Chapter 15
PGPR-Induced Systemic Resistance (ISR) in Plant Disease Management

K. Annapurna, Amod Kumar, L. Vithal Kumar, V. Govindasamy, Pranita Bose, and D. Ramadoss

15.1 Introduction

Plant growth promoting rhizobacteria (PGPR) are bacteria that colonise the plant root and act as an additional source of hormones, vitamins, and growth factors that are helpful to improve plant growth and yield (Kloepper and Schroth 1978; Babalola 2010). PGPR are non-pathogenic and known to posses several mechanisms to suppress the plant pathogens like competing for fundamental niche (Elad and Baker 1985; Elad and Chet 1987), antibiosis by producing antibiotics and hydrogen cyanide (HCN) (Senthilkumar et al. 2007a, b; Pierson and Thomashow 1992) and also acting as a good source of siderophores which chelate the iron in the root vicinity to limit the availability of iron necessary for the growth of phyto-pathogens (Kloepper et al. 1980; Lemanceau et al. 1992; Compant et al. 2005).

Induced resistance is a physiological "state of enhanced defensive capacity" elicited by non-pathogenic organisms (Van Loon et al. 1998) or specific environmental stimuli, whereby the plant's innate defences are potentiated against subsequent biotic challenges (Van Loon et al. 1998). Generally, induced resistance is systemic because the defensive capacity is increased not only in the primary infected plant parts, but also in non-infected, spatially separated tissues. Thus, induced systemic resistance (ISR) is a state of increased defensive capacity developed by plants when appropriately stimulated, through activation of latent resistance induced by diverse agents including rhizobacteria (Van Loon et al. 1998; Mariutto et al. 2011). The utilisation of pathogenic organisms as inducing agents is less promising under field conditions, because the induction of ISR with pathogen inoculation will give less duration for the protection than that with PGPR-mediated ISR because prior inoculation of a pathogen might act as a good source of secondary inocula (Wei et al. 1991).

K. Annapurna (✉) • A. Kumar • L.V. Kumar • V. Govindasamy • P. Bose • D. Ramadoss
Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110 012, India
e-mail: annapurna96@yahoo.co.in

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Induced systemic resistance or ISR may become localised sometimes and is known as localised acquired resistance (LAR) when the boosting of resistance occurs to some specific tissues against a primary invader. The mode of action of both the LAR and systemic resistance seems to be similar in their effect against various types of pathogens, but in case of LAR, only localised effect of resistance develops and is not propagated throughout the plant. There are major differences in ISR when compared to other mechanisms. First, the action of ISR is based on the defence mechanism that is activated by inducing agents. Second, ISR expresses multiple potential defence mechanisms that include increase in activity of chitinase, β-1,3 glucanase and peroxidase; accumulation of antimicrobial low molecular substances such as phytolexins and formation of protective biopolymers viz., lignin, callose and hydroxyproline-rich glycoprotein (Archana et al. 2011). Third, an important aspect of ISR is the wide spectrum of pathogens that can be controlled by a single inducing agent (Dean and Kuc 1985; Hoffland et al. 1996). Thus ISR appears to be the result of several mechanisms, which together are effective against a wide range of fungal, bacterial and viral pathogens. For successful disease management, it is important to find more effective, practical and economical ways to protect plants from various pests and diseases. The utilisation of natural PGPR strains as inducers of plant defence responses may increase the chance of their applicability and offer a practical way to deliver immunisation.

15.2 Defence Mechanisms in Plants

In response to the pathogen (fungi, bacteria, viruses, nematodes and insects) attack, the plant undergoes biotic stress and develops some type of defence mechanism to cope with the situation. Plant has two types of defence mechanisms: passive or constitutive and active or inducible.

Passive or constitutive defence mechanism—attack of the pathogen on the outer layer of the plant leads to damage in the cuticle or lignin of the plant surface. Secretion of plant metabolites such as phenols, resins, tannins and alkaloids at the damaged sites of the plant surface are found to be pathogenic to some pathogens. This mode of plant defence is known as passive or constitutive defence mechanism.

Active or inducible defence mechanism—in response to the attack of the pathogen, the plant acquires some changes like thickening of the outer layer known as wall opposition so that it would be tough for the pathogen to invade through the plant surface. In addition, plants also show active defence by developing hypersensitive responses. In hypersensitive response, the cells near the site of pathogen infection become necrotic and become metabolically inert. Cells start to accumulate toxic compounds and also initiate the secretion of phytoalexins as immune response.

15.3 PGPR-Induced Systemic Resistance in Plants

The importance of PGPR was realised as an off shoot of biological control of soil-borne pathogens. Systemic resistance induced by exogenous chemical agents and pathogenic organisms is termed as systemic acquired resistance (SAR), whereas PGPR-mediated protection is generally referred to as ISR (Kloepper et al. 1992). All plants possess active defence mechanisms against pathogen attack. If defence mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease incidence can be reduced. Induced resistance is not the creation of resistance where there is none, but the activation of latent resistance mechanisms that are expressed upon subsequent, so-called challenge inoculation with a pathogen (Van Loon 1997). The terms "induced" and in some cases "acquired" systemic resistance were used interchangeably by different research groups until Ryals et al. (1996) defined the type of resistance induced by pathogenic organisms and/or chemicals involving salicylic acid as mediator of SAR as a tribute to Ross, disregarding many earlier publications describing entirely the same phenomenon using ISR as a synonym. But it was Van Loon's research group that used ISR as the term solely to describe resistance mediated by PGPR (Pieterse et al. 1996, 1998, 2000, 2002; Van Loon et al. 1998).

