

## MOLECULAR MECHANISM OF DISEASE RESISTANCE IN PLANTS: A REVIEW

SAHNI, S.,<sup>1</sup> PRASAD, B. D.,<sup>2</sup> KUMAR, G.<sup>1</sup> AND RANJAN, T.<sup>2</sup>

<sup>1</sup>Department of Plant Pathology, T.C.A., Dholi, Dr. Rajendra Central Agricultural University, Pusa (Bihar);

<sup>2</sup>Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur (Bihar). E. mail: [dev.bishnu@gmail.com](mailto:dev.bishnu@gmail.com). (829) 806-2075

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**Abstract:** *Plants are extremely important sources of food or energy for human beings. For this reason, reducing the pre- and post-harvest crop loss due to numerous diseases constitutes one of the most serious challenges for catching up with the nutritional needs of the continuously growing world population. Ever since Harold Flor, a geneticist who worked with flax plants and flax rust (a foliar disease), first outlined the gene-for-gene concept in the 1940s and 1950s, immense efforts have been made to develop low cost, environmentally-friendly approaches through engineering durable disease resistance in economically important crops instead of applying environmentally-harmful pesticides. Unfortunately, many of these attempts have failed. Until now, the control of plant disease has indeed been mainly dependent on pesticide application. Nevertheless, the development of sustainable agriculture requires better strategies for controlling plant diseases. The most promising way to generate a disease-resistant crop is most likely the manipulation of the target genes implicated in the induced resistance to pathogens or the signal transduction pathways controlling the expression of the defense-related genes.*

**Key words:** Disease resistance plants

### INTRODUCTION

Plants are constantly confronted with a wide variety of potential pathogens within their environment. Plant pathogenic microorganisms such as fungi, bacteria, phytoplasmas, viruses, and viroids cause harmful and economically important diseases in a very broad range of plant species worldwide. Damage is often sufficient to cause significant yield losses in cultivated plants. The two major effects of pathogens on agriculture are decremented production and, in a less direct way, the need of implementation of extravagant management, control procedures, and strategies. Until now, the control of plant disease has indeed been mainly dependent on pesticide application. Nevertheless, the development of sustainable agriculture requires better strategies for controlling

plant diseases. The most promising way to generate a disease-resistant crop is most likely the manipulation of the target genes implicated in the induced resistance to pathogens or the signal transduction pathways controlling the expression of the defense-related genes. Ever since Harold Flor, a geneticist who worked with flax plants and flax rust (a foliar disease), first outlined the gene-for-gene concept in the 1940s and 1950s, immense efforts have been made to develop low cost, environmentally-friendly approaches through engineering durable disease resistance in economically important crops instead of applying environmentally-harmful pesticides [1]. Unfortunately, many of these attempts have failed.

**Defense mechanisms in plants:** The sessile nature requires plants to adjust their metabolic processes to

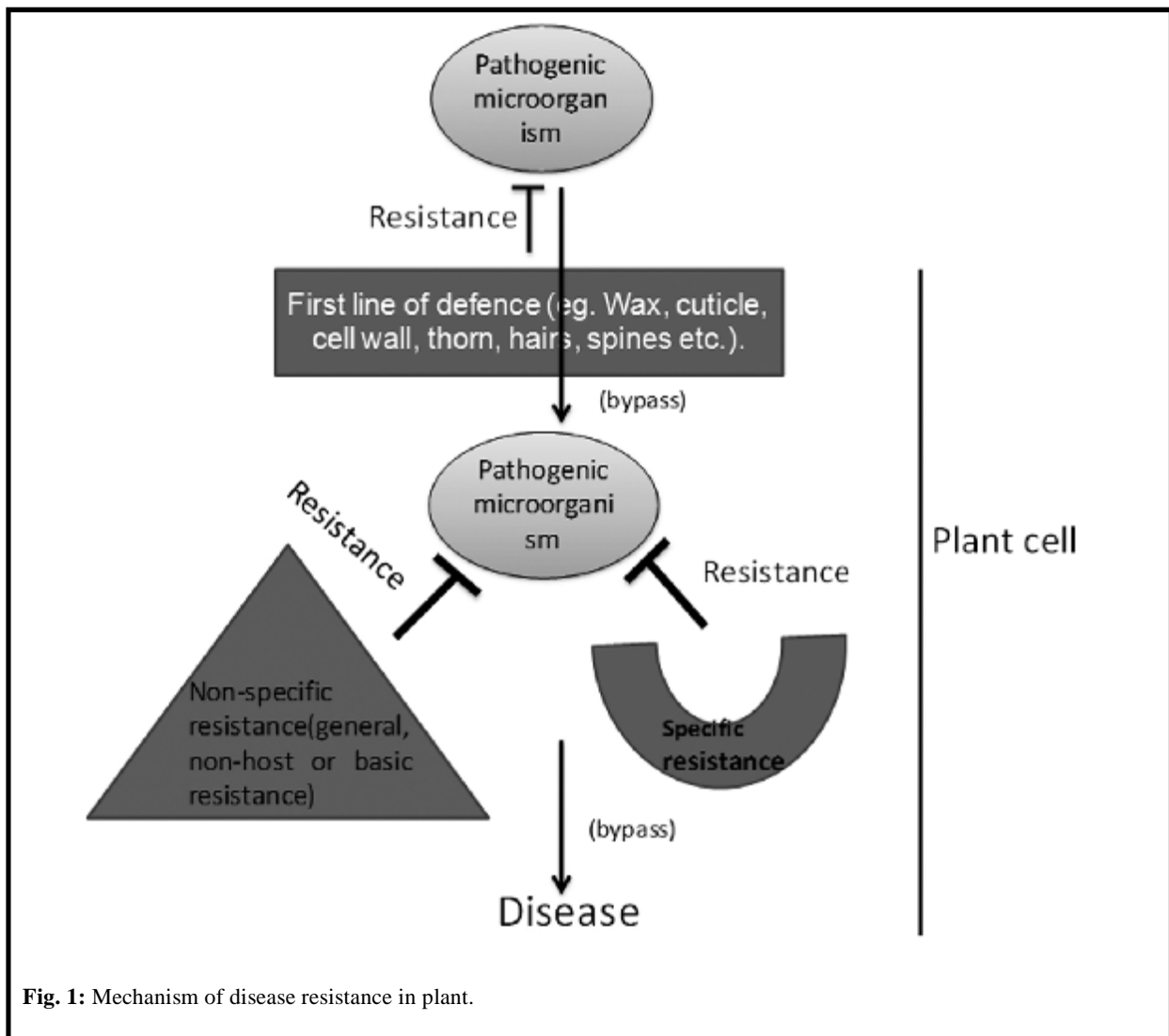
the various biotic stresses caused by intruding organisms including viruses, bacteria, fungi, nematodes, and herbivorous insects. The various defense mechanisms containing constitutive physical barriers as well as a battery of inducible defense responses must all be adapted to combat different types of intruders. Necrotrophic pathogens secrete plant cell wall-degrading enzymes (PCWDEs) and/or toxic metabolites to destroy the infected cells directly upon invasion or to produce elicitors to trigger host cell death that facilitates pathogen colonization [2,3]. In contrast, hemibiotrophic or biotrophic pathogens keep the cells in the infected tissue alive for a significant fraction of the pathogen's life cycle [2]. The utter diversity of pathogen infection or attacking mechanisms and the complexity of defense systems involving the synergism or antagonism of multiple hormone-signaling pathways against different pathogens are the consequence of the constant coevolution between plants and intruders [3].

