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Review-

SYNTHETIC PLANT ACTIVATORS FOR CROP DISEASE MANAGEMENT

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ABSTRACT:

In a resistant cultivar, the defense system of the host is activated properly in time and to a sufficient magnitude necessary to suppress the advancement of the pathogen resulting in decreased or no disease. Whereas in a susceptible cultivar, in spite of the presence of general defense genes, are unable to block the advancement of the pathogen and disease development, since these are activated either late or their magnitude of expression is of a lower amount. However, with the use of certain biotic or abiotic defense inducers even susceptible cultivars can be made resistant by activating their disease defense response system. Plant activators are chemicals that activate the defense genes in plants by providing signals via the signal transduction pathway mediated by salicylic acid. Since plant activators do not have any pesticidal or antibiotic activity, their adverse effects on human health and environment are minimal. In addition, since they do not interact directly with the pathogens, it is unlikely that plant pathogens will develop resistance to these chemicals. Among the plant activators, important ones are acibenzolar-S-methyl, 2, 6-dichloroisonicotinic acid, β-aminobutyric acid, probenazole, salicylic acid, riboflavin, prohexadione- Ca, potassium phosphonate, harpin and methyl jasmonate. The success of defense inducers for plant disease control depends on our ability to manage their phytotoxicity either by chemical modification of the compound or by modifying their formulation. Since, plant activators would never be able to provide complete protection; they could be more suited as a component of integrated disease management.

KEYWORDS: Plant activators, Systemic acquired resistance, Signal transduction, Induced systemic resistance, Defense response.

INTRODUCTION:

In nature plants survive in the face of attack by many microbes that threaten their survival. Still most of them are rendered harmless due to passive as well as active defense barriers employed by the plant. In a resistant cultivar, defense system of host is activated properly in time and to a sufficient magnitude necessary to suppress the advancement of the pathogen resulting in decreased or no disease. Susceptible cultivars, in spite of the presence of general defense genes, are unable to block the advancement of the pathogen and disease development, since these are activated either late or their magnitude of expression is of a lower amount. However, with the use of certain biotic or abiotic defense inducers even susceptible cultivars can be made resistant by activating their disease defense response system.

Plant activators:

Plant activators are chemicals that activate the defense genes in plants by providing signals via the signal transduction pathway mediated by salicylic acid (Vidhyasekharan 2004). A chemical will be considered as a 'plant activator' only if, neither the agent nor its metabolites have direct antifungal/antibacterial activity *in vitro* or *in planta*. The agent should modify the plant – pathogen interaction so that it resembles phenotypically an incompatible interaction, which include defense related mechanism prior to or after challenge and the agent should protect a plant against broad spectrum of pathogens.

Plant activators render plants resistance to a wide spectrum of pathogens by activating systemic acquired resistance (SAR). Since plant activators do not have pesticidal or antibiotic activity, their adverse effects on human health and environment are minimal. In addition, since they do not interact directly with the pathogens, it is unlikely that plant pathogens will develop resistance to these chemicals (Huang and Hsu 2003).

Genetic basis of induced disease resistance:

The natural resistance of plants to pathogens and herbivorous insects is based on the combined effects of preformed barriers and induced mechanisms. In both cases, plants use physical and antimicrobial defenses against the invaders. In contrast to constitutive resistance, induced resistance relies on recognition of an invader and subsequent signal transduction events leading to the activation of defenses. In many cases, localized infection by pathogens induces resistance directed at a broad spectrum of widely different pathogens such as fungi, bacteria or viruses. This resistance is expressed locally at the site of pathogen attack and systemically, in uninfected parts of the plant. The defense mechanisms involved include a combination of physical changes such as cell wall lignification, papilla formation or the induction of various pathogenesis-related proteins (PRs) (Van – Loon and Van – Strein, 1999). Systemic acquired resistance implies the production by the plant of one or several translocated signals that are involved in the

activation of resistance mechanisms in uninfected parts (Alvarez *et al.*1998). Thus, a first infection predisposes the plant to resist further attacks. Recently, a number of reports have indicated that plant growth promoting rhizobacteria (PGPR) can induce systemic acquired resistance that operates independently of SA (Pieterse *et al*. 1998). The nature of the systemic signal involved in PGPR-induced resistance is not known, but it does not require SA. To distinguish systemic SA-dependent defenses resulting from pathogen pretreatments (or pretreatments with SA or SA like compounds) from other systemic responses that operate without SA, the former reactions are termed as systemic acquired resistance (SAR) and the latter reactions as induced systemic resistance (ISR).