Strains of the genera such as *Aeromonas*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconobacter*, *Klebsiella*, *Pseudomonas* and *Serratia* have been identified as PGPR and efforts are being made to identify more and more rhizobateria having PGP traits (Dey et al. 2004; Jaizme-Vega et al. 2004; Joo et al. 2004; Tripathi et al. 2005). The diversity of PGPR in the rhizosphere largely varies according to the plant type and nutrients available (Tilak et al. 2005). *Pseudomonas* and *Bacillus* spp. have a wide distribution among this diversity of PGPR and are the extensively studied genera for PGP and biological disease control.

In recent years, the use of PGPR as an inducer of systemic resistance in crop plants against different pathogens has been demonstrated under field conditions (Wei et al. 1991, 1996; Vidhyasekaran and Muthamilan 1999; Viswanathan and Samiyappan 1999). Several studies have been carried out to elicit ISR by PGPR in plants. ISR by PGPR has been achieved in a large number of crops including *Arabidopsis* (Pieterse et al. 1996), cucumber (Wei et al. 1996), tomato (Duijff et al. 1998), potato (Doke et al. 1987), radish (Leeman et al. 1996), carnation (Van Peer et al. 1991), sugarcane (Viswanathan and Samiyappan 1999), chilli (Bharathi et al. 2004), brinjal (Chakravarty and Kalita 2011), tomato and hot pepper (Ramamoorthy et al. 2002), rice (Vidhyasekaran et al. 2001; Nandakumar et al. 2001) and mango (Vivekananthan et al. 2004) against a broad spectrum of pathogens including fungi (Leeman et al. 1995; Doke et al. 1987), bacteria (Liu et al. 1995a), nematodes (Siddiqui et al. 2007), insects (Tomczyk 2006) and viruses (Khalimi and Suprapta 2011).

In carnation, application of *Pseudomonas fluorescens* induces systemic resistance against an array of plant pathogens reported through a number of studies (Wei

Table 15.1 List of some studies conducted to show PGPR-induced systemic resistance

Organism	Host plant	Pathogen	Author
P. putida (89B-61) *Serratia marcescens* (90-166) *Flavomonas oryzihabitans*(INR-5) *Bacillus pumulus* (INR-7)	*Arabidopsis*	*P. syringae* pv. Lachrymans	Wei et al. (1996)
P. fluorescens (WCS417r) *Fusarium oxysporum* (Fo47[a])	Tomato	*F. oxysporum* (lycopersici race-2)	Duijff et al. (1998)
P. fluorescens (WCS 374)	Radish	Fusarium wilt	Leeman et al. (1996)
P. fluorescens (WCS417r)	Carnation	*F. oxysporum* (dianthi)	Van Peer et al. (1991)
P. fluorescens (Pf1)	Rice	*X. oryzae*	Vidhyasekaran et al. (2001)
P. fluorescens (Pf1 and Pf7)	Rice	*R. solanii*	Nandakumar et al. (2001)
P. putida(KKMI) *P. fluorescens* (VPT4)	Sugarcane	*Colletotrichum falcatum*	Viswanathan and Samiyappan (2001)
P. fluorescens (Pf1) *P. putida* (PFATR and KKM1)	Tomato and Hot pepper	*Pythium aphanidermatum*	Ramamoorthy et al. (2002)
P. fluorescens (Pf1) *Bacillus subtilis*	Chillies	*Colletotrichum capsici*	Bharathi et al. (2004)
P. fluorescens (PFV, PFP, PSV) *Bacillus subtilis*(BSV, BSP)	Tea	*Exobasidium vexaus*	Saravanakumar et al. (2007)
P. fluorescens (PFMMP) *T. viridae* (TVUV10) *Bacillus subtilis* (BSG3)	Peppermint	*R. solani*	Kamalakaman et al. (2003)
P. fluorescens (Pfl-94)	Chickpea	*F. oxysporum* fsp *ciceri*	Saikia et al. (2006)
P. fluorescens	Brinjal	*Ralstonia solanacearum*	Chakravarty and Kalita (2011)
P. putida (MTCC no.-493)	Lentil	*M. javanica* (nematode)	Siddiqui et al. (2007)
P. aeruginosa	Soyabean	*Soyabean stunt virus*	Khalimi and Suprapta (2011)
P. fluorescens (P-112)	Cucumber	*Tetranychus urticae* (insect)	Tomczyk (2006)
P. fluorescens (FP7)	Mango	*Colletotrichum gloeosporioides*	Vivekananthan et al. (2004)

[a]Known non-pathogenic strain of *Fusarium* (Alabouvette et al. 1993)

et al. 1996; Duijff et al. 1998; Leeman et al. 1996; Van Peer et al. 1991). Duijff et al. (1998) used *P. fluorescens* wcs417r and non-pathogenic *Fusarium* strain Fo47 against the fungal pathogen *Fusarium oxysporum lycopersici* race-2 for the development of ISR in tomato. The induction of resistance in radish with *P. fluoresens*

wcs417r was strategically analysed and found that inoculation of bacteria and pathogen on alternate days gave the best result (Leeman et al. 1995) (Table 15.1).