**Plant perception systems for pathogen recognition:**

Plants have evolved multiple defense strategies including both the preformed and inducible defense systems for combating potential pathogens. To successfully infect plants, microbes must first access the plant interior either by directly penetrating the tissue surface, by entering through wounds, or through natural openings such as stomata. Once a pathogen overcomes or bypasses the preformed defense system, it has to face a two-branched innate immunity system, where the central component is non-self recognition [4,5]. The first branch is cultivar-specific, as in the gene-for-gene type of interactions, while the second nonspecifically recognizes the presence of a pathogen by those molecules common to many classes of microbes (Fig. 1).

**Gene-for-gene recognition:** The most effective and efficient way to reduce disease losses in crops is to use resistant plants or cultivators. Often, the plant disease resistance described is cultivar- or accession specific and is referred to as the gene-for-gene type of plant-pathogen interactions [6,7]. The 'Gene-for-Gene' hypothesis proposed by Flor [6] suggests that for each avirulence gene product synthesized by the pathogen, the resistance host carries a complementary, single, dominant R gene whose product recognizes the Avr product. During infection an interaction between these two components induce a defence response (Fig. 2).

A typical, visible feature of R-Avr interactions is the hypersensitive reaction (HR; rapid, localized cell death at the site of an attempted infection), which is accompanied by an oxidative burst and an increased expression of defense-related genes [e.g. pathogenesis-related (PR) genes] and is thought to restrain pathogen growth and spreading in planta [8]. Since isolation of the Pto resistance gene of the tomato with a positional cloning strategy, which confers resistance against the *P. syringae* pv. tomato bacteria expressing the Avr gene AvrPto [9], many Avr gene-specific R genes have been isolated and characterized from various species [10-13]. The majority of the R proteins contain a nucleotide binding site (NBS) and leucine-rich repeats (LRR). Such NBS-LRR R proteins have been classified into different groups according to the distinct N-terminal domains of either a coiled-coil (CC) or a TIR domain sharing similarity with the cytoplasmic domain of the *Drosophila* Toll and mammalian interleukin-1 receptor protein [14]. Of the other LRR-containing R-protein structural classes, some R genes encode proteins containing kinase or the WRKY domains such as Xa21 and RRS1-R [15, 16]. Interestingly, the Pto gene encodes a serine-threonine kinase without the extracellular LRR domain [17], which is the most common feature of all the R protein classes and thought to mediate protein-protein interactions [18]. However, genetic analysis has uncovered that Pto-mediated resistance depends on the NBS-LRR Prf protein [19,20]. The other atypical R protein is RPW8, which contains a putative N-terminal transmembrane domain and a CC domain only [21]. The RPW8 functionality requires EDS1, an R-gene signaling component [21, 22]. In contrast to the striking degree of similarity in the structural components of the R proteins, most of pathogen-derived Avr proteins show little or no homology to one another and have no functions that are deduced or experimentally defined [23]. A direct interaction between the avr products and R proteins has been demonstrated in only a few cases [16]. As a matter of fact, many Avr proteins have been shown to act as virulence factors that contribute to disease development on the susceptible hosts lacking the corresponding R gene. Clearly the simplified ligand-receptor theory for gene-for-gene interaction [6, 24] does not provide a clear explanation for all types of the R controlled disease resistance in plants. To solve this dilemma, Dangl and Jones [10] have proposed the guard hypothesis that R proteins have evolved to recognize the activities of what is

