; Maathuis 2009; Nieves-Cordones et al. 2014). The K^+-sensing mechanism in plants seems to be facilitated by various factors such as cell membrane potential, ROS, Ca^{2+}, and

phytohormones (e.g., ethylene, auxin, jasmonic acid, or cytokinins) as well as by direct sensing K^+ level by potassium channels (Armengaud et al. 2004; Jung et al. 2009; Nam et al. 2012; Nieves-Cordones et al. 2014). Low potassium signal causes a hyperpolarization of root cell membrane potential and ROS production, which is followed by the expression of genes involved in potassium transport such as HAK5 and those involved in the root system architecture (Amtmann et al. 2005; Nieves-Cordones et al. 2008; Kim et al. 2010; Hernandez et al. 2012; Yang et al. 2013; Nieves-Cordones et al. 2014).

ROS accumulation in roots under potassium stress condition affects the root hair growth and elongation (Foreman et al. 2003; Liszkay et al. 2004; Shin et al. 2005; Schachtman and Shin 2007). Root ROS production in potassium-stress plants can be blocked by ethylene inhibitors, and the expression of genes related to the ethylene biosynthesis in plants is induced in the root tissue under potassium stress, suggesting that ethylene works upstream of ROS in low-potassium signaling pathway (Shin et al. 2005; Jung et al. 2009; Nieves-Cordones et al. 2014). In addition to ethylene, low potassium stress affects the signaling pathways mediated by auxin, jasmonic acid, and cytokinin which affects root system architecture and the expression of potassium transporters, suggesting that the phytohormone signaling pathway may form a complex regulatory network under low-potassium stress condition (Nam et al. 2012; Dolan 2013; Rigas et al. 2013; Schachtman 2015; Wang and Wu 2017).

It has been shown that several transcription factors induced by potassium-deficient condition bind the promoter region of HAK5, suggesting their involvement of HAK5 transcription under low-potassium stress condition (Kim et al. 2012; Hong et al. 2013; Nieves-Cordones et al. 2014). For instance, while under potassium-sufficient condition, *Arabidopsis* ARF2 transcription factor binds to HAK5 promoter resulting in the repression of HAK5 transcription, potassium stress causes phosphorylation of AFR2 which relieves the repression of HAK5 transcription (Zhao et al. 2016; Wang and Wu 2017).

In addition to AKT1 and HAK5, two major potassium uptake channels in *Arabidopsis*, AtKUP7, whose affinity is lower than HAK5 (10 $\sim$ 20 μM), was identified as a potential potassium transporter that may be involved in potassium xylem loading facilitating potassium translocation to the shoot (Han et al. 2016; Nieves-Cordones et al. 2016; Wang and Wu 2017). Another Shaker-type potassium channel, SKOR is also involved in the xylem loading of K^+ (Pilot et al. 2003; Maathuis 2009). In addition, the data showing that SKOR interacts with GORK, another Sharker family channel, suggested that SKOR and GORK form a functional heteromeric channel that may mediate the potassium efflux (Dreyer et al. 2004; Ashley et al. 2006). *Arabidopsis* AKT2 and AKT3 are predominantly expressed in phloem parenchyma cells and direct bidirectional K^+ transport, which may be involved in phloem loading/unloading of K^+ in vascular tissue (Marten et al. 1999; Lacombe et al. 2000; Deeken et al. 2002). The presence of potassium transporters functioning on potassium loading/unloading through the vascular tissue implicates that the potassium translocation from root to shoot through xylem followed by its

redistribution through phloem tissue plays a critical role in maintaining K^+ homeostasis in a plant (Maathuis 2009).

12.3.4 Sulfur Sensing and Signaling

Inorganic sulfur is present mainly as sulfate ($SO4^{2-}$) in the soil under aerobic condition, which is taken up by the plant through sulfate transporters mediated by H^+ gradient (proton/sulfate cotransporters) (Maathuis 2009). Once sulfate is transported into root tissue, it is quickly mobilized to the shoot tissue where it is reduced mainly in the chloroplast ($SO4^{2-}$ to $SO3^{2-}$ to S^{2-}) and then assimilated into amino acid cysteine (Schachtman and Shin 2007; Maathuis 2009). In cysteine, sulfur presents as a thiol (-SH) group that can form a covalent -S-S- bond if another -SH group is present, assisting the formation of protein tertiary and quaternary structure for its functional activity (Maathuis 2009). Although the regulation mechanism of sulfate metabolism in plants has been well characterized, the majority of sulfur sensing and signaling mechanism is still unknown (Schachtman and Shin 2007).