Similarly Van Peer et al. (1991) have observed that bacterisation of the plant 1 week before the inoculation of *F. oxysporum* gave best result than simultaneous bacterisation and inoculation. PGPR strains applied as a seed treatment resulted in a significant reduction in anthracnose disease caused by *Colletotrichum orbiculare* in cucumber (Wei et al. 1991, 1996). The induction of systemic resistance by *P. putida* strain 89B-27 and *S. marcescens* strain 90-166 reduced *Fusarium* wilt of cucumber incited by *F. oxysporum* (Liu et al. 1995b).

The use of a mixture of PGPR not only developed resistance towards anthracnose disease in cucumber plants but also improved plant growth promotion by an increase in the main runner length and in leaf number (Wei et al. 1996). Seed and root treatment of rice with Pseudomonad Pf1 and FP7 enhanced the resistance for the sheath blight pathogen *Rhizoctonia solani* (Vidhyasekaran and Muthamilan 1999). Similarly, in sugarcane, Viswanathan and Samiyappan (1999) established PGPR-mediated ISR against *C. falcatum* causing red rot disease in sugarcane.

PGPR is also reported to develop systemic resistance against bacterial diseases. Alstrom (1991) treated the bean seeds with *P. fluorescens* 97 and observed development of resistance against halo blight disease caused by *Pseudomonas syringae*. He also pointed that the optimum level of the inoculum of PGPR strain Pseudomonad strain 97 lie between 4.6×10^8 cfu/ml and 4.6×10^7 cfu/ml. The treatment of cucumber seed with *P. putida 89B-61* and *S. marcescens* strain 90-166 decreased the incidence of bacterial wilt disease (Kloepper et al. 1993). Angular leaf spot of cucumber, caused by *P. syringae* pv. Lachrymans, was controlled through PGPR-mediated resistance after the inoculation of combined inoculum of *Bacillus pumilus* INR7, *Curtobacterium flaccumfaciens* MEl and *Bacillus subtilis* GB0 (Raupach et al. 2000). Similar type of systemic resistance was observed in Cucumber after seed treatment with *P. putida* strain 89B-61, *Flavomonas oryzihabitans* INR-5, *S. marcescens* strain 90-166 and *B. pumilus* INR-7 against the angular leaf spot caused by *P. syringae* by reducing total lesion diameter compared with non-treated plants (Liu et al. 1995a; Wei et al. 1996).

Development of systemic resistance against viruses by the use of PGPR has also been reported in a number of important plants. A mixture of *P. putida* strain 89B-61 and *S. marcescens* strain 90-166 treated seeds of cucumber and tomato plants respectively developed the systemic resistance against cucumber mosaic virus (CMV) wherein the virus-induced symptoms got delayed (Raupach et al. 1996). Likewise, *S. marcescens* strain 90-166 and *B. pumilus* SE34 had significantly reduced severity by CMV (Murphy et al. 2000). *P. fluorescens* CHAO-induced systemic protection reduced the incidence of leaf necrosis in tobacco after the challenge of tobacco necrosis virus (TNV) (Maurhofer et al. 1994, 1998). Application of *B. cereus* (I-35) and *Stenotrophomonas* sp (II-10) through seed treatment and soil drenching reduced the effect of TMV, and chilli veinal mottle virus (ChiVMV) in hot pepper (*Capsicum annuum*) (Damayanti and Katerina 2008). Murphy et al. (2000) observed *Bacillus amyloliquefaciens* 937a, *B. subtilis* 937b and *B. pumilus* SE34 mediated significant enhancement of the resistance in tomato against tomato mottle virus (ToMoV). Similarly, inoculation of *Pseudomonas* B-25 also enhanced plant

growth through increase in NPK uptake and reduced the effect of TMV-mediated pathogenesis in tomato (Kirankumar et al. 2008).

A number of studies reported the efficacy of PGPR-mediated ISR in the control of insect pests. Zehnder et al. (1997) observed lower level of cucurbitacin, a cucumber beetle feeding stimulant, in the PGPR-treated than non-treated plant, and the choice of feeding in the cucumber beetle (*Diabrotica undecimpunctata howardii*) also shifted from treated to non-treated plants. Similarly, Tomczyk (2006) also reported the efficacy of *P. fluorescens* in inducing resistance in cucumber against the spider mites. The relative growth rate, consumption rate and digestibility of feed by *Helicoverpa armigera* have been affected when larvae fed on cotton plants treated with *Pseudomonas gladioli* due to an increase in their polyphenol and terpenoid content (Qingwen et al. 1998). Pseudomonads are good endophytic rhizospheric colonisers. Hence, efforts have been made to transfer the insecticidal crystal protein from *Bacillus thuringiensis* to *P. fluorescens* and in some studies a positive result came out (Herrera et al. 1994). The *cry* gene transformed *P. fluorescens*, suppressed the sugarcane borer *Eldana saccharina* in a greenhouse study on sugarcane. Transgenic *P. cepacia* 526 with the crystal protein gene has consistently shown insecticidal activity against tobacco hornworm (Stock et al. 1990).