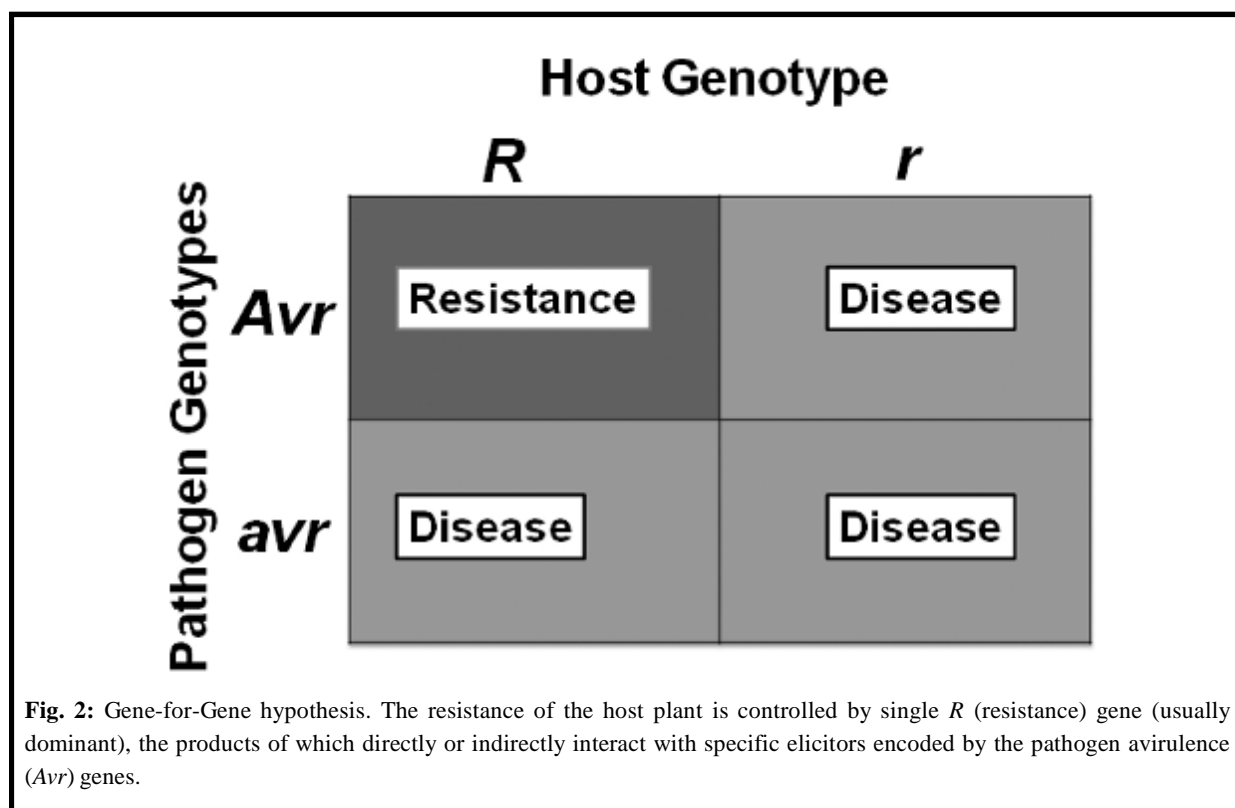


**Fig. 1:** Mechanism of disease resistance in plant.

referred to as the multiple Type III effector proteins (Avrs) instead of directly physically matching the pathogen-derived cognate Avr proteins (Fig. 3). This model proposes that the R proteins interact, or guard, a protein known as the guardee, which is the target of the Avr protein. When it detects interference with the guardee protein, it activates resistance. As a consequence, the R proteins might “guard” a set of key cellular targets of the pathogen effector proteins by detecting physiological changes in the host cells [10,25,26]. Recent biochemical evidence to support the guard hypothesis centers on the Arabidopsis RIN4 protein functioning as a general component of the host defense [4]. Two NBS-LRR R proteins, RPM1 and RPS2, have been shown to interact with RIN4 in normal living cells, respectively. The Type III effector proteins AvrRpt2, AvrRpm1 and AvrB are able to target RIN4 upon pathogen infection. The proteolytic activity of AvrPto2 causes RIN4

degradation. Furthermore, loss of RIN4 function confers the constitutive activation of the RPS2-mediated defense responses. These results together indicate that both RPM1 and RPS2 guard the same cellular target RIN4 and monitor the Avr-mediated modifications of RIN4 upon pathogen infection.

**Pathogen-associated molecular pattern recognition:** In addition to recognizing the pathogen-derived Avr-products, recent work has revealed that plants express another defense mechanism against potential pathogens through the receptor mediated recognition of highly conserved microbial structures called pathogen-associated molecular patterns (PAMPs) that often trigger a plant response in a noncultivar-specific manner [27-30]. Such conserved microbial structures including lipopolysaccharides, chitins, cellulose binding elicitor lectins, the necrosis-inducing protein NPP1, flagellin, harpin (hrpZ), the



