Chapter 15
Induction of Systemic Resistance for Disease Suppression

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15.1 Introduction

Plants are dependent on nutrient acquisition from soil. Plant roots secrete a considerable measure of chemicals into the rhizosphere which influences growth, development, and acclimatization to environmental stresses (Vallad and Goodman 2004; Van Loon and Glick 2004). The microbial population within the rhizospheric region will similarly contribute chemical constituents that affect the microbial population as well as the plant. The dynamic nature of the rhizospheric microflora allows for an interplay between pathogenic and beneficial microorganisms. This therefore results in the organisms interacting via synergistic or antagonistic interactions (Beardon et al. 2014) where signals are being exchanged between the microorganisms and the root systems that effectively form an active belowground association (Weller et al. 2002; Van Loon and Bakker 2005). These belowground interactions are functional as long as the microbial–plant systems are kept alive to buffer the activity in the rhizospheric environment. These root microbe interactions can result in variation in effect against soil pathogens, microbial propagation, and colonization of the roots (Somers et al. 2004; De Vleesschauwer et al. 2009).

Beneficial organisms such as PGPR and plant growth promoting fungi (PGPF) control plant diseases through suppression of pathogenic soil organisms and induction of host systemic resistance. The presence of these organisms consistently induces resistance in the host beyond basal levels which acts to protect against a host of non-beneficial organisms in its surrounding. *Acinetobacter*, *Azospirilium*, *Rhizobium*, *Pseudomonas*, and *Bacillus* have been reported as efficient inducers of systemic resistance in leguminous and nonleguminous plants. In addition, *Trichoderma* spp., *Penicillium simplicissimum, Piriformospora indica*, *Phoma* sp.,

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non-pathogenic *Fusarium oxysporum*, and arbuscular mycorrhizal fungi have been listed as PGPF that have successfully suppressed diseases in several plant systems (Bakker et al. 2013).

While a large number of these strains interact and produce beneficial outcomes on varying hosts, certain strains have shown specificity indicating that the plant–microbial interaction are regulated by host variety, soil conditions, and microbial populations. Certain rhizospheric organisms produce determinants such as lipopolysaccharides, siderophores, lytic enzymes, exopolysaccharides, lipopeptides, and others (Nadarajah 2016a). These determinants trigger pathways that result in the activation of defense-related genes and responses downstream. Jasmonic acid (JA) and ethylene (ET) regulate rhizobacterial-induced systemic resistance (ISR), while systemic acquired resistance (SAR) is controlled by SA (Van Loon and Glick 2004; Haas and Défago 2005). Though both mechanisms induce host systemic resistance, they remain distinct.

While an array of microorganisms have been identified as potential biocontrol agents, only a handful have had their mechanisms elucidated (Heil and Bostock 2002; Choudhary et al. 2007). Mutants have proven to be a wonderful tool in studying the role of determinants in the mechanism of disease suppression as seen in the repression of *F. oxysporum* f. sp. *raphani* by *P. putida* WCS358 (Raaijmakers et al. 1995). Similarly cucumber plants challenged with *Colletotrichum orbiculare* showed inhibition of anthracnose symptoms post inoculation with several strains of PGPR (Wei et al. 1991). These experiments imply that the area of antagonistic influence of PGPR is not confined to the rhizosphere, but develops from below ground into above ground defense elicitations. Hence various studies and experimentations have concluded that the heightened level of resistance *in planta* was mediated through an immune response that was activated in response to rhizobacteria-ISR. As extensive reviews of these organisms have been presented elsewhere (Van Loon et al. 1998; Pozo and Azcon-Aguilar 2007), this chapter ventures to present the mechanisms, signaling pathways, comparisons between ISR and SAR, in addition to differences between these defense mechanisms that collectively work to defend plants against their hostile environment (Kloepper et al. 2004; Van Loon and Bakker 2006; De Vleesschauwer et al. 2008, 2009; Walters et al. 2013).

15.2 Induced Systemic Resistance (ISR): The Mechanism

ISR and SAR are two major players in induced plant resistance. While both contribute towards resistance, one major difference between these systems lies in the inducers, where contrary to ISR, SAR is induced in response to pathogens which results in subsequent protection from infections against a broad host of attackers (Walters et al. 2013; Pieterse et al. 2014). Further ISR and SAR are not just expressed within the locality of induction but are transmitted to distant tissues through systemic spread of signal molecules (Van Loon et al. 2008). ISR like SAR is regulated by

signaling pathways that are interlinked and regulated by signal molecules/hormones (Pieterse et al. 2012). In the following segments, ISR and the contribution of ISR in agricultural practices, specifically in disease suppression, will be discussed.