Signal transduction for systemic acquired resistance:

The first step in the development of SAR is the recognition of pathogen infection by a plant. Once the plant reacts to the pathogen, signals are released that trigger resistance in adjacent as well as distant tissues (Figure 1). Importantly, not all plant pathogen interactions lead to SAR induction. Compatible interactions can lead to SAR induction; thus, the pathogen need not induce a gene-for-gene resistance reaction. SA has been proposed as one signal leading to SAR because its concentration rises dramatically after a pathogenic infection. The most compelling evidence that implicates SA as a signal in SAR comes from experiments using transgenic tobacco to express the enzyme salicylate hydroxylase, encoded by the nahG gene from *Pseudomonas putida*. This enzyme catalyzes the conversion of SA to catechol, which is not an active SA.R inducer. The NahG-expressing plants do not accumulate SA in response to pathogen infections and are unable to induce an SAR response to viral, bacterial or fungal pathogens. These experiments implicate the direct involvement of SA in SAR signaling, but they do not address whether SA is the long-distance, phloem-mobile signal for SAR (Conrath, 2006).

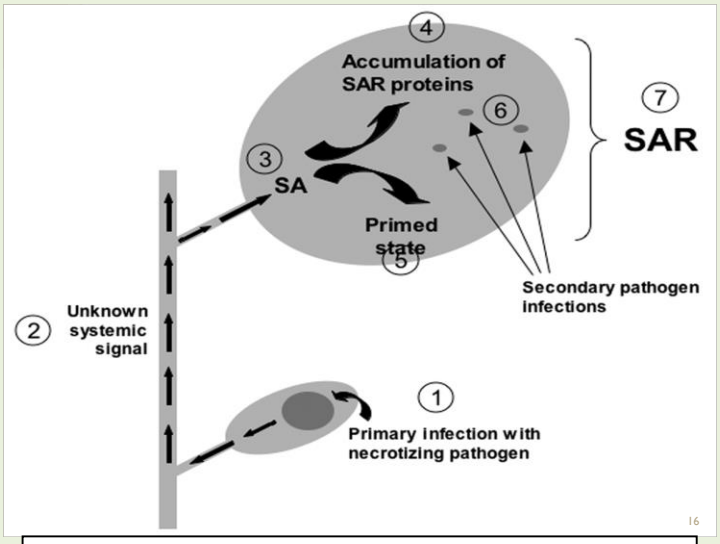

Figure. 1. Signal transduction in SAR

Biosynthesis of salicylic acid:

In higher plants SA has been proposed to be synthesized from trans-cinnamic acid to SA, via the intermediates orthocoumaric acid or BA. Such a pathway provides a link between pathogen induction of

phenylpropanoid biosynthesis and SAR signal production. The final step in SA synthesis is the conversion of BA to SA by benzoic acid 2-hydroxylase, a probable Cyt P450 enzyme. Moreover, benzoic acid 2-hydroxylase activity is induced approximately 10-fold by pathogen infection and is blocked by a protein synthesis inhibitor. Thus, one apparent pathway for in vivo SA production appears to be the conversion of trans-cinnamic acid to BA followed by ortho-hydroxylation to SA. However, this does not exclude the possibility that other pathways for the biosynthesis of SA may exist, including via iso-chorismate or even via polyketide biosynthesis, as occurs in bacteria. Once synthesized, the fate of SA in the cell is not clear. Like other phenolics in plants, SA is rapidly conjugated to an O-glucoside. The role of this conjugate is not clear, but it has been reported to be inactive as an inducer of PR-1 in tobacco. It seems likely that the conjugate may serve either as a storage form that can be hydrolyzed as needed or as an inactive form targeted for catabolism.

SAR Genes:

In tobacco and *Arabidopsis*, establishment of SAR is associated with the expression of a set of so-called SAR genes, which include some of those encoding pathogenesis related (PR) proteins. Some PR proteins have been identified as acidic β-1,3-glucanases (BGL2) and chitinases (PR-3), possibly able to hydrolyze microbial cell wall components. Therefore, the accumulation of PR proteins has often been proposed as the molecular basis for SAR. However, over the past few years it became widely appreciated that the accumulation of PR proteins does not per se explain the SAR phenomenon. For instance, cloning of PR genes and plant transformation by now have not provided a single example in which an inducible acidic glucanase or chitinase, alone or in combination, enhances resistance to fungal pathogens. Thus, the contribution of PR proteins to SAR appears to be minor.