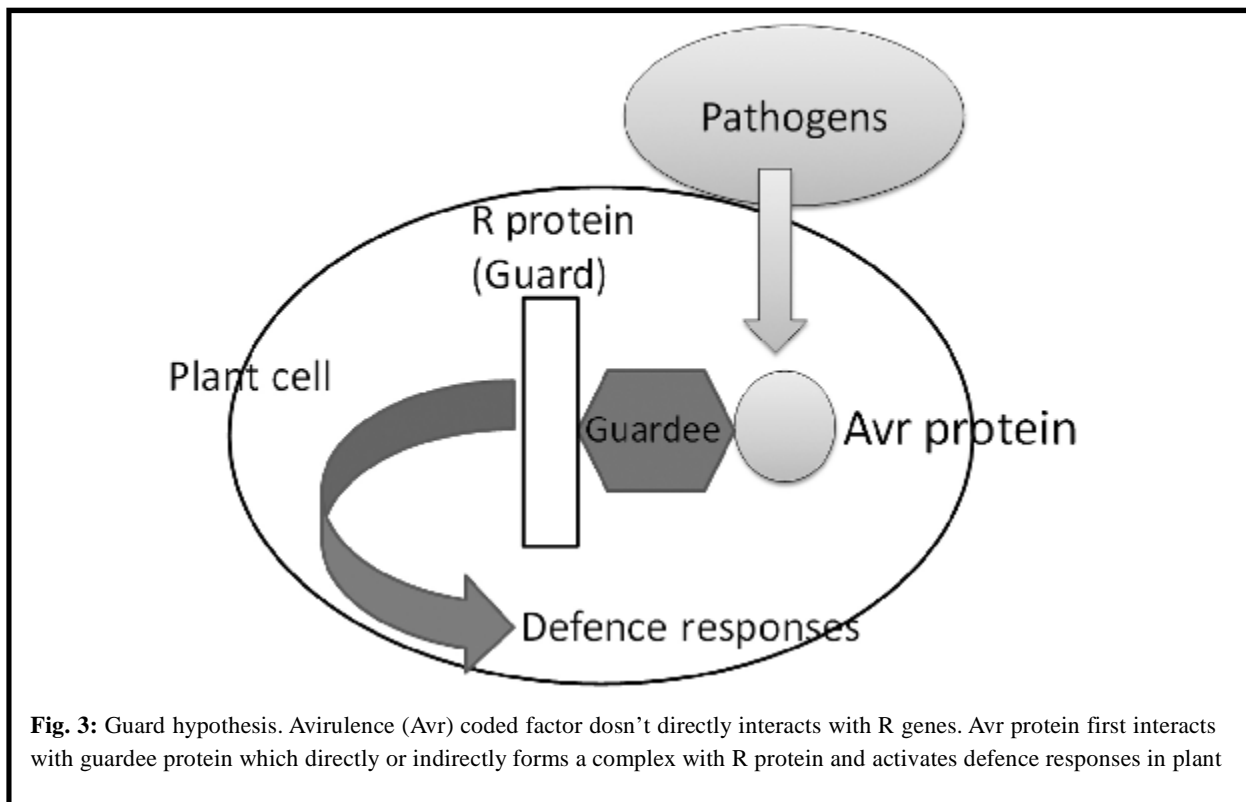
elongation factor Tu, cold-shock proteins, and many others are also classified as general elicitors of plant defense [31-33]. Some of these PAMPs are only perceived by a narrow range of plant species, whereas others trigger defense responses in many species. In addition, some plant-derived molecules can also act as general elicitors, such as oligosaccharides and glycopeptides released by the action of PCWDEs from attacking phytopathogenic microorganisms [34,35].

Like mammals, plants have evolved plasma-membrane-localized pattern recognition receptors (PRRs) and these function to recognize certain PAMPs [3]. For example, the Arabidopsis genome contains more than 400 receptor-like kinases (RLKs), 235 of which carry a LRR domain and are designated LRR-RLKs. A significant number of these putative transmembrane receptor kinases with an extracellular domain are assumed to be involved in PAMP perception [36]. The PRR activation triggers signaling events including the rapid alteration of cytoplasmic  $Ca^{2+}$  levels, the generation of ROS and NO, and the activation of post-transcriptionally regulated mitogen-activated protein kinase (MAPK). These signaling events lead to the upregulation of numerous genes encoding transcription factors, hormone-related proteins, RLKs, phosphatases, proteins involved in

protein degradation, and defense-related proteins associated with cell-wall reorganization [33,37]. The PAMP-mediated non-self recognition and signal transduction is assumed to activate the first line of inducible defense responses. This defense may eventually stop the attempted invasion of pathogens [3,36]. To advance our understanding of the PAMP-triggered defense responses, the most studied example is the perception of flagellin flg22, which is a conserved 22 amino acid (aa) peptide of the protein subunit of bacterial flagella, which are required for bacterial motility [29].

**Plant disease resistance:** Although plants do not have the benefit of a circulating antibody system, the existence of the preformed physical or chemical obstacles and the evolution of the plant immune response have culminated in a highly effective defense system that is able to resist potential attack by potential invaders [5]. The former mechanisms are in place irrespective of whether or not the plant tissue is challenged by microbes, whereas the latter are activated in response to a pathogen attack. Following pathogen or elicitor recognition, systemic signals emanating from the local sites of infection are responsible for the systemic responses.

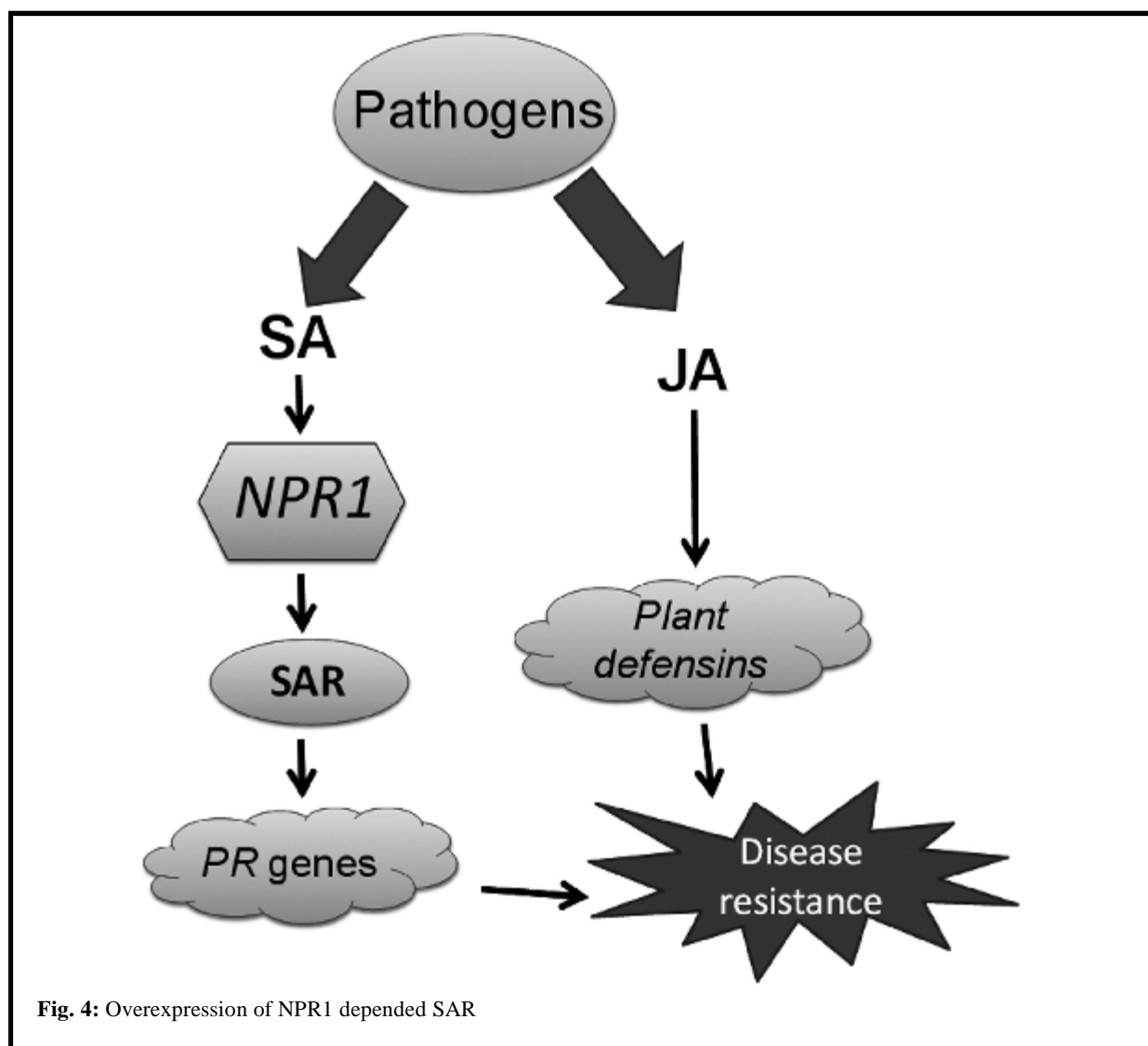
**Non-host Resistance:** Non-host resistance defines