15.3 Pathogen-Induced SAR

SAR has been studied locally and systemically in various plant systems. The local response includes the production of physical and chemical responses such as structural modification to the cell walls, production of phytoalexins and pathogenesis-related (PR) proteins, and hypersensitive response (HR) (Hunt and Ryals 1996; Lamb and Dixon 1997; Van Loon 1997; Van Loon and Van Strien 1999; Métraux 2002; Durrant and Dong 2004; Conrath et al. 2006). Although HR is produced in both compatible and incompatible gene-for-gene interactions (Hammond-Kosack and Jones 1997; Ellis et al. 2002), at the molecular and cellular level, HR is dispersed through uninfected tissues to trigger systemic resistance in the whole plant (Stone et al. 2000). While changes such as lignification and callous deposition are brought about post infection, the systemic transmission results in PR protein production prior to infection (Sticher et al. 1997; Dong 2004). This rapid response of distant tissue is referred to as conditioning which involves systemic signal molecule(s) such as SA. SA and other related inducers (2,6-dichloroisonicotinic acid [INA] or benzothiadiazole [BTH]) are able to promote superoxide production in the cell resulting in the production of reactive oxygen species (ROS), which ultimately activates downstream host defense enzymes such as phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX) (Katz et al. 1998; Thulke and Conrath 1998; Kauss et al. 1999; Conrath et al. 2002). Another player in the induction of pathogen-derived resistance, β-aminobutyric acid (BABA), retains effective induction even in plants with impaired SA, JA, and ET pathways (Zimmerli et al. 2000). However BABA is only able to protect mutants insensitive to JA and ET but remains ineffective in rescuing mutants defective in SAR signaling.

15.3.1 SAR Signaling

Endogenous SA has been experimentally proven to induce SAR (Van Loon and Antoniw 1982; Van Loon et al. 2008) resulting in increase of SA post induction in local and distant tissues through phloem transport (Malamy et al. 1990; Métraux et al. 1990; Verberne et al. 2003; Durrant and Dong 2004; Van Loon et al. 2008). The salicylate hydrolase defective mutant, *NahG*, that reduces SA to catechol leaving it incapable of inducing SAR was used to study the role of SA in SAR. The lack of SAR in these plants may be "rescued" through the application of exogenous INA and BTH (Ryals et al. 1996; Sticher et al. 1997; Conrath et al. 2002). Subsequently

in establishing the mobile signal(s) involved in SAR, there are two possibilities: (1) SA is not the mobile signal as the rootstock-scion experiment showed induction of SAR despite no accumulation of SA in the NahG rootstock; and (2) SA as a versatile signal that is transported to distal tissues ensuing SA generation in distant tissues. Further, the presence of SA in the phloem of plants has been linked to the transport of this signal molecule within the plant to distal organs thus lending towards SAR. The overexpression of salicylate hydroxylase focused in phloem tissue of tobacco resulted in diminished SAR thus supporting a role for SA in systemic signaling (Mur et al. 2000). Another compound, methyl salicylate (MeSA) was observed in tobacco to elicit defense response. As such, MeSA was proposed as a component that acts with SA in *in planta* communication and signaling. It is therefore likely that SA as well as other systemic signals (azelaic acid, diterpenoid dehydroabietinal, glycerol-3-phosphate-dependent factor, pipecolic acid) could be involved in SAR (Shulaev et al. 1995; Seskar et al. 1998; Pieterse et al. 2014). The effective function of SA is dependent on the presence of an ankyrin protein that changes the oligomeric state of NPR1 to monomers (Cao et al. 1997). Pathogenesis-related (PR) proteins are produced from the interaction between NPR1 and transcription factors (Dong 2004). PR proteins are affected by SAR and therefore are suitable markers to study induced resistance (Kessmann et al. 1994) and remain the hallmark of SAR induction.

15.3.2 SA Mode in SAR

Catalase and ascorbate peroxidase act as SA-binding proteins that result in the formation of phenolic radicals involved in lipid peroxidation. Lipid peroxidation remains a crucial process in ensuring defense gene activation (Farmer and Mueller 2013), hence requiring the proper execution of its production at the right location and time. Other SA-binding proteins (SAPs) that demonstrate a higher affinity for SA and its analogs were identified (Bakker et al. 2014). While the biological significance of these SAPs remains unresolved, they provide an interesting view in comprehending the method of SA activity. SA- and pathogen-inducible protein kinase (SIPK), a MAP kinase member was isolated and studied in tobacco (Zhang and Klessig 1997; Zhang et al. 2002). Various studies have focused on the upstream regulatory sequences (URS) of *PR-1* promoter, one of the terminating reactions in SAR. The TGACG sequence in the URS of *PR-1* is perceived by a bZIP family TGA transcription factor (Lebel et al. 1998). TGAs were likewise found to interact with the NPR1 protein, providing a connection between *NPR1* and SA-induced *PR-1* expression (Lebel et al. 1998; Zhang et al. 1999; Després et al. 2000; Zhou et al. 2000). PR expression is suppressed when *SNI1* (negative regulator) binds to the DNA or transcription factors (Li et al. 1999). Other research groups have looked into a SA- and pathogen-inducible WRKY DNA-binding elements that recognize specific sequences on the promoter sequence of chitinase gene in tobacco. This study discovered that protein phosphorylation is essential for the function of WRKY DNA-binding components, thus underscoring the function of kinases in SA signaling (Yang et al. 1999).