NPR1: A key component for SAR:

Over the past decade, a variety of mutants with compromised activation of SAR have been identified. The *Arabidopsis* mutant *npr1* is probably the most prominent of these mutants. *Npr1* accumulates wild-type SA levels in response to infection with avirulent pathogens but is unable to activate PR genes, or establish the primed state, or develop biologically or chemically induced SAR. Thus NPR1 is a likely key regulator of SAR and priming. This assumption has further been supported by two studies demonstrating that constitutive over expression of NPR1 in transgenic plants did not lead to enhanced SA levels or constitutive expression of *PR* genes. Rather, these plants showed stronger *PR* gene expression after pathogen infection and they also expressed greatly enhanced disease resistance. Interestingly, *npr1* shows enhanced susceptibility to some virulent pathogens and seems to be involved also in R gene-mediated disease resistance. In addition, NPR1 seems to play a key role in the SA-independent induced systemic resistance (ISR) response. ISR is triggered by selected strains of saprophytic rhizobacteria and confers broad-spectrum disease resistance in the aerial parts of the plant. Impressively, in *Arabidopsis* activation of the NPR1-dependent ISR state is not associated with major changes in defense gene expression before

pathogen infection. Rather, a plethora of defense-related genes shows augmented expression after pathogen attack, suggesting that NPR1-dependent priming is a major mechanism also in ISR (Dong, 2004).

Induced systemic resistance (ISR):

ISR is potentiated by plant growth-promoting rhizo-bacteria (PGPR), of which the best characterized are strains within several species of *Pseudomonas* that cause no visible damage to the plant's root system. Unlike SAR, ISR does not involve the accumulation of pathogenesis-related proteins or salicylic acid but instead, relies on pathways regulated by jasmonate and ethylene. However, these molecular characterizations are based on a limited number of ISR systems. Other examples of ISR are linked to the production of siderophores or salicylic acid by PGPR strains and, therefore, have more in common with SAR. Neither the nature of the eliciting agent nor the site of elicitor action on the plant is as critical in the classification of induced resistance phenomena as the biochemical responses incited within the plant. Finally, SAR is effective across a wide array of plant species, whereas there is demonstrated specificity in the ability of PGPR strains to elicit ISR on certain plant species and genotypes (Yan *et al.* 2002).

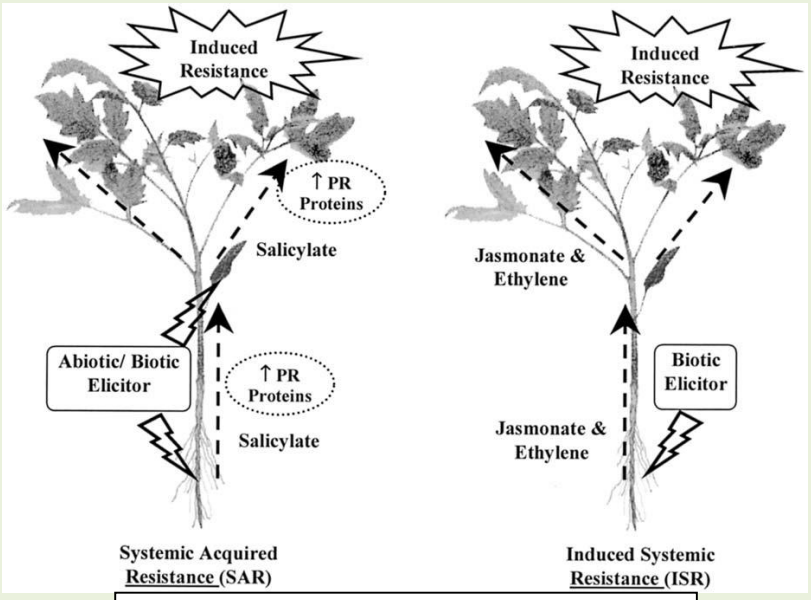

Figure. 2. Comparison of SAR and ISR

Priming – A plant's memory:

After infection by a necrotizing pathogen, colonization of the roots with certain beneficial microbes, or after treatment with various chemicals, many plants establish a unique physiological situation that is called the 'primed' state of the plant (Goellner and Conrath, 2008). In the primed condition, plants are able to 'recall' the previous infection, root colonization or chemical treatment. As a consequence, primed plants respond more rapidly and/or effectively when re-exposed to biotic or abiotic stress, a feature that is frequently associated with enhanced disease resistance. Though priming has been known as a component

of induced resistance for a long time, most progress in the understanding of the phenomenon has been made over the past few years (Conrath *et al*. 2008) (Figure 3).