the nonspecific resistance against all members of a given pathogen species throughout an entire plant species [38,39]. This type of resistance is the most common and durable form of plant resistance to disease causing organisms [38,40,41] and classified into Type I without visible symptoms and Type II related to the HR often resulting from PAMP-induced defense responses [39]. A pathogen that cannot cause disease on a nonhost plant is referred to as a nonhost or heterologous pathogen. Nonhost resistance, which is also referred to as heterologous plant-microbe interactions or basic incompatibility, comprises a variety of distinct mechanisms, of which some are preformed and others are inducible [4,10].

The metabolites and their derivatives may be constitutively present in healthy plants or alternatively undergo enzyme-catalyzed transformations in response to a pathogen attack [42]. In many cases, the preformed structural or chemical barriers effectively halt pathogen colonization or the establishment of infection structures following an attempted attack by nonhost pathogens. However, when nonhost pathogens or their elicitors enter the apoplast of plant cells by bypassing or circumventing constitutive obstacles, the plants immediately initiate a PAMP induced defense referred to as basal resistance. The basal defense responses activated

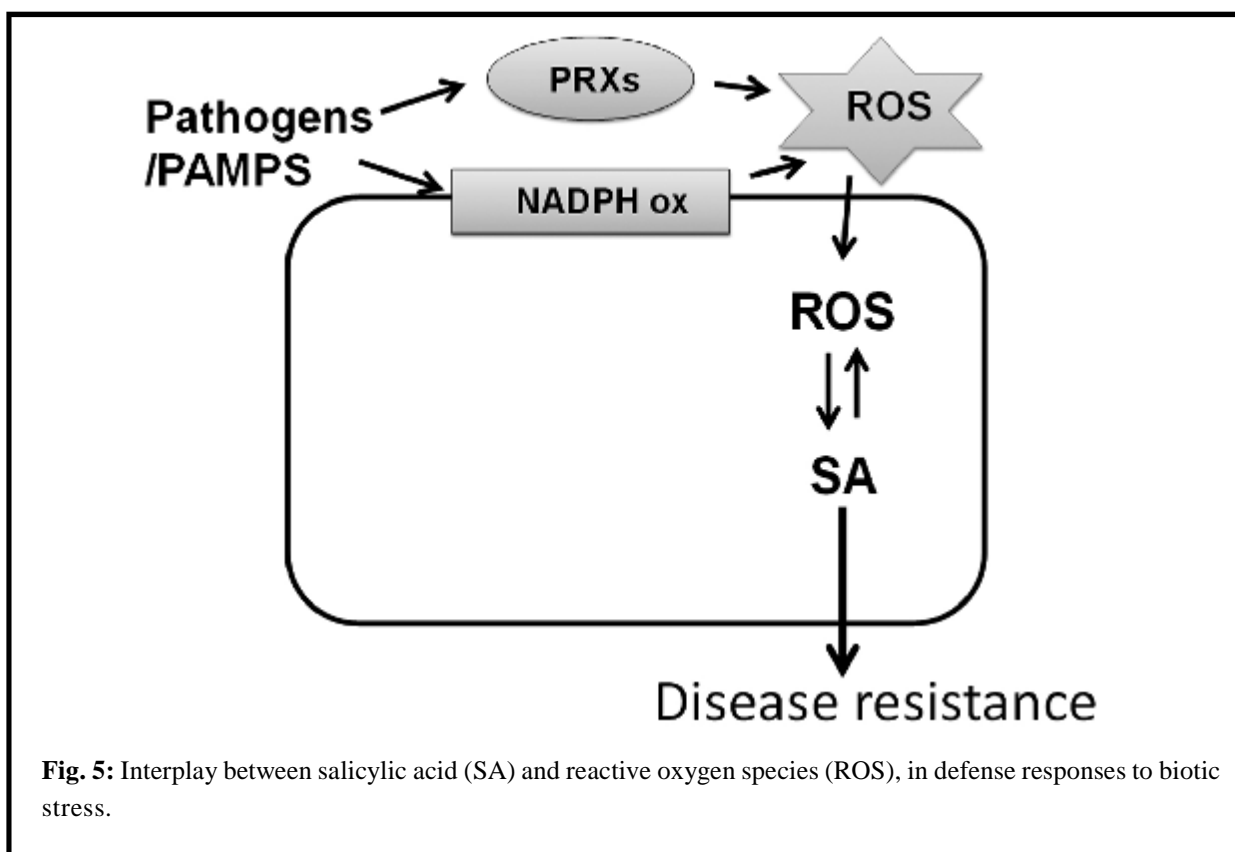
during basic incompatible interactions are often sufficient to restrict the invasion or growth of nonhost pathogens [4,43]. The systemic protection against subsequent infection with virulent pathogens can be obtained through infiltration of PAMPs such as HrpZ and flg22 into plants [35,36], indicating that the PAMP-based recognition events might not only trigger local defense responses, but also potentiate systemic defense responses in the natural environment. In contrast to the considerable progress made in understanding host resistance, it is genetically ill-defined as to why a particular plant species is typically resistant to potential pathogens that successfully infect other plant species [38,40]. Yet, a recent series of mutational analysis revealed that several genes such as PAD3, EDS1 and NHO1 are required for a nonhost resistance against nonhost pathogens. The nonhost resistance of Arabidopsis to the necrotrophic pathogen *Alternaria brassicicola* is compromised in the phytoalexin-deficient mutant pad3-1 [44]. PAD3 encodes a putative cytochrome P450 monooxygenase required for the biosynthesis of camalexin, demonstrating an important role for the inducible production of the antimicrobial compounds in plant species resistance to one specific necrotrophic fungus. A combination of the loss of actin cytoskeleton function and EDS1 activity resulted in a severe loss of nonhost resistance in Arabidopsis against the