15.3.3 SAR-Signaling Network

Ding et al. (2015) conducted a genetic screen of SA mutants via biosensor technique (Marek et al. 2010) which identified upstream (*EDS1, PAD4, NDR1*) and downstream components (e.g., *NPR1*), transcription factors (*CBP60g, SARD1*), and metabolic enzymes (*EPS1, PBS3*) that are crucial for SA signaling (Cao et al. 1997; Ryals et al. 1997; Jirage et al. 1999; Zhang et al. 2010; Wang et al. 2011; Yezhang et al. 2015). These screens identified two leucine rich repeats (LRR—NBS; LRR and TRI:NBS:LRR) as effectors of signal transduction post infection by avirulent pathogens (Glazebrook 1999). These pathways in the end merge at the DND1 protein which controls the development of HR cell death (Clough et al. 2000) (Fig. 15.1). In the event of being induced by a virulent pathogen, *PAD4* is activated resulting in phytoalexin production (Jirage et al. 1999). This activation further results in the downstream activation of *SID1* and *SID2* that controls SA generation (Nawrath and Metraux 1999). Studies on the *SID1* gene has shown that it is associated with the SOS response of the cell. The SOS response is elicited upon introduction of stress into the cell system. While *EDS5/SID1* expression is independent of SA, *EDS5/SID2* genes encode a MATE transporter and an ICS1 enzyme which are crucial in the SA synthesis (Ding et al. 2015) (Fig. 15.1). The *sln* mutants together with *eds5*, *sid2*, and *pad4* are involved in SA accumulation (Ryals et al. 1997; Jirage et al. 1999; Nawrath and Métraux, 1999; Nawrath et al. 2002; Ding et al. 2015). NPR1 acts as a feedback inhibitor of SA biosynthesis following accumulation of SA in response to infection and infestation in *NPR1* gene mutants. In addition to components upstream of SA biosynthesis (Clarke et al. 2000; Zhang et al. 2010), NPR1-independent defense responses is triggered by *EDS5*, *PAD4, SID2*, and *SLN* genes (Glazebrook 1999, 2001). The *sln1* mutant influences the PR protein expression; hence this goes against the proposed involvement of SA-independent pathway in the regulation of *PR* gene expression.

15.4 Rhizobacteria-ISR

The microorganisms within the rhizosphere of the soil have an important role in the general well-being and health of plants. The bacteria and fungi within the rhizosphere can either be in a beneficial relationship or negatively affect the plant or microbial population (Nadarajah 2016a, b). Among the functions attributed to these organisms are the ability to participate in the nutrient cycles, nutrient acquisition, and management of biotic (Bakker et al. 2007; Van der Heijden et al. 2008; Khan et al. 2009; Kraiser et al. 2011; Berendsen et al. 2012) and abiotic stresses (Yang et al. 2004). These functions are associated with a staggeringly dynamic and complex microbiome within the rhizosphere (Berendsen et al. 2012; Hartmann et al. 2009; Raaijmakers et al. 2009). Through the utilization of molecular techniques, it is expected that the repertoire of microbiome identified that are in