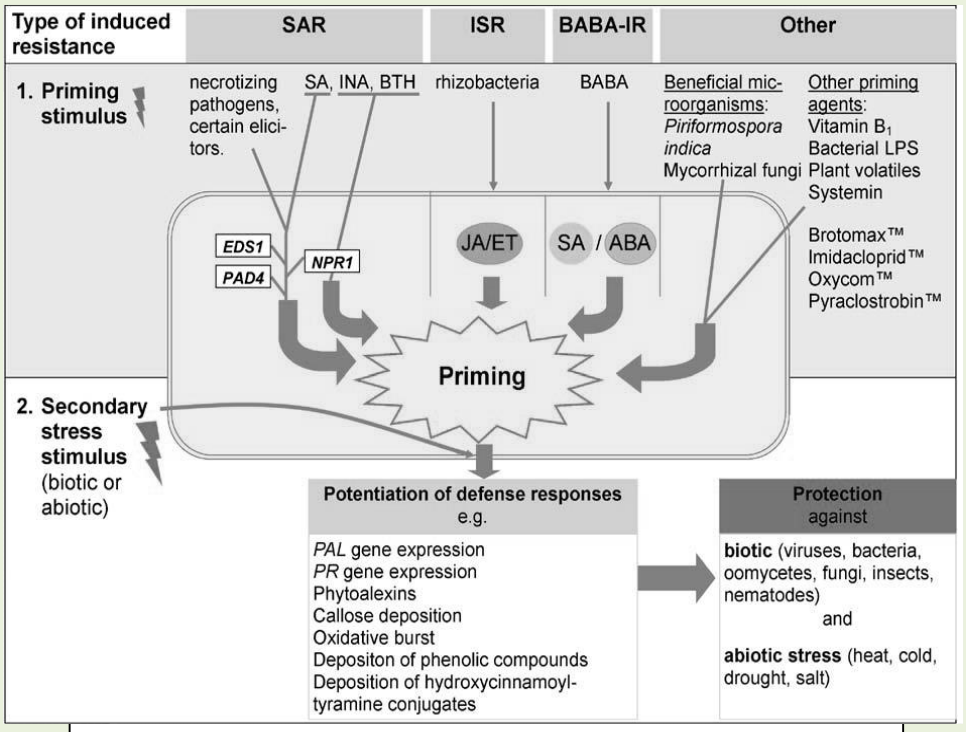

Figure 3. Events associated with induced resistance in plants

Induction of induced resistance by chemicals:

Many different organic and inorganic compounds have been shown to activate induced resistance in plants. When SA was identified as an essential endogenous signal for the SAR response, an intensive search was initiated in order to identify synthetic chemicals able to mimic SA in SAR induction. 2, 6-dichloroisonicotinic acid and its methyl ester (both are named INA) were the first synthetic compounds reported to activate the bonafide SAR response in plants (Kessmann *et al.* 1994). Later, benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) became an attractive synthetic SAR activator. SA, INA and BTH are assumed to activate SAR via the same signaling pathway. Among the plant activators, important ones are acibenzolar-S-methyl, 2,6-dichloroisonicotinic acid, β-aminobutyric acid, probenazole, salicylic acid, riboflavin, prohexadione- Ca, potassium phosphonate, harpin and methyl jasmonate.

Acibenzolar-S-Methyl:

Acibenzolar-S-methyl is chemically benzo (1,2,3) thiadiazole-7-carbothioic acid-S-methyl ester (BTH). It was developed by Syngenta Crop Protection, Inc. in USA and is marketed as Bion® and Actigard® and is effective against downy mildew of vegetable crops, bacterial spot of tomatoes and blue mold of tobacco. It acts as a substitute for salicylic acid in SAR (Gent and Schwartz, 2005; Ziadi *et al.* 2001).