The effectiveness of PGPR-mediated ISR against nematode pests is also well documented (Oostendorp and Sikora 1990; Sikora 1992; Sikora and Hofmann-Hergarten 1992; Siddiqui and Shaukat 2004). *P. fluorescens* has ISR and inhibited early root penetration by *Heterodera schachtii*, the cyst nematode in sugar beet (Oostendorp and Sikora 1989, 1990). Similarly, *B. subtilis* induced protection against *Meloidogyne incognita* and *M. arenaria* in cotton (Oostendorp and Sikora 1989). Though attempts to use PGPR for nematode control are limited, the use of PGPR as biological control agents of plant parasitic nematodes, especially for sugar beet and potato cyst nematode, has been reported as a successful strategy in management of these nematodes (Sikora 1992). Treatment of rice seed with PGPR alone or in combination with chitin and neem cake has reduced the root and soil population of the rice root nematode *Hirschmanniella oryzae* (Swarnakumari and Lakshmanan 1999; Swarnakumari et al. 1999). The level of infestation of root-knot nematode *M. incognita* in tomato was reduced with fewer galls and egg masses in the soil following root dipping with *P. fluorescens* strain Pf1 (Santhi and Sivakumar 1995). Similarly, application of *P. chitinolytica* reduced the root-knot nematode infection in tomato crop (Spiegel et al. 1991). These experiments showed that PGPR-mediated ISR is effective in both dicotyledonous plants, viz., arabidopsis, bean, carnation, cucumber, radish, tobacco tomato, etc., and certain monocotyledonous plants, viz., rice, maize and sugarcane.

15.4 Rhizobacterial Determinants in Triggering ISR

Usually a large number of Rhizobacteria are found to be present on the root surface, where they get their nutrients from plant exudates and lysates (Lynch and Whipps 1991). Some of these rhizobacteria exhibit direct antibiosis with the soil-borne

pathogens (Wei et al. 1996). PGPR-induced systemic resistance can be proved experimentally through the spatially separated inoculation of pathogens and PGPR to avoid any antagonistic reaction between plant pathogens and PGPR. Some biochemical compounds of PGPR affect the complimentary receptors on the plant surface for the successful elicitation of systemic resistance. Treatment of tobacco roots with *P. fluorescens* CHAO triggered accumulation of SA-inducible PRs in the leaves (Maurhofer et al. 1994). He suspected that siderophore pyoverdin might be associated with the increase in the level of SA and acts as a systemic resistance elicitor against TNV. A SA-deficient mutant of *Pseudomonas aeruginosa* 7NSK2 failed to induce resistance in bean and tobacco, whereas two mutants affected in other siderophores were still capable of inducing resistance, so these studies suggested the elicitation of IRS against *B. cinerea* due to the production of bacterial SA (De Meyer and Höfte 1997).

Earlier, several structural and metabolic compounds have been detected which are associated with elicitation of rhizobacteria-mediated ISR (Van Loon et al. 1998). Purified lipopolysaccharides (LPS) and flagella of some non-pathogenic *Pseudomonas* strains have been shown to induce systemic resistance as well (Leeman et al. 1995; Van Peer and Schippers 1992; Van Wees et al. 1997). Some plants have been shown to possess a sensitive perception system for bacterial flagellins (Felix et al. 1999). N-terminal of bacterial flagellin f15 acts as a strong elicitor which led to alkalisation that initiated systemic resistance in tomato and some other plants (Felix et al. 1999; Gomez-Gomez and Boller 2000). These examples ascertain that the bacterial flagella or LPS is directly involved in elicitation of a defence-signalling pathway (Van Peer and Schippers 1992; Van Wees et al. 1997). But, Van Wees et al. (1997) contradict the finding by using bacterial mutants lacking flagella or the O-antigenic side chain of the LPS and showed that these are still able to elicit ISR in Arabidopsis. So, in addition to LPS and flagellin, some more determinants are possibly involved in the elicitation of PGPR-mediated ISR. In *P. putida* BTP1, an unknown iron-regulated metabolite casamino acid appears to be responsible for ISR in bean against *Botrytis cinerea* (Ongena et al. 2002).

Ongena et al. (2007) showed that *Bacillus subtilis* strain 168 producing lipopeptides surfactins and fengycins elicited the systemic resistance in bean. Some reports of implication of antibiotics in the elicitation of ISR are also available. Iavicoli et al. (2003) demonstrated that 10–100 μM of 2,4-diacetylphloroglucinol (DAPG) applied to roots of *Arabidopsis* mimicked the ISR against *Peronospora parasitica*. Audenaert et al. (2002) concluded that phenazine antibiotic pyocyanin in combination with SA or the SA-containing siderophore pyochelin produced by *P. aeruginosa* 7NSK2 acts as a determinant for induced resistance against *B. cinerea*. On the basis of above discussion, it can be concluded that the PGPR determinants responsible for ISR elicitation can be divided into three classes: cell surface components, such as flagella or outer membrane LPS; iron-regulated metabolites with siderophore activity like casamino acid or pyoverdine and other inhibitory metabolites like DAPG and phenazine (Table 15.2).

Table 15.2 List of reported ISR determinants of PGPR

Resistance elicitor compound	Host plant	Pathogen	Author
Siderophore, pyoverdin	Tobacco	Tobacco mosaic virus	Maurhofer et al. (1994)
Bacterial SA	Bean	*B. cinerea*	De Meyer and Höfte (1997)
Fucose and rhamnose (Lipopolysaccharide)	Radish	*Fusarium*	Leeman et al. (1995)
Flagellins	Tomato	*Pseudomonas syringae* pv *tabaci*	Felix et al. (1999)
Casamino acid	Beans	*Botrytis cinerea*	Ongena et al. (2002)
DAPG	Arabidopsis	*Peronospora parasitica*	Iavicoli et al. (2003)
Phenazine and pyocyanin	Tomato	*B. cinerea*	Audenaert et al. (2002)

15.4.1 Signalling in PGPR-Induced ISR

Plants have the ability to develop an enhanced defensive capacity upon stimulation by pathogenic and non-pathogenic microorganisms. This induced disease resistance is generally expressed as a restriction of pathogen growth and reduction of symptom development (Hammerschmidt 1999). Induced resistance can be triggered by certain chemicals, non-pathogens, avirulent forms of pathogens, incompatible races of pathogens, or by virulent pathogens under circumstances where infection is stalled owing to environmental conditions.