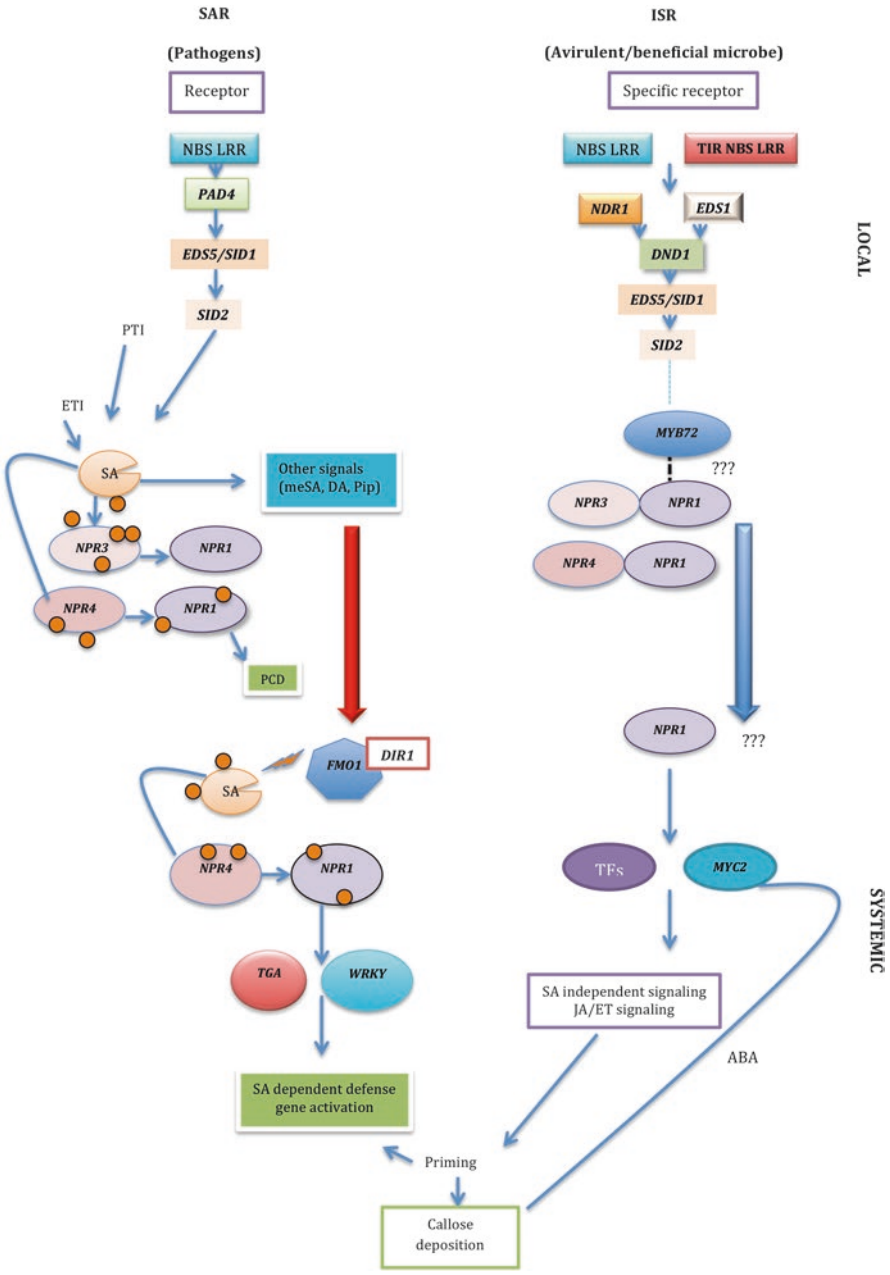

Fig. 15.1 Schematic representation of the components involved in the activation of SAR and ISR. These protein perceive and transmit the signal in SAR and ISR. *Solid lines* and *arrows* are confirmed connections. *Dashes* imply further study required. *Solid arrows* indicate the transmission from local site of elicitation to distant tissue transmission. *Abbreviations*: *NB-LRR* Nucleotide-Binding-Leucine-Rich Repeat, *PCD* Programmed Cell Death, *PRR* Pattern-Recognition Receptor, *PTI* PAMP-Triggered Immunity, *SA* Salicylic Acid, *TF* Transcription Factor, *PAD* Phytoalexin Deficient, *EDS* Enhanced Disease Susceptibility, *SID* SA Induction-Deficient, *DND* Defense, No Death, *NDR* Non-Race Specific Disease Resistance, *DIR* Defective In Induced Resistance, *FMO* Flavin-Dependent Monooxygenase, *NPR* Non-Expressor PR, *TGA* TGACG-binding protein (TF), *MYB* Myeloblastosis (TF), and *MYC* TF regulator

interaction with plants and associated with ISR against biotic and abiotic stresses will increase. Studies will not be limited to only identification of new rhizospheric microorganisms but to the mechanisms, key players, and pathways involved in these processes.

15.4.1 Beneficial Microbes and the SAR Pathway

While SAR is a complex mechanism of systemic resistance in plants, ISR prompts a more complex defense in response to non-pathogenic rhizobacteria. Due to the systemic response elicited by ISR, it was at one point assumed to be SAR. This misconception was debunked by Hoffland et al. (1995) who established that ISR against *F. oxysporum* was induced by *P. fluorescens* WCS417r in radish without the trademark PR production as seen in SAR. Similar findings were described by Pieterse et al. (1996) in Arabidopsis. This was further corroborated when *NahG* resulted in an ISR response post treatment with WCS417r-ISR, indicating the involvement of an SA-independent pathway and separating this process from SAR (Pieterse et al. 1996). The same is seen in response to *P. putida* WCS358r (Raaijmakers et al. 1995; Van Wees et al. 1999) and *Serratia marcescens* 90-166 where the loss of SA production induced resistance against *Colletotrichum orbiculare* and *P. syringae* pv. tomato (Press et al. 1997). However when strain 7NKS2 was used in treatment of *NahG* mutants in Arabidopsis and tomato, ISR was abolished against TMV and *Botrytis cinerea* (De Meyer et al. 1999a; Audenaert et al. 2002). Ryu et al. (2003) also encountered similar findings in Arabidopsis, with *B. pumilus* SE34 against *P. syringae pv maculicola*. Further, Maurhofer et al. (1998) observed SA-dependent SAR elicitation post treatment with *P. fluorescens* P3 overexpressing SA biosynthesis gene cluster of *P. aeruginosa* PAO1. Additionally *P. fluorescens* SS101, *Paenibacillus alvei* K165 (Tjamos et al. 2005; Van de Mortel et al. 2012), and *Trichoderma* PGPF (Mathys et al. 2012; Martínez-Medina et al. 2013) were also reported to induce SA-dependent SAR. The requirement for SA in 7NKS2 was substantiated through utilization of bacterial mutants defective in SA production (De Meyer and Höfte 1997; De Meyer et al. 1999b; Audenaert et al. 2002). However following this finding, further experiments were conducted by Van Loon and Bakker (2005) who inferred that SA-independent pathways is the main regulatory pathway in rhizobacteria-mediated ISR (De Meyer et al. 1999a). Although some PGPR produce SA but it is still not the main signal involved in elicitation of ISR (Ran et al. 2005; Djavaheri et al. 2012). The SA produced however binds with siderophores in iron-limiting condition and thus is not directly involved in SAR. Meanwhile, in cases where SA is produced by PGPR, ROS is an elicitor required to activate SAR. Further these responses are not dependent on accumulation of SA but rather on increasing the sensitivity of tissue to SA (Van Loon and Bakker 2003).