heterologous fungal pathogen wheat powdery mildew *Blumeria graminis* f. sp. *tritici*. The Arabidopsis NHO1, which encodes a glycerol kinase, is required for resistance against the nonhost pathogens *Botrytis cinerea* and *P. syringae* isolates. Like *eds1*, *nh1* mutation also compromises gene-for-gene resistance mediated by various R genes. These observations suggest that nonhost resistance and host resistance might share a common pathway. In addition, nonhost resistance against fungal pathogens is associated with the penetration process [38]. The isolation and functional characterization of several PEN mutants [36,38] provides a mechanistic link between the nonhost and basal penetration resistance at the plant cell wall. Numerous gram negative bacterial pathogens deliver virulence factors (also referred to as effector proteins) directly into the plant cells via the Type III secretion system (TTSS). Some

pathogen species may secrete the exoenzymes involved in degrading plant cell walls via the Type II secretion system or produce toxins. Such pathogens render plants susceptible to disease and are considered homologous pathogens. Furthermore, these plants turn out to be hosts sharing a basic compatibility with a homologous pathogen [36]. The basal resistance triggered by PAMP in susceptible hosts is insufficient to stop a pathogen infection. It is believed that a strong, selective pressure on host plants posed by virulent pathogens has ultimately resulted in the coevolution of plant R genes. Correspondingly, R proteins directly or indirectly recognize strain- or race-specific effectors and allow for the establishment of a plant cultivar specific disease resistance [14,36].

**Host resistance:** Cultivar resistance is restricted



**Fig. 5:** Interplay between salicylic acid (SA) and reactive oxygen species (ROS), in defense responses to biotic stress.

to a particular pathogen species and is often referred to as a host resistance, which is tightly associated with the gene-for-gene recognition and accompanied by the HR [10,14,39]. When a plant is resistant, the interaction is then called incompatible, and when a plant is susceptible, the interaction is called compatible. Since R genes can be manipulated by plant breeders to raise the resistance in normally susceptible cultivars, the host resistance has been extensively studied for decades. This host resistance consists of the local resistance at the site of infection and the systemic acquired resistance (SAR) in the distal, noninoculated parts of plants following an activation of a local resistance [45]. Local resistance has been associated with a number of biochemical and physiological features. These include the rapid induction of the ion fluxes of  $H^+$ ,  $K^+$ ,  $Cl^-$ , and  $Ca^{2+}$  across the plasma membrane, protein phosphorylation or dephosphorylation, oxidative burst, deposition of callose and lignin, biosynthesis of proteins involved in the production of signal molecules such as ET, JA and SA as well as the accumulation of PR proteins and protective secondary metabolites [46]. The local HR, the most recognizable form at the site of infection, is often associated with the onset of SAR. SAR has been recognized as a typical response to

plant pathogen infection for almost 100 years. Currently, SAR refers to a distinct, integrated set of signal transduction pathways, which is triggered by a local pathogen challenge. This is also associated with the activation of many plant genes that ultimately makes the plant not only locally, but also systemically, more refractory to subsequent infections by a wide variety of unrelated pathogens [45,47]. When the SAR is activated, a normally compatible plant-pathogen interaction can be converted into an incompatible one [48]. Conversely, when the SAR is incapacitated, a normally incompatible interaction becomes compatible [48]. SAR can be distinguished from other disease-resistance responses by the spectrum of pathogen protection [45]. The induction of what is referred to as the SAR-marker genes is tightly correlated with the onset of the SAR in an uninfected tissue [49]. Over the past decade, considerable effort has led to the identification of the several components with distinct properties involved in the establishment of SAR [47]. The requirement for SA in SAR was shown using transgenic plants expressing the NahG gene [50]. This gene encodes a salicylate hydroxylase degrading the SA to catechol, the SA-insensitive *npr1* mutants [50], and the SA induction-deficient mutants *sid1* and