The signalling pathways controlling pathogen-induced SAR and rhizobacteria-mediated ISR are relatively well studied. Pathogen-induced SAR is controlled by a signalling pathway that depends on endogenous accumulation of salicylic acid (SA), and is associated with the accumulation of pathogenesis-related (PR) proteins (Ryals et al. 1996; Sticher et al. 1997; Van Loon 1997). In some cases, rhizobacteria have been shown to activate the SAR pathway by producing SA at the root surface (Maurhofer et al. 1994, 1998; De Meyer and Höfte 1997; De Meyer et al. 1999). However, in arabidopsis, ISR is triggered by *P. fluorescens* by transcriptional activation of PR genes (Pieterse et al. 1996; Van Wees et al. 1997). However, both SAR and ISR pathways must diverge downstream of NPR1. This indicates that NPR1 differentially regulates defence responses depending on the pathway that is activated upstream of it (Pieterse et al. 1998) (Fig. 15.1). Induction of PRs is invariably linked to necrotising infections giving rise to SAR, and has been taken as a marker of the induced state (Kessmann et al. 1994; Uknes et al. 1992; Ward et al. 1991). Some of these PRs are β 1,3-glucanases and chitinases and capable of hydrolysing fungal cell walls. Other PRs have more poorly characterised antimicrobial activities or unknown functions. The association of PRs with SAR suggests an important contribution of these proteins to the increased defensive capacity of induced tissues.

Non-pathogenic, rhizosphere-colonising *Pseudomonad* trigger a form of induced resistance, phenotypically similar to SAR, called rhizobacteria-mediated ISR.

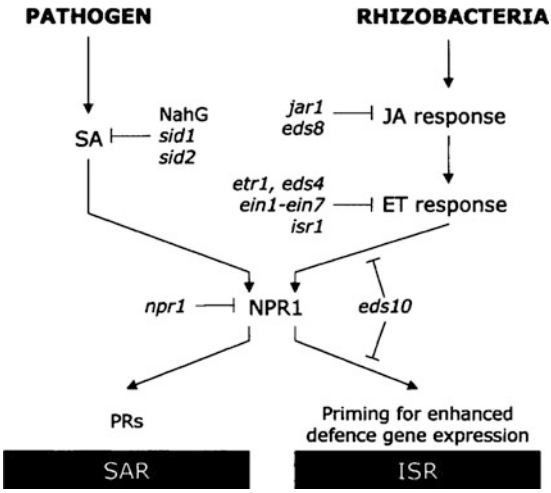

Fig. 15.1 Schematic model of pathogen-mediated SAR and rhizobacteria-mediated ISR signal transduction pathways in Arabidopsis (Courtesy: Pieterse et al. 2002)

P. fluorescens strain WCS417r (WCS417r) has been shown to activate ISR in several plant species (Duijff et al. 1998; Pieterse et al. 1996; van Peer et al. 1991). In *Arabidopsis*, WCS417r-mediated ISR is effective against different types of fungal and bacterial pathogens (Pieterse et al. 1996; van Wees et al. 1997). Interestingly, SAR and ISR are regulated by distinct signalling pathways. In contrast to SAR, WCS417r-mediated ISR functions independently of SA and PR gene activation (Pieterse et al. 1996; van Wees et al. 1997), but requires JA and ethylene signalling. The JA response mutant jar1 (Staswick et al. 1992) and the ethylene response mutant etr1 (Bleecker and Kende 1988) do not express ISR upon treatment with WCS417r, indicating that the ISR-signalling pathway requires components of the JA and ethylene response (Knoester et al. 1999; Pieterse et al. 1998). Although SAR and ISR follow distinct signalling pathways, they are both blocked in the regulatory mutant npr1 (Cao et al. 1994; Pieterse et al. 1998). Thus, NPR1 is not only required for the SA-dependent expression of PR genes during SAR, but also for the JA- and ethylene-dependent activation of unidentified defence responses resulting from rhizobacteria-mediated ISR.

The ability to develop ISR in response to rhizobacteria has been documented for many plant species (Van Loon et al. 1998) and appears to depend on the host–rhizobacterium combination (Leeman et al. 1995; Van Peer et al. 1991; Van Peer and Schippers 1992; Van Wees et al. 1997). A specific recognition between the plant and the ISR-inducing rhizobacterium is required for the induction of ISR. For instance, *P. putida* and *P. fluorescens* perform differently on different plant species: *Arabidopsis* is responsive to *P. putida*, whereas radish and carnation are not (Leeman et al. 1995; Van Peer et al. 1991; Van Peer and Schippers 1992; Van Wees et al. 1997). Conversely, radish is responsive to *P. fluorescens*, whereas Arabidopsis is not (Leeman et al. 1995; Van Wees et al. 1997). This suggests that specific recognition between the plant and the ISR-inducing rhizobacterium is required for the induction of ISR. Research on the rhizobacterial determinants

involved in the elicitation of ISR revealed several bacterial traits as potential inducers of ISR, including outer membrane LPS and iron-regulated siderophores (Leeman et al. 1995; Van Loon et al. 1998; Van Peer and Schippers 1992).