15.4.2 NPR1 as a Common Component of ISR and SAR

Transmission of SAR to distal organs requires mediators. The transmitted SAR signal is chaperoned by one such mediator, Defective In Induced Resistance1 (*DIR1*) (Champigny et al. 2013) which assist Flavin-Dependent Monooxygenase 1 (*FMO1*) in receiving and amplifying signals for long distance SAR signaling (Mishina and Zeier 2007) (Fig. 15.1). The well-characterized transcriptional co-regulator, *NPR1*, plays a role in SA accumulation and in the SAR-signaling pathway (Dong 2004; Vlot et al. 2009; Pajerowska-Mukhtar et al. 2013). Pieterse et al. (1998) had implicated *NPR1* in ISR through studies conducted on Arabidopsis. In studying the activation of ISR post treatment with *P. fluorescens* WCS417r and numerous other PGPR and PGPF, Pieterse et al. (1998) found a connection between *NPR1* and the JA/ET-signaling pathways (Lavicoli et al. 2003; Ryu et al. 2003; Stein et al. 2008; Weller et al. 2012). This therefore demonstrates that SA signaling in response to either an avirulent pathogen or rhizobacteria can activate *NPR1*. While the role of *NPR1* in SA signaling has been connected to nuclear function, recent studies have provided information that the *NPR1* component of the JA/ET signaling is within the cytosol (Spoel et al. 2003; Stein et al. 2008; Ramirez et al. 2010). Both ISR and SAR defense mechanisms have additive effect within the host. At this juncture, it is difficult to ascertain the specific molecular mechanism involved in the *NPR1* mediated JA/ET based ISR induction in host (Van Wees et al. 2000). Pieterse et al. (2014) reported that plant roots expressed high levels of *NPR1, NPR3,* and *NPR4* suggesting a crucial role for these genes in belowground interactions. Both NPR3 and NPR4 together with Cullin 3 (CUL3) ubiquitin E3 ligase are involved in the degradation of NPR1. NPR3 degrades NPR1 when the levels of SA are high causing localized cell death during effector triggered immunity (ETI), while at lower SA levels, NPR4 maintains NPR1 during pathogen-associated molecular patterns triggered immunity (PTI) and thus results in PR expression (Fig. 15.1). In ISR, NPR1 itself acts to mediate the systemic response together with MYC and TFs and the JA/ET pathways. Though *NPR1* is a shared component of ISR and SAR, the mechanism downstream of *NPR1* perception is different as SAR results in PR accumulation while ISR does not. This could perhaps be due to lower levels of SA-induced ISR that perhaps was insufficient to result in PR production. Studies with the *npr1* mutant plants that did not express ISR post cultivation with WCS417 indicated that the expression was dependent on regulation and sensitivity and not towards the SA levels within the host. However, further research is required to understand the role of *NPR1* and the possible involvement of other regulatory factors in the SA-NPR1 interaction in ISR (Pieterse et al. 2012).

15.4.3 Other Pathways That Control ISR

As mentioned in the above sections, the JA/ET-signaling pathway is crucial in the induction of ISR in plants. Arabidopsis JA (*jar1, coi1, jin1*) and ET (*ein2, etr1, eir1, ein3*) mutants were utilized to establish the function of JA/ET in the plant immune

system (Thomma et al. 2001). When these mutants were treated with PGPR (*Pseudomonas* CHA0, *P. fluorescens* WCS417r–ISR, *P. syringae* pv. maculicola, *P. fluorescens* Q2-87, *S. marcescens* 90–166) (Pieterse et al. 1998; Ryu et al. 2003; Pozo et al. 2008) and PGPF (*P. indica, Penicillium* sp. GP16-2, *Trichoderma harzianum* T39) they failed to induce ISR confirming the role of JA and ET in ISR (Ryu et al. 2004, Stein et al. 2008, Weller et al. 2012; Pieterse et al. 2014). Similar observations were also made in other plant systems, thus supporting the notion that in SA-independent ISR, JA/ET are the main regulators of plant immunity (Yan et al. 2002; De Vleesschauwer et al. 2008; Van der Ent et al. 2009). These pathways are also effective against necrotrophs and herbivores (Van Loon et al. 2008; Van Wees et al. 2008; De Vleesschauwer et al. 2009; Ding et al. 2015; Yezhang et al. 2015).

The *Jar1* gene encodes JA-amino acid synthetase which activates the JA signaling. Treatment of wild-type plants with meJA and the ET precursor1-aminocyclopropane-1-carboxylate (ACC) did elicit a response similar to rhizobacterial colonization in plants. However, when treated with these beneficial organisms, endogenous JA levels did not increase which led to the conclusion that the signaling was dependent on JA responsiveness (Pieterse et al. 2000; Staswick and Tiryaki 2004; Van Loon and Bakker 2005). Further, Knoester et al. (1999) using ethylene insensitive mutants demonstrated impaired ISR implicating the requirement of complete and functional ET pathway for proper ISR function.

15.5 Elicitor Molecules in Rhizobacteria-ISR

The organisms that result in ISR do not cause any damage to host. Hence this has resulted in the early conclusion that the chemical compounds resulting in ISR and those resulting in SAR/HR are different. Unlike SAR, ISR is not dependent on localized cell death but rather on the production of elicitors/determinants that trigger the mechanism (Ebel and Mithöfer 1998). A host of chemical determinants have been identified as capable of inducing resistance either individually or in combination (Bakker et al. 2003). These determinants however seem to share similarities in the defense reaction elicited within the host (Gómez-Gómez 2004; Nürnberger et al. 2004). For instance, crude cell wall extracts and lipopolysaccharides (LPS) of *P. fluorescens* WCS358 resulted in the activation of defense-associated reactions in Arabidopsis (Raaijmakers et al. 1995; Van Wees et al. 1999; Meziane et al. 2005; Nadarajah 2016a) and reduced disease symptoms in pathogen challenged plants. Mutant analysis displayed a redundancy in elicitors as the lack of either O antigenic side chains in Lipopolysaccharide (LPS) or flagellin in these mutants still resulted in induced resistance, as the presence of either one elicitor compounds was sufficient to elicit a response. However, not all strains of *P. fluorescence* can elicit resistance in Arabidopsis or other plants (Van Wees et al. 1997). This variation may be due to differences in the chemical composition or structure of their determinants. For instance, it was reported that the O-antigenic side-chain of LPS differs from strain to strain probably resulting in perception specificity towards different plant