Nair and Anith (2009) evaluated the influence of Acibenzolar-S-Methyl, a chemical activator, and four Plant Growth Promoting Rhizobacteria (PGPR; *Pseudomonas fluorescens* PN026R, *P. putida* 89B61,

Bacillus pumilus SE34, and *B.subtilis* GB03) on amaranth (*Amaranthus tricolor* L.) foliar blight (*Rhizoctonia solani* Kuhn) suppression. *In vitro* and *in vivo* experiments were conducted both under sterile and non-sterile soil conditions in which the PGPR and activator were tried both individually and in combination. Results indicated that PGPR induced resistance against *R. solani* in a susceptible amaranth variety, 'Arun'. A native isolate, *P. fluorescens* PN026R was particularly effective in suppressing the disease and promoting plant growth. Plants treated with PN026R showed lower disease incidence and disease severity; 67 and 35 % respectively compared to 92 and 52 % for plants inoculated with pathogen alone. Combined application of PGPR and ASM was more effective with disease incidence and disease severity of 42 and 21 % respectively (Nair *et al*. 2007). Ability of acibenzolar-S-methyl to induce resistance in pepper plants against *Xanthomonas campestris* pv. *vesicatoria* was investigated in both growth chamber and open field conditions. Growth chamber experiments showed that acibenzolar-S-methyl (300 µM) treatment protects pepper plants systemically and locally against *X. campestris* pv. *vesicatoria*. Evidence for this was a reduction in the number and diameter of bacterial spots and bacterial growth *in planta*. Systemic protection was also exerted by the acibenzolar-S-methyl acid derivative, which may be produced by hydrolysis in the plant. The efficacy of acibenzolar-S-methyl was also found in open field conditions, where both leaves and fruit were protected from the disease. The highest efficacy (about 67%) was obtained by spraying the plants 6–7 times every 8–12 days with a mixture of acibenzolar-S-methyl and copper hydroxide. Persistence and translocation data obtained from the growth chamber experiments revealed a persistence of acibenzolar-S-methyl lasting five days after treatment with rapid translocation and negligible levels of acid derivative formation. Since the protection exerted by acibenzolar-S-methyl against bacterial spot disease was observed when the inducer was completely degraded, it would appear to be due to SAR activation.

Pradhanang *et al*. (2005) investigated the chemical elicitor acibenzolar-S-methyl (ASM; Actigard 50 WG), which induces systemic acquired resistance (SAR), to determine the effect on bacterial wilt of tomato caused by *Ralstonia solanacearum* on moderately resistant cultivars under greenhouse and field conditions. In greenhouse experiments, ASM was applied as foliar spray and/or soil drench (3µg/ml) before and as foliar spray (30 µg/ml) after transplanting. The chemical elicitor was ineffective in reducing bacterial wilt incidence on susceptible tomato cultivars Equinox and FL 47 when plants were inoculated with *R. solanacearum*. However, greenhouse studies indicated that ASM significantly enhanced resistance in cultivars with moderate resistance to bacterial wilt such as Neptune and BHN 466. It appeared that ASM-mediated resistance was partially due to prevention of internal spread of *R. solanacearum* toward upper stem tissues of tomato plants. The effect of ASM on moderately resistant cultivars was consistent in field experiments conducted in 2002 and 2003 in Quincy, FL, where bacterial wilt incidence was significantly reduced in ASM-treated BHN 466 (in 2002), FL 7514 (in 2003), and Neptune (both years) plants. ASM treated BHN 466 and FL 7514 produced significantly higher tomato yield than the untreated

controls. This is the first report of ASM-mediated control of bacterial wilt under field conditions, which suggests that use of this treatment for moderately resistant genotypes may be effective for control of bacterial wilt of tomato.

Baysal *et al*. (2005) evaluated the leaves of pepper (*Capsicum anuum* L.) were inoculated with *Phytophthora capsici* 3 day after treatment with acibenzolar-S-methyl benzo [1,2,3]thiadiazole-7-carbothioic acid-S-methyl ester (ASM) and resistance to *Phytophthora* blight disease. Results showed that *P. capsici* was significantly inhibited by ASM treatment by up to 45 % *in planta*. The pepper plants responded to ASM treatments by rapid and transient induction of L-phenylalanine ammonia-lyase (PAL), increase in total phenol content and activities of chitinase and β-1,3-glucanase. No significant increases in enzyme activities were observed in water-treated control plants compared with the ASM-treated plants. Therefore it may be suggested that ASM induces defense-related enzymes, PAL activity, PR proteins and phenol accumulation in ASM-treated plants and contribute to enhance resistance against *P. capsici*.