One of the parallels between rhizobacteria-mediated ISR and pathogen-induced SAR is that both types of induced resistance are effective against a broad spectrum of plant pathogens (Kuc 1982; Van Loon et al. 1998). To compare the spectrum of effectiveness of ISR and SAR, a range of viral, bacterial, fungal and oomycete pathogens of *Arabidopsis* was tested. Both *P. fluorescens*-mediated ISR and SAR induced by an avirulent strain of the pathogen *P. syringae* in tomato appeared to be effective against bacterial speck and black rot disease caused by the bacterial pathogens *P. syringae* and *X. campestris* respectively (Pieterse et al. 1996; Ton et al. 2002). Also fusarium wilt disease caused by the fungus *F. oxysporum* was equally affected by defence responses expressed during ISR and SAR (Pieterse et al. 1996; Van Wees et al. 1997). Moreover, disease caused by the downy mildew pathogen *P. parasitica* was reduced in both cases, although SAR was significantly more effective than ISR (Ton et al. 2002). Besides these similarities in effectiveness, there are also clear differences. For instance, ISR-expressing plants showed enhanced resistance against infection by the fungus *A. brassicicola*, whereas SAR is not effective against this pathogen. Conversely, expression of SAR inhibits multiplication of turnip crinkle virus and strongly reduces disease symptoms caused by this virus, whereas ISR has no effect at all (Ton et al. 2002). Thus, the spectrum of effectiveness of ISR and SAR partly overlaps but is clearly divergent, suggesting that the defence responses activated during both types of induced resistance are, at least partly, dissimilar.

15.4.2 SAR Signal Transduction Pathway

Early research on molecular mechanisms involved in induced disease resistance was mainly focussed on pathogen-induced SAR in tobacco, cucumber and bean plants. It was demonstrated that the onset of SAR is accompanied by a local and systemic increase in the endogenous levels of SA (Malamy et al. 1990; Metraux et al. 1990) and the concomitant up-regulation of a large set of genes (Ward et al. 1991), including ones encoding pathogenesis-related (PR) proteins (Van Loon and Van Strien 1999). Several PR proteins possess antimicrobial activity and are thought to contribute to the state of resistance attained. Exogenous application of SA, or functional SA analogues, such as 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH), induced SAR and activates PR genes (Ryals et al. 1996). Conversely, transgenic NahG plants expressing the bacterial salicylate hydroxylase gene *nahG* were unable to accumulate SA and were compromised in SAR (Gaffney et al. 1993), demonstrating that SA is both necessary and sufficient for induction of SAR.

15.4.3 ISR Signal Transduction Pathway

Research on the molecular mechanism of rhizobacteria-mediated ISR was initially focussed on the role of PR proteins, as the accumulation of these proteins was considered to be strictly correlated with induced disease resistance. However, radish plants of which the roots were treated with ISR-inducing *P. fluorescens* did not accumulate PR proteins, although these plants clearly showed enhanced resistance against fusarium wilt disease (Hoffland et al. 1995). Similarly, *Arabidopsis* plants expressing *P. fluorescens*-mediated ISR showed enhanced resistance against *F. oxysporum* and *P. syringae*, but this did not coincide with the activation of the SAR marker genes *PR-1, PR-2* and *PR-5* (Pieterse et al. 1996; Van Wees et al. 1997). After refuting the dogma that systemically induced disease resistance strictly coincides with accumulation of PR proteins, Pieterse et al. (2002) reviewed ISR signalling pathway in more detail in *Arabidopsis*. The data regarding the role of SA in ISR are available in SA non-accumulating Arabidopsis NahG plants. In contrast to pathogen-induced SAR, *P. fluorescens*-mediated ISR against *P. syringae* was normally expressed in these plants (Pieterse et al. 1996; Van Wees et al. 1997). Likewise, the SA induction deficient mutants sid1-1 and sid2-1 (Nawrath and Metraux 1999) expressed normal levels of ISR. Moreover, determination of SA levels in ISR-expressing Arabidopsis plants revealed that, in contrast to SAR, ISR is not associated with increased accumulation of SA (Pieterse et al. 2000). This led to the conclusion that *P. fluorescens*-mediated ISR is an SA-independent resistance response, and that ISR and SAR are regulated by distinct signalling pathways. Apart from *P. fluorescens*, *P. putida* induced the SA-independent ISR pathway in *Arabidopsis* (Van Wees et al. 1997).

In addition, the biological control strain *S. marcescens* 90-166 has been shown to induce protection in both wild-type and transgenic NahG tobacco plants against *P. syringae* (Press et al. 1997), indicating that the ability to trigger an SA-independent pathway controlling systemic resistance is not uncommon among ISR-inducing rhizobacteria. However, not all resistance-inducing rhizobacteria trigger an SA-independent resistance. For instance, an SA-overproducing mutant of *P. aeruginosa* and a genetically modified, SA-overproducing *P. fluorescens* strain have been shown to trigger the SA-dependent SAR pathway by producing SA at the root surface (De Meyer and Höfte 1997; Maurhofer et al. 1998).