species. Examples of specificity can be seen in application of LPS from *Burkholderia cepacia* against *Phytophthora nicotianae* strain ASP B 2D in tobacco (Coventry and Dubery 2001) and the efficient control of the nematode, *Globodera pallida* with LPS from *Rhizobium etli* strain G12 in potato (Reitz et al. 2002). Different species or strains of these rhizobacteria resulted in either induction or no response in the host.

Siderophore is another determinant that is involved in the induction of ISR. As there is a redundancy of determinants in rhizobacteria, ISR may be induced by different components in different plant species as exhibited by 7NSK2 in bean and tomato where SA and siderophores were implicated in the response (Audenaert et al. 2002). As siderophores are produced under iron-limiting conditions, it not only inhibits the pathogens within the soil but also induces systemic reaction within the host. However, while all siderophores are able to utilize iron, not every siderophore elicits ISR due to the differences in their chemical structure. Some siderophores produced by the rhizobacteria are pseudomonine, pyochelin, and pseudobactin (Nadarajah 2016a). Some examples of siderophore utilizing rhizobacteria are WCS374 (Leeman et al. 1996; Djavaheri et al. 2012), *P. aeruginosa* 7NSK2 (Audenaert et al. 2002), *Serratia marcescens* 90-166 (Press et al. 1997), and *P. fluorescens* CHA0 (Maurhofer et al. 1994; Weller et al. 2004).

Antibiotics play dual function in the rhizosphere as a microbial antagonist and a defense activator (Fernando et al. 2005). PGPR have been associated with producing more than one antibiotic which relates to its usefulness against phytopathogens (Glick et al. 2007). Diffusible (e.g., phenazines, pyoluteorin, pyrrolnitrin, cyclic lipopeptides (CLP)) and volatile (HCN) antimicrobial products are classified into six groups and interact effectively against microorganisms, nematodes, and plants (Haas and Défago 2005; Raaijmakers et al. 2010). The pyocyanin and pyochelin siderophore from 7NSK2 elicit ISR in conjunction with the 2,4-diacetylphloroglucinol (DAPG) antibiotic (Lavicoli et al. 2003) in tomato. DAPG likewise acts as an inducer in Q2-87 and CHA0 inducing resistance in tomato against *Meloidogyne javanica* (Siddiqui and Shaukat, 2003; Weller et al. 2004). These reports on DAPG suggest that there may be other rhizobacteria and antibiotics capable of eliciting ISR in plants. Pyrrolnitrin produced by the *P. fluorescens* (BL915) prevents damping-off by *Rhizoctonia solani* in cotton while phenazine producing pseudomonads possess redox potential with the ability to suppress various pathogens (Chin-A-Woeng et al. 2003). Phenazine-1-carboxamide that was isolated and studied from roots of tomato was able to mobilize iron from soil (*P. chlororaphis* PCL1391) (Haas and Défago 2005). A large number of Pseudomonads and *Bacillus* spp. have been reported to produce various antimicrobial compounds that have selective effect against various host and environments (Beneduzi et al. 2013).

Studies have shown that the interaction between these rhizobacteria and plant roots are dependent on plant variety, environmental conditions, and soil community (Ton et al. 1999; Nadarajah 2016b). While certain strains are perfect inducers of resistance in various plant species, most show tight specificity to root cell surface receptors (Van Loon et al. 1998). For example, WCS358 stimulates resistance in tomato, Arabidopsis, and bean (Raaijmakers et al. 1995; Meziane et al. 2005),

and fails to do so in carnation or radish (Leeman et al. 1995). Other strains such as WCS374 induced a powerful response in radish (Leeman et al. 1995) while another, WCS417, could successfully elicit a response in all the above five species of plants (Leeman et al. 1995; Bakker et al. 2013, 2014). Over the course of the last two

Table 15.1 Example of beneficial microbes and their determinants involved in disease suppression