There are five gene families encoding sulfate transporters in *Arabidopsis* whose expressions are up-regulated by sulfate deficiency to facilitate the sulfate uptake from soil (Smith et al. 1995; Takahashi et al. 1997; Schachtman and Shin 2007; Yi et al. 2010). These transporters have different degrees of sulfur affinities, suggesting that the presence of sulfate transporters with different biochemical properties ensures the sulfate uptake efficiency under various sulfate availability in the environment (Takahashi et al. 2000; Kataoka 2004; Yi et al. 2010). *Arabidopsis* mutant line, sulfur limitation 1 (slim1) that does not respond to low sulfur condition has a mutation in an EIL family transcription factor, ethylene-insensitive-like 3 (EIL3) that regulates the expression of genes required for sulfate acquisition under low sulfur condition (Maruyama-Nakashita et al. 2006; Schachtman and Shin 2007).

Phytohormones cytokinin, auxin, and jasmonic acid are known to be involved in sulfur signaling mechanism under low sulfur condition (Schachtman and Shin 2007). Application of cytokinin down-regulates the expression of high-affinity sulfate transporter, SULTR1;2 which is induced by low sulfur stress (Maruyama-Nakashita et al. 2004; Schachtman and Shin 2007). The cytokinin-mediated regulatory mechanism via cytokinin response receptor (CRE1) is likely redundant since the application of cytokinin on cre1-1 mutant line only partially reduces sulfate uptake (Maruyama-Nakashita et al. 2004; Schachtman and Shin 2007). As auxin- and jasmonic acid-inducible genes are also up-regulated by low sulfur condition, it is likely that both phytohormones are also parts of the sulfur response regulatory network (Maruyama-Nakashita 2003; Nikiforova et al. 2003; Schachtman and Shin 2007).

The expression of sulfate transporter genes (e.g., SULTR1 and 2) are regulated by the 16-bp sulfur-responsive element (SURE) in their promoter regions, and many sulfur-responsive genes are regulated by the SURE element in their promoters (Maruyama-Nakashita et al. 2005; Schachtman and Shin 2007).

Identification of cis-acting elements and their corresponding transcription factors that are involved in the regulation of sulfur-responsive genes will provide more information about the signaling cascade taking place in the plant under sulfur stress condition.

12.3.5 Calcium Sensing and Signaling

Calcium (Ca^{2+}) functions as a secondary messenger involved in various cellular signal transduction pathways as well as a structural component facilitating cell wall cross-linking (Maathuis 2009). Although calcium is abundant in the soil, the concentration of calcium available for plants can be decreased due to weather and leaching-out from the soil leading to calcium deficiency (Maathuis 2009). Calcium uptake at the root tissue is thought to be taking place through Ca^{2+}-permeable channels which are either Ca^{2+} selective or non-selective, the identity of the specific protein mediating Ca^{2+} uptake is still not fully characterized (Demidchik and Maathuis 2007; Maathuis 2009). Within the plant, Ca^{2+} is sequestered in the vacuole of mature cells, which is mediated by cation exchanger (CAX) H^+/Ca^{2+} antiporter family and by ATP-driven P-type ATPase (Blumwald and Poole 1985; Shigaki et al. 2006; Demidchik and Maathuis 2007; McAinsh and Pittman 2009). No calcium transporters involved in xylem loading/unloading have been identified although apoplastic calcium transfer to xylem may take place (White 2001). Since calcium level in xylem is low, those fast-growing tissues that calcium demand is relatively high may suffer from the outcome (e.g., black heart disease in celery) of low calcium stress (Maathuis 2009). The free calcium concentration in cytoplasm is kept extremely low at around 100 nm of which concentration in cytoplasm can be rapidly changed by various stimuli, making calcium as an ideal secondary molecule in diverse cellular signal transduction pathways including responses to biotic/abiotic stresses (Mahouachi et al. 2006; Maathuis 2009; McAinsh and Pittman 2009).