sid2 [51]. SA was originally thought to be the mobile transducer of SAR [45]. However, results obtained from the detachment experiments on *P. syringae* infected cucumber leaves and the grafting experiments on tobacco plants indicate that SA does not appear to function as the systemically transported signal [52]. Recently, the genetic characterization of Arabidopsis defective in induced resistance1-1 (*dir1-1*) suggests that an essential mobile signal during SAR is a lipid-based molecule rather than SA [53]. The *dir1-1* mutant exhibits wild-type local resistance and a normal accumulation of SA in either inoculated (local) or uninoculated (systemic) leaves following pathogen infection but fails to develop SAR and to express the PR genes in systemic leaves. Importantly, *dir1-1* is deficient in the mobile signal for the SAR and the DIR1 gene product is a putative apoplastic lipid transfer protein. These observations suggest that DIR1 may interact with a lipid-based signal molecule and promote long-distance signaling during SAR [53]. Several other lines of evidence also support the hypothesis that a lipid-derived small molecule may be a mobile signal for SAR [54]. For example, the Arabidopsis EDS1 and PAD4 genes encode lipase-like proteins [55], suggesting that they may initiate the release of the lipid metabolites involved in regulating the biosynthesis or accumulation of the SA in local and systemic tissues. Intriguingly, EDS1 is required to generate the mobile signal in the local leaves and its perception in the systemic leaves. The analysis of another Arabidopsis mutant, *sfd1*, also indicates a role for a lipid-derived signal in the establishment of SAR. The SFD1 encodes a glycerol-3-phosphate dehydrogenase involved in glycerolipid biosynthesis and the mutation in this gene decreases the SA accumulation, partially blocks the PR1 expression, and compromises the SAR [56]. Additional evidence for a role of lipid signaling in the SAR comes from studies on tobacco plants [57]. For instance, the tobacco SA-binding protein SABP2 is a lipase and its lipase activity is significantly increased by the addition of SA. Conversely, silencing the SABP2 gene diminishes the SA-inducibility of the *PR-1* gene, local resistance, and the development of SAR. Besides SA, other signal molecules including ET, JA, NO and H<sub>2</sub>O<sub>2</sub>, which are originated from the local site of the attempted infection, may also be responsible for host resistance [2]. Indeed, a growing body of evidence suggests that host resistance results from a sophisticated signaling network involving crosstalk among the different signal transduction pathways [58]. In addition, the defense pathways

involved in the basal resistance and the R gene-mediated resistance are probably linked to each other [4]. A recent breakthrough in understanding the molecular mechanisms behind plant innate immunity is the discovery that the RPM1-interacting protein RIN4 is not only a convergence point for different R gene-mediated signaling pathways, but also a regulator of the PRR-mediated signaling [4].

**Induced systemic resistance:** In addition to the well-documented SAR, there is a second type of systemic resistance which is referred to as induced systemic resistance (ISR). This ISR is potentiated by some growth-promoting rhizobacteria. The best characterized of these rhizobacteria are the strains within several species of fluorescent *Pseudomonas* spp. that do not cause any visible disease symptoms to the plant's root system [59]. Although it does not involve the accumulation of the known PR proteins that are characteristic of the SAR in Arabidopsis, ISR is effective against a broad range of diseases caused by viruses, bacteria, and fungi [59]. In contrast to SAR across a wide array of the plant species, the elicitation of the ISR by specific rhizobacterial strains is restricted to certain plant species or genotypes [59]. The onset of ISR does not depend on SA but on ET and JA [62]. Interestingly, NPR1, the central regulatory protein of SAR, is required for developing ISR [52].

Furthermore, ISR and SAR can be activated simultaneously, resulting in an additive level of protection against plant pathogens. However, these molecular characterizations are based on a limited number of ISR systems. Other examples of the ISR linked to the production of SA or siderophores, therefore have more in common with the SAR [63].

**Defense signalling pathways:** In general, from the initial stage of recognition by the plant to the successful confinement or death of the pathogen, the distinct signaling pathways mediated by the small, signaling molecules, such as SA, JA, ethylene (ET), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), nitric oxide (NO), and abscisic acid (ABA), constitute the complex signal transduction network controlling plant defense and thereby endowing the plant with a more sophisticated capacity for the highly complex, multifaceted defense response [59]. The relative contribution of such signaling molecules to an inducible defense depends on the particular intruder [2]. Furthermore, a growing body of evidence regarding cellular signaling



transduction and the regulation of expression of defense-related genes suggests that the defense signaling pathways do not function in a linear, independent fashion. Instead, each pathway can influence other pathways through positive or negative regulatory interactions [58].

#### **NPR1-dependent SA signaling in plant defense:**

The Arabidopsis genetic screens based on *PR* gene expression or disease resistance in response to SA led to identifying the multiple alleles of a single gene, NPR1 [50], suggesting that this gene is an essential, positive regulator of the SAR response. The overexpression of NPR1 enhances disease resistance to various pathogens but does not constitutively activate the expression of the SAR markers, indicating that the activation of the NPR1 protein is a prerequisite for the establishment of SAR (Fig. 4). The NPR1 homologs have also been identified in many other plant species such as rice and tobacco [64], suggesting that NPR1 function is conserved across the plant kingdom. The NPR1 protein has two protein-protein interaction domains, an ankyrin-repeat and a BTB/POZ domain, as well as a putative nuclear localization signal and phosphorylation sites [65]. Application of SA or its analogs stimulates the translocation of the NPR1 into the nucleus, which is required for the activation of downstream signaling. Changes in the cellular redox status after a pathogen infection or SA treatment play a key role in this regulation. The NPR1 is present as a cytosolic oligomer in the uninduced state. Upon SAR induction, the reduced monomeric NPR1 protein accumulates in the nucleus and physically interacts with members of the transcription factor TGA family to activate the expression of the *PR* genes and the protein secretory pathway genes essential for the SAR [66]. In addition to the NPR1-mediated enhancement of transcriptional activator(s) described above, the NPR1 may also exert its function by removing a negative regulator(s) or inhibiting transcriptional repressor(s) during SAR. The screens for suppressors of the *npr1* led to the identification of the Arabidopsis recessive *sni1* (suppressor of *npr1* inducible) mutant, which restores the inducible *PR* gene expression and pathogen resistance in the *npr1-1* background [67]. The lack of SAR induction in the *SNI1/npr1* plants and the restoration of SAR in the *sni1/npr1* double mutant suggest that the wild-type *SNI1* protein is a negative regulator of the *PR* gene expression and SAR and that the role of the NPR1 in SAR is probably to remove the *SNI1* repression [67]. *SNI* contains

no DNA-binding domain and presumably interacts with the WRKY factor(s) to repress the transcription of the *PR* genes [47].