Host	Pathogen	Beneficial microbe	Determinant	Reference
Arabidopsis	*Erwinia caratovora*	*B. amyloliquefaciens* IN937a	2,3-Butanediol	Ryu et al. (2003, 2004)
	P. syringae pv maculicola	*B. substilis* GB03	2,3-Butanediol	Lavicoli et al. (2003)
		B. pumilus SE34;T4	SA	Weller et al. (2004)
		P. fluorescens CHA0	2,4-DAPG	Meziane et al. (2005)
	Peronospora parasitica	*P. fluorescens* Q2-87	2,4-DAPG	
	P. syringae pv tomato	*P. putida* WCS358	LPS, siderophore	
Tobacco	TNV	*P. fluorescens* CHA0	Siderophore	Maurhofer et al. (1994)
	Peronospora tabacina	*B. pumilus* SE34	SA	De Meyer et al. (1999a)
	TMV	*P. aeruginosa* 7NKS2	SA	De Meyer et al. (1999b)
Tomato	*Botrytis cinerea*	*P. aeruginosa* 7NKS2	Pyochelin, pyocyanin	Audenaert et al. (2002)
	Meloidogyne javanica	*P. fluorescens* CHA0, *P. putida* WCS358	2,4-DAPG, LPS, siderophore	Siddiqui and Shaukat (2003)
	P. syringae			Meziane et al. (2005)
Bean	*P. syringae*	*P. aeruginosa* 7NKS2	SA	De Meyer et al. (1999a)
		P. putida WCS358	LPS, siderophore	Meziane et al. (2005)
		P. putida BTP1	Iron regulated metabolite, hexanal	
Rice	*Magnaporthe oryzae*	*P. aeruginosa* 7NSK2	SA, LPS, siderophores	De Vleesschauwer et al. (2006, 2008, 2009)
	Rhizoctonia solani	*P. fluorescens* WCS374r	LPS, siderophores	
	Magnaporthe oryzae	*Serratia plymuthica* IC1270	SA, LPS, siderophores	
	Cochliobolus myiabeanus			

decades, many ISR determinants have been identified in certain rhizobacterial species. Some examples are provided in Table 15.1.

15.5.1 Key Early Root-Specific Regulator in ISR

Although signaling for ISR starts at the root–microbe interface, not much research has been done to investigate the signaling components involved at eliciting ISR at the root level. Knoester et al. (1999) in studying the root interaction in ISR used a root ET insensitive mutant (*eir1*) which exhibited the involvement of ET in the transmission of ISR, which was aided perhaps by some other regulatory elements. Further *MYB72* was identified as a transcription factor that is involved in the signal transduction from the root as observed in response to *P. fluorescens* WCS417r in Arabidopsis (Verhagen et al. 2004; Pieterse et al. 2014). *MYB72* shows high levels of expression in PGPR-induced roots and no expression was detected in the phloem of uninduced plants. PGPR (*P. putida* WCS358, *P. fluorescens* WCS417r) and PGPF (*Trichoderma* spp.) treated mutant *MYB72* plants showed no ISR response hence indicating a significant role for this factor in ISR (Segarra et al. 2009). However, these studies showed that an overexpression of *MYB72* did not result in enhanced ISR but rather is dependent on iron-limiting conditions making a connection between iron equilibrium and ISR induction (Van der Ent et al. 2008; Palmer et al. 2013). Treatment with ISR-inducing *Pseudomonas* strains resulted in the co-regulation of iron deficient marker genes (*FRO2, IRT1*) and *MYB72* in Arabidopsis (Zamioudis et al. 2013). Transcriptome profiling of mutant *myb72* and wild-type Arabidopsis provided evidence that iron deficiency response genes were the most dominant species found in roots. PGPR and PGPF however are known to produce siderophores which result in iron uptake from environment therefore resulting in the iron deficient environment. In order to establish if the siderophores are responsible for the deficiency, a siderophore mutant was utilized which exhibited normal *MYB72*, *FRO2,* and *IRT1* gene activity confirming the role of these microbes in iron deficiency. This interaction requires further study for a better understanding of the connection between iron limitation and siderophore function in ISR (Zhang et al. 2007).

15.6 Expression of ISR

The consequence of ISR expression leads to reduced disease incidence as well as severity post treatment. While ISR executes its defense from belowground, SAR spreads its defense to distal organs from site of pathogenic infection. While both share some overlap in the mechanism of defense moderation, their signals differ. Studies have also shown that due to these differences in signals and moderation, SAR is more effective against biotrophic pathogens while ISR are active against

necrotrophs (Bakker et al. 2013). Therefore through the activation of JA and SA-dependent pathways, plants defends themselves against different pathogens in different plant species. This preparatory state of the plant to defend against invasion is called "priming" where there is enhanced level of cellular defense resulting in improved resistance (Van Wees et al. 2008). Various studies conducted on PGPR and PGPF have shown a role for priming in ISR defense (Van Loon and Bakker 2005; Wang et al. 2005). Priming is an important biological and chemical process that is fit and cost effective in adapting plants to its hostile environment (Pozo et al. 2008; Conrath 2011). In addition to the chemical changes observed within the plants in defense, there are structural changes such as callose deposition observed at the site of pathogen entry as seen in *P. fluorescens* WCS417r treated Arabidopsis (Van der Ent et al. 2009). Abscisic acid (ABA) has been indicated as essential in primed response against insects and pathogens in ISR (Corné et al. 2013; Vos et al. 2013). Besides callose deposition, *Bacillus subtilis* FB17 was observed to aggregate around the roots of plants infected by *P. syringae*. The presence of this organism induced stomatal closure and thence reduced the potential of invasion by foliar pathogens through the stomata (Walters et al. 2008). Transcription factors have a responsibility in signaling and regulating the primed state. These factors remain inactive in a non-induced stage but are rapidly activated when the host is affected by pathogens or insects. One transcriptional factor that has been linked to regulation and signaling of ISR is a member of the AP2/ERF family (Van der Ent et al. 2009). These factors have been linked with JA/ET regulated genes and are directly linked to ISR expression (Verhagen et al. 2004). Promoter region analysis of these ISR-related genes revealed the presence of cis-acting G box motif. These motifs are linked to a regulator of JA dependent response, MYC2 (Pozo et al. 2008; Stein et al. 2008) (Fig. 15.1) which is required for proper execution of this pathway. Out of the genes expressed in Arabidopsis, only ~1% of these genes are expressed at the root level and there is no constancy in the expression level observed in the distal leaves (Verhagen et al. 2004).