Besides SA, jasmonic acid (JA) and ethylene (ET) have repeatedly been implicated in the regulation of primary resistance responses in plants (Pieterse and Van Loon 1999; Pieterse et al. 2001). In many cases, infection by microbial pathogens and attack by herbivorous insects are associated with enhanced production of these hormones and a concomitant activation of distinct sets of defence-related genes. Moreover, exogenous application of these compounds often results in an enhanced level of resistance. To investigate the role of JA and ET in rhizobacteria-mediated ISR, the Arabidopsis JA response mutant jar1-1 and the ET response mutant etr1-1 were tested for their ability to express ISR. Both mutants were unable to mount resistance against *P. syringae* pv. tomato after colonisation of the roots by

P. fluorescens WCS417r (Pieterse et al. 1998), indicating that ISR requires responsiveness to both JA and ET. In addition to etr1-1, a set of other well-characterised *Arabidopsis* mutants that are affected at different steps of the ET signalling pathway were tested for their ability to express ISR. None of the mutants developed ISR against *P. syringae* (Knoester et al. 1999), indicating that an intact ET signalling pathway is required for the expression of ISR. To elucidate the sequence of the signalling events, the resistance-inducing ability of methyl jasmonate (MeJA) and 1-aminocyclopropane-1-carboxylate (ACC), the natural precursor of ET, was tested in wild-type, NahG, jar1-1 and etr1-1 plants. Like *P. fluorescens*, MeJA and ACC were effective in inducing resistance against *P. syringae* in SA non-accumulating NahG plants, suggesting that both inducers activate the SA-independent ISR pathway. Moreover, MeJA-induced protection was blocked in both jar1-1 and etr1-1, whereas ACC-induced protection was affected in etr1-1, but not in jar1-1 plants. Hence, it was postulated that *P. fluorescens*-mediated ISR follows a signalling pathway in which components from the JA and ET response are successively engaged (Pieterse et al. 1998). ISR is dependent on NPR1, and NPR1 has been shown to be an important regulatory factor in the SA-dependent SAR response (Cao et al. 1994). To know whether NPR1 is also involved in the SA-independent ISR response, *Arabidopsis* mutant npr1 was tested for the induction of ISR. Surprisingly, mutant npr1 plants were blocked in their ability to express *P. fluorescens*-mediated ISR, indicating that, like pathogen-induced SAR, rhizobacteria-mediated ISR is an NPR1-dependent defence response (Pieterse et al. 1998). Elucidation of the sequence of ISR signalling events revealed that NPR1 functions downstream of JA and ET in the ISR signalling pathway. Evidently, NPR1 is not only required for the SA-dependent expression of PR genes that are activated during SAR, but also for the JA- and ET-dependent activation of defence responses resulting from rhizobacteria-mediated ISR. This demonstrates that NPR1 is able to differentially regulate defence gene expression, depending on the signalling pathway that is activated upstream of it.

15.4.4 Expression of PGPR-Induced ISR

A large number of defence enzymes that have been associated with ISR include phenylalanine ammonia lyase (PAL), chitinase, β-1,3-glucanase, peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase (CAT), lipoxygenase (LOX), ascorbate peroxidase (APX) and proteinase inhibitors (Koch et al. 1992; Schneider and Ullrich 1994; Van Loon 1997). These enzymes also bring about liberation of molecules that elicit the initial steps in induction of resistance, phytoalexins and phenolic compounds (Keen and Yoshikawa 1983; Van Loon et al. 1998). The state of pathogen-induced SAR is characterised by the concomitant activation of a set of PR genes. In SAR-expressing plants, PR-gene products accumulated systemically to levels from 0.3 % to 1 % of the total mRNA and protein contents (Lawton et al. 1995). Although some PRs possess anti-microbial activity, a relationship between accumulation of PRs and the broad-spectrum

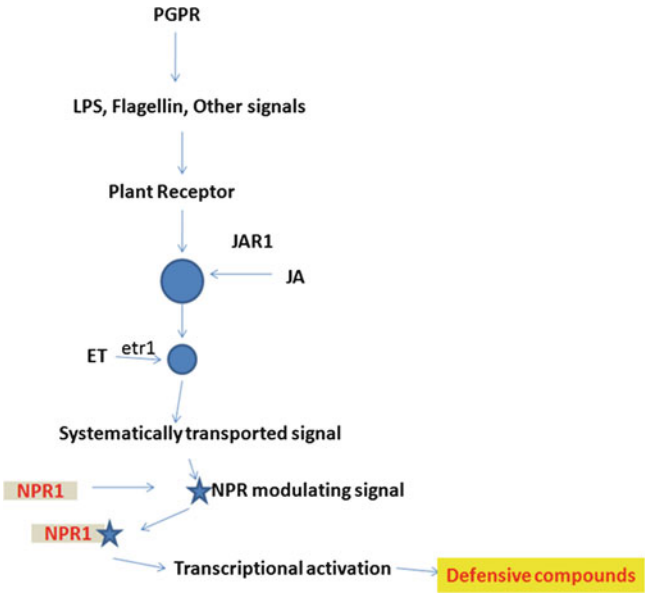

Fig. 15.2 Schematic model of PGPR-mediated pathway for production of defensive compounds in plants

resistance characteristic of SAR has never been convincingly demonstrated (Van Loon 1997) (Fig. 15.2).