#### **Roles of reactive oxygen species (ROS) in plant defense:**

The ROS consist of hydrogen peroxide ( $H_2O_2$ ), superoxide ( $O_2^{\bullet-}$ ), hydroxyl radical ( $OH^{\bullet}$ ), perhydroxyl radical ( $O_2H^{\bullet}$ ) and singlet oxygen ( $^1O_2$ ), which are generated endogenously during certain developmental transitions such as seed maturation and as a result of photosynthetic and respiratory metabolism [68]. Under unstressed conditions, the ROS are rapidly removed by both enzymatic and nonenzymatic scavenging systems confined to various cell compartments and thus plant cells maintain a normal redox homeostasis [68]. Any circumstance in which the cellular redox homeostasis is disrupted can lead to the accelerated generation of ROS or oxidative stress [8,68]. However, charged  $O_2^{\bullet-}$  is impermeable through phospholipid membranes and relatively nontoxic against biological macromolecules. At physiological pH, the  $O_2^{\bullet-}$  disproportionates to  $H_2O_2$ , a relatively stable form of ROS, and  $O_2$ , either spontaneously or by the action of superoxide dismutases. The  $H_2O_2$  has the ability to pass through membranes and hence to reach cellular components distant from the initial sites of its generation [8]. Since Doke [69] first reported the generation of  $O_2^{\bullet-}$  during incompatible interactions between the potato and the late blight pathogen *Phytophthora infestans*, an accumulation of ROS (mainly  $O_2^{\bullet-}$  and  $H_2O_2$ ) has been associated with both a local and a systemic defense response in the host-pathogen interactions [70]. The bi-phasic production of apoplastic ROS, which is the oxidative burst during the incompatible interactions, is a central feature in successfully recognizing plant pathogens [8]. Increasing the endogenous levels of  $H_2O_2$  either by overexpressing GOX in potato and rice [71] or GTP-binding protein OsRac1 in rice are suggestive of a link between elevated levels of  $H_2O_2$ , cell death and disease resistance. Apoplastic  $H_2O_2$  generation can be mediated by cell-wall peroxidases, germin-like oxalate oxidases or by amino oxidases under abiotic and biotic stresses [68]. However, genetic studies indicate that the membrane bound NADPH oxidases are the main source of the pathogen-driven production of ROS in the defense responses [72]. Several plant defense roles have been proposed for ROS. For instance,  $H_2O_2$  may be directly toxic to pathogens in its ability to give rise to the extremely reactive  $OH^{\bullet}$  in the presence of iron. Alternatively,  $H_2O_2$  may contribute

to the structural reinforcement of the plant cell walls either by cross-linking the various cell wall proteins or by increasing the rate of lignin polymer formation, leading to preventing the microbes from penetrating or containing the microbial spreading [8]. In addition, the ROS have been proposed to act as signaling molecules for the induction of defense-related genes [73].

**ROS-mediated signaling in plant defense:** How the ROS are integrated into the signaling network of the plant defense responses is largely unknown, but some details of several key players implicated in the ROS signal transduction pathways have been elucidated. For example, the  $\text{Ca}^{2+}$  fluxes appear to function not only in the induction of the oxidative burst after pathogen infection but also in the downstream of the ROS production by the activation of the plant NADPH oxidase [72]. The  $\text{Ca}^{2+}$  binding motifs presented in all plant Rboh (Respiratory burst oxidase homolog) gene products could account for the direct regulation of the NADPH oxidase by  $\text{Ca}^{2+}$ . Recently, a central role of the MAPKs in the onset of the plant pathogen defense has been firmly established [46,73].

The ROS modulate the expression of the numerous genes including those encoding the antioxidant enzymes and regulatory proteins involved in the  $\text{H}_2\text{O}_2$  production [74]. However, specific ROS-regulatory DNA sequences and their cognate transcription factors have not yet been isolated and characterized. In general, three modes of action have been proposed as to how the ROS signaling could affect gene expression [75]. The ROS sensors, which are supposed to be unidentified receptor proteins, could trigger specific signaling cascades. Alternatively, ROS might directly inhibit the activity of phosphatases and result in the activation of particular kinases, thus triggering the downstream signaling events. Finally, ROS might change gene expression via targeting and modifying the activity of the redox-sensitive transcription factors or regulatory proteins.

**Crosstalk between SA and ROS signaling pathways:** The coordination of SA and ROS signaling pathways has been documented in a variety of experimental systems. The enhancement of the SA signal can occur through a signal amplification loop involving ROS, where SA binds  $\text{H}_2\text{O}_2$  scavenging enzymes such as catalases and ascorbate peroxidases and thereby inhibits their activities [76].

This suggests that elevated levels of  $\text{H}_2\text{O}_2$  may function upstream of SA to trigger defense responses. On the other hand, SA has also been shown to potentiate the production of  $\text{H}_2\text{O}_2$  and HR cell death. The addition of low concentrations of SA in soybean cells that have been inoculated with pathogens dramatically enhances the oxidative burst and cell death, indicating that the accumulation of low levels of SA together with the development of oxidative microbursts could amplify responses to secondary infections and contribute to SAR [77]. Although  $\text{H}_2\text{O}_2$  is a poor inducer for the PR gene expression, combined applications of  $\text{H}_2\text{O}_2$  and SA boost the PR-1a expression and provide a greater protection of tobacco against subsequent infection by the wildfire pathogen, *P. syringae* pv. *tabaci*, than treatments with SA alone would provide. These examples support the synergism between  $\text{H}_2\text{O}_2$ - and SA-mediated defense pathways. Fig. 5 interplay between salicylic acid (SA) and reactive oxygen species (ROS), in defense responses to biotic stress.

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