15.7 Disease-Suppressed Soil

Disease suppressive soil has been described as soil that shows suppression of pathogen through competition for nutrient, antagonism, lytic enzymes, quorum sensing, and various other means by which a non-beneficial microbial population is kept at check (Weller et al. 2002; Loper et al. 2012; Philippot et al. 2013). Through these belowground activities, damage is reduced significantly to the host or the establishment of disease becomes less important over time in a particular soil (Mazzola 2002). Beneficial microbes have been used to control agriculturally significant organisms such as *Gaeumannomyces graminis* var. *tritici* through the production of DAPG on Take All Disease. Over successive events of take all in a particular location, the soil eventually became suppressive towards the pathogen. This has been seen in events where monocultures were grown over a period of time resulting in

inhibition of the pathogen due to eventual multiplication and high dosage levels of the beneficial microbes (*Pseudomonas fluorescens*) within the soil (Weller et al. 2002). This disease suppressive soil can also be used in amending condusive soil to reduce disease incidences (Raaijmakers and Weller 1998). Another example in disease suppresiveness is against Fusarium wilt (Alabouvette 1999), and *Rhizoctonia solani* infestations (Mendes et al. 2011). The competition for nutrient and the production of phenazines appear to reduce the wilt symptom in infected soil (Mazurier et al. 2009). In each incident, it has been reported that while there may be a dominant microbe facilitating this suppression, in most cases it will involve a consortium. This consortium may be made up of microbes from the groups: *Azospirillum, Bulkholderia, Comamonas, Gluconacetobacter, Pseudomonas*, and *Sphingomonadaceae* genus (Kyselkova et al. 2009). Through Chip technology, 17 taxa of β and γ-proteobacteria and firmicutes were linked to disease suppressiveness (Mendes et al. 2011). Most often, disease suppressiveness has been linked to antibiosis (Raaijmakers et al. 2002), siderophore producing Pseudomonaceae (Duijff et al. 1998; Zhang et al. 2007) and ISR (Bakker et al. 2007) subsequently resulting in reduced disease incidence and severity (Pieterse et al. 2013).

15.8 Concluding Remarks and Future Prospects

Much research has been devoted towards understanding the role of beneficial microbes in the elicitation of plant immunity and its specific role in ISR since its discovery more than two decades ago. The plant immune system is unique in a way that it is activated to fend off enemies while it remains suppressed to support beneficial interactions. Both these interactions of the plant immune system are in play in ISR to benefit the host. It remains to be deciphered how a phenomenon that enhances plant immunity towards both biotic and abiotic stresses can at the same time contribute towards improved growth and development in the host. One would expect that the initial approach would be to try and determine or understand the "message" transmitted at the point of contact and how this message is then amplified and transmitted to the rest of the plant. We should also look at how both the ISR and SAR mechanisms overlap and what are the shared or different points of regulation between these mechanisms in incurring an effective immune response in host. The perception of the signal by the receptors as well as the regulators involved in the long distance signaling and perception of the signal in both ISR and SAR is still not completely understood. We believe through the use of the "omics" platforms, a wealth of information will surface not only to enable us to better understand the key players in these processes but to add on and gap fill on issues such as regulators, receptors, signal molecules, pathways, and other participants of the complex system of plant–microbe interactions in ISR and SAR. While we may have acquired sufficient knowledge on how the soil microbiomes improve plant heath and development, we are still vague on how the host is able to shape the microbial community

surrounding the roots to best benefit it. Likewise, while we may know the key processes involved in the perception and signaling of SAR in plant, there are still gaps in our knowledge with regards to the signals, the regulation, the perception and the mode of transmission of signals long distance.

As a major societal challenge is producing sufficient agricultural produce to meet the market and population demands, any development in science that lends towards this is a positive contribution. In this chapter, we see how both SAR and ISR are two main contributors of the plant defense mechanism and how a better knowledge and understanding of these can assist us with the challenge. ISR has been used in the past decades as biocontrols and in soil amendments all with the hope of reducing disease incidence and severity and at the same time contributing towards better yield and development. A better understanding of SAR on the other hand will likewise contribute towards the knowledge to enhance plant immunity through external stimuli, breeding and the utilization of transgenics towards generating crops with heightened defense mechanisms.

Some answers that may assist with obtaining a clearer and more well-defined picture of SAR and ISR in plants may arise from addressing the following questions:

1. How does the plant facilitate the colonization of a suitable community to enhance growth and immunity? How do these communities play a dual role in growth and immunity?
2. What are the long distance signal(s) involved in both ISR and SAR? How are they transmitted and how do they trigger ISR/SAR?
3. Are there any other regulators than *MYB72* in the root for ISR?
4. Are there any other transcription factors or regulators that are involved in ISR and SAR? What are their function and contribution in these processes?
5. Is there a role for autoregulation of mutualism in ISR?
6. How exactly does *NPR1* regulate ISR?
7. What are the differences and similarities in genes triggered by ISR and SAR in plants?

While the above questions are not the only areas left with gaps to fill, the constant inquisition on the above mechanisms will only increase our knowledge. However there is always a possibility that with new knowledge comes new questions and new issues to address.

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