Of many defence-related genes tested in Arabidopsis (e.g. the SA-inducible genes PR-1, PR-2 and PR-5 and the ethylene- and/or JA-inducible genes Hel, ChiB, Pdf1.2, Atvsp, Lox1, Lox2 and Pal1), none were found to be up-regulated in plants expressing ISR (Van Wees et al. 1999). Moreover, neither standard differential screening of a cDNA library of WCS417r-induced plants, nor 2D-gel analysis of proteins from induced and non-induced plants yielded significant differences (Van Wees et al. 1999). Thus, in contrast to SAR, the onset of ISR is not associated with major changes in gene expression. Nevertheless, ISR-expressing plants are clearly more resistant to different types of pathogens. Therefore, plants must possess as yet undiscovered defence-related gene products that contribute to broad-spectrum disease resistance.

15.4.5 PGPR-Mediated ISR: Molecular Approach

In general, induced resistance can be triggered in three ways: (1) by a predisposing infection with a necrotising pathogen (Ross 1961a, b; Kuc 1982); (2) by treatment with certain chemicals, such as salicylic acid (White 1979; Malamy and Klessig 1992) and dichloroisonicotinic acid (Metraux et al. 1991) or (3) by colonisation of

the rhizosphere with selected PGPR (Alstrom 1991; van Peer et al. 1991; Wei et al. 1991). Selected PGPR, mainly fluorescent *Pseudomonas* spp, have been demonstrated to control plant diseases effectively by suppressing pathogens and deleterious microorganisms through siderophore-mediated competition for iron, or antibiosis (Thomas et al. 2004; Thomashow and Weller 1995).

The studies related on mechanisms of biological control by PGPR revealed that some PGPR strains protect plants against pathogen infection through induction of systemic resistance, without provoking any symptoms themselves. Recently, a flagellin receptor of *Arabidopsis* was characterised as a receptor kinase sharing structural and functional homology with known plant resistance genes (Gomez-Gomez and Boller 2000). Alstrom (1991) demonstrated *P. fluorescens*-mediated ISR in bean against halo blight caused by *P syringae pv phaseolicola*, van Peer et al. (1991) in carnation against *Fusarium* wilt and Wei et al. (1991) in cucumber against *Colletotrichum orbiculare* infection. Maurhofer et al. (1994) showed that ISR induced by strain CHAO of *P. fluorescens* in tobacco against TNV was accompanied by an increase in PR protein accumulation, suggesting that PGPR-mediated ISR and pathogen-induced SAR are manifestations of a similar defence mechanism. However, Hoffland et al. (1995) were unable to establish an accumulation of PR proteins in radish displaying substantial ISR against *Fusarium oxysporum* when plants were treated with strain WCS417r of *P. fluorescens*. Therefore, it is unclear whether PGPR-mediated ISR and pathogen-induced SAR share a common signal transduction pathway. With the goal of addressing whether a common pathway is shared, two bioassays for PGPR-mediated ISR were developed by using Arabidopsis as the host plant and a rifampicin-resistant mutant of the non-pathogenic, root-colonising PGPR strain WCS417 of *P. fluorescens* (*P. fluorescens* WCS417r) as an inducer. *P. fluorescens* WCS417 is an effective biocontrol agent of the take-all disease in wheat caused by *Gaeumannomyces graminis* pv tritici (Lamers et al. 1988) and has been demonstrated to be a strong inducer of ISR against vascular wilt caused by *F. oxysporum* in carnation and radish (van Peer et al. 1991; Leeman et al. 1995). It has been proved that, in contrast to classic SAR, induction of *P. fluorescens* WCS417r-mediated ISR is independent of both endogenous SA accumulation and PR gene activation.

Ward et al. (1991) found a set of plant genes expressed during the onset of SAR in tobacco; they have pronounced those genes as SAR markers which consist of at least nine families comprising acidic forms of PR-1 (PR-1a, PR-1b and PR-1c), β-1,3-glucanase (PR-2a, PR-2b and PR-2c) resistance, class II chitinase (PR-3a and PR-3b, also called PR-Q), hevein-like protein (PR-4a and PR-4b), thaumatin-like protein (PR-5a and PR-5b), acidic and basic isoforms of class III chitinase, an extracellular β-1,3-glucanase (PR-Q) and the basic isoform of PR-1. A basic protein family called SAR 8.2 that is induced during the onset of SAR but which shows a pattern of gene expression distinct from that of the other SAR genes has also been described (Ward et al. 1991). In Arabidopsis, the SAR marker genes are PR-1, PR-2 and PR-5 (Uknes et al. 1992). The genes encoding these SAR marker proteins have been cloned and characterised and have been used extensively to evaluate the onset of SAR (Ward et al. 1991; Uknes et al. 1992). In order to identify genes associated with

PGPR-induced systemic resistance, a number of microarray-based study have been performed (Cartieaux et al. 2003, 2008; Verhagen et al. 2004; Wang et al. 2005).

15.5 Conclusion

The nature has provided us with PGPR which are becoming a powerful weapon for the chemical-free protection of crops from pathogens. It is an eco-friendly strategy for crop protection against plant pathogens. Among the many defence mechanisms, the induction of resistance in plants (ISR) through the application of PGPR seems to have transgressed boundaries or limitations to any particular groups of pathogens, e.g. it is effective against a broad range of pathogens of viz., bacterial, viral, nematodes, arthropods, etc. Experiments have also shown that a consortia of PGPR strains play a synergistic role in the induction of resistance. In conclusion, the exploration for more non-pathogenic strains with plant defence/resistance inducing capacity needs to be promoted. The other major challenges in the research on induced resistance are to identify signalling components from the ISR and SAR pathway that confer this specificity in NPR1-dependent defence gene activation.

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