12.3.6 Magnesium Sensing and Signaling

Magnesium (Mg^{2+}) is easily leached out from soil due to its low absorption rate in soil, which can cause frequent magnesium deficiency condition (Deng et al. 2006; Maathuis 2009). Although magnesium is an important component in the photosynthetic system and functions as a cofactor with ATP in various enzymatic reactions, only recently, a part of the magnesium uptake mechanism, transport, and homeostasis in plant is being characterized (Axelsson et al. 2006; Deng et al. 2006; Sirijovski et al. 2008; Maathuis 2009). Magnesium uptake in root tissue is mediated by transporters of the MGT family (e.g., AtMGT1), a homolog of bacterial CorA magnesium transporters (Berezin et al. 2008; Maathuis 2009). Overexpression of AtMGT1 in heterologous plant system increased the magnesium uptake as well as the growth under low magnesium condition (Berezin et al. 2008). Vacuole stores

magnesium which is likely mediated by Mg^{2+}/H^{+} antiporters that contribute to the turgor maintenance (Maathuis 2009).

12.4 Micronutrient Signaling

Plants require a little amount of micronutrients because the high amount of these elements is toxic to plant cells (Giehl et al. 2009). Plants developed a sophisticated mechanism to adjust the acquisition of micronutrients for the maintenance of micronutrient homeostasis through the use of various signaling pathways mediated by phytohormones, ions, and metabolites at various cellular/tissue levels (Giehl et al. 2009). The cellular level homeostasis of micronutrients is regulated by controlling uptake, efflux, and storage steps (Giehl et al. 2009). For instance, boron homeostasis is regulated at uptake and efflux stages through NIP5; 1 transporter and boron exporters, respectively (Miwa et al. 2007; Giehl et al. 2009). On the other hand, Fe homeostasis includes up-regulation of genes required for Fe acquisition from the soil as well as those required for Fe mobilization from vacuoles (Giehl et al. 2009). Nitric oxide (NO) is known to be involved in the early Fe signaling pathway and the modulation of Fe deficiency in root tissue (Arnaud et al. 2006; Graziano and Lamattina 2007; Giehl et al. 2009). In the case of copper (Cu), miRNAs, ROS, and Ca^{2+} are involved in the maintenance of Cu homeostasis in plants (Yeh et al. 2007; Abdel-Ghany and Pilon 2008). Micronutrient deficiency can cause morphological changes in root tissue. Both manganese (Mn) and Fe deficiency cause deformation of root hair formation (Schikora 2001; Muller and Schmidt 2004; Wei Yang et al. 2008). The transfer of micronutrient-derived signals from the root tissue to the shoot can be mediated by various factors (e.g., phytohormones and ROS) (Lucena et al. 2006; Russo et al. 2008; Séguéla et al. 2008). In addition to root-derived signals, plants can generate and transmit shoot-derived signals to other parts of a plant responding to Fe (Grusak and Pezeshgi 1996; Giehl et al. 2009). The presence of systemic signals communicating between shoot and root in response to zinc (Zn) is also speculated based on the study in *A. thaliana* (Gustin et al. 2009).

The effect of macro- and micronutrients on plant growth and productivity has been well studied and characterized in various plant species. The plant response network toward the fluctuating the nutrient level in the rhizosphere is a continuum of multiple signaling networks (e.g., phytohormones, ROS, transcription factors, and small RNAs), each of which can influence other signaling mechanism that imposes a great difficulty on the identification of a key regulatory element that can be targeted for the improvement of nutrient use efficiency. In recent years, there has been and is being made a significant technological advancement that can provide the scientific community with more elaborated methodologies and data analysis. The systems' biology approach paired with computational machine learning strategy may provide a new insight to understand how plant responds to various

nutrient-generated signals that will eventually lead to design a better strategy for the improvement of nutrient use efficiency.

Although the application of fertilizers to supplement the required nutrients is an essential agricultural practice to maximize the crop productivity, the excess amount of nutrients can exert an adverse effect on both crop quality and the environment (Brunetto et al. 2015; Jat et al. 2015). To balance the chemical fertilization and the crop productivity, the concept of integrated nutrient management system has been developed where nutrient supply is adjusted, both spatially and temporally, depending on the demand of a crop species and the soil condition (Jat et al. 2015). The incorporation of integrated nutrient management system in the agricultural practices will require the continuous monitoring the soil and plant nutrient conditions that can be facilitated by smart farming techniques where modern technologies such as information and communication technologies for data management and Internet of Things (IoT) play a major role (Wolfert et al. 2017).

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