Tomato spotted wilt virus (TSWV) is an economically important virus of flue-cured tobacco. Mandal *et al.* (2008) studied the activation of systemic acquired resistance (SAR) by acibenzolar-S-methyl (ASM) in flue-cured tobacco under greenhouse conditions by challenge inoculation with a severe isolate of TSWV. ASM restricted virus replication and movement, and as a result reduced systemic infection. Activation of resistance was observed within 2 days after treatment with ASM and a high level of resistance was observed at 5 days onwards. Expression of the pathogenesis-related (PR) protein gene, PR-3, and different classes of PR proteins such as PR-1, PR-3, and PR-5 were detected at 2 days post-ASM treatment which inversely correlated with the reduction in the number of local lesions caused by TSWV. Tobacco plants treated with increased quantities of ASM (0.25, 0.5, 1.0, 2.0, and 4.0 g a.i./7,000 plants) showed increased levels of SAR as indicated by the reduction of both local and systemic infections by TSWV. The highest level of resistance was at 4 g a.i., but this rate of ASM also caused phytotoxicity resulting in temporary foliar spotting and stunting of plants. An inverse correlation between the TSWV reduction and phytotoxicity was observed with the increase of ASM concentration. ASM at the rate of 1 to 2 g a.i./7,000 plants activated a high level of resistance and minimized the phytotoxicity.

2,6-dichloroisonicotinic acid (INA):

The plant activator 2,6-dichloroisonicotinic acid (INA) has been reported to induce systemic resistance against many diseases in agricultural and horticultural crops. INA enters a common pathway downstream of salicylic acid synthesis. It was found to protect rice, bean, barley, cucumber, sugar beet and rose against several pathogens and was also effective in inducing resistance against *Pseudomonas.syringae* pv. *tabaci* in tobacco and *Alternaria macrospora* leaf spot in cotton (Colson-Hanks and Deverall, 2000).

Colson-Hanks and Deverall (2000) evaluated the wettable powder (WP) formulation providing 5–25 mg/ml of 2,6-dichloroisonicotinic acid (INA) and 15–75 mg/ ml of WP applied to cotton cotyledons significantly increased the resistance of the next two leaves to challenge inoculation by *Alternaria*

macrospora. The wettable powder alone at 15–75 mg/ml had a lesser effect. A wettable granule (WG) formulation supplying 35 mg/ ml of benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) and 35 mg/ ml of WG, applied as a cotyledonary treatment, significantly reduced the formation of lesions on the subsequent two leaves when challenged with *A. macrospora*. The WG control had no effect. Each treatment except for the WG control also raised the activities of β-1, 3-glucanase in unchallenged leaf and stem tissue. Each of the components of the wettable powder without INA applied to cotyledons raised enzyme activities in the next leaves. Individual components, as suspensions of silicic acid and kaolin and solutions of the detergent Attisol II, the wetting agent Ultravon W300 and pure INA, applied to cotyledons increased the resistance of the next leaves to *A. macrospora*.

β-aminobutyric acid (BABA):

β-aminobutyric acid (BABA) acts through a pathway other than the salicylic acid, jasmonic acid and ethylene signaling pathways and enhance disease defense against late blight of tomato, downy mildew of grape vine and *Phytophthora* blight of pepper (Reuveni et al., 2001; Seigrist et al., 2000; Silue *et al*., 2002; Zimmerli et al., 2000).

Ammour *et al*. 2003 studied induced resistance in the model pathosystem Arabidopsis- *Phytophthora brassicae* in comparison with the agronomically important late blight disease of potato caused by *Phytophthora infestans*. For the quantification of disease progress, both *Phytophthora* species were transformed with the vector p34GFN carrying the selectable marker gene neomycine phosphotransferase (nptII) and the reporter gene green fluorescent protein (gfp). Eighty five per cent of the transformants of *P. brassicae* and *P. infestans* constitutively expressed GFP at high levels at all developmental stages both *in vitro* and *in planta*. Transformants with high GFP expression and normal *in vitro* growth and virulence were selected to quantify pathogen growth by measuring the *in planta* emitted GFP fluorescence. This non destructive monitoring of the infection process was applied to analyse the efficacy of two chemical inducers of disease resistance, a functional SA-analogue, benzothiadiazole (BTH), and β- aminobutyric acid (BABA) which is involved in priming mechanisms of unknown nature. BABA pre-treatment (300 µM) via soil drench applied 24 h before inoculation completely protected the susceptible Arabidopsis accession Landsberg erecta (Ler) from infection with *P. brassicae*. A similar treatment with BTH (330 µM) did not induce resistance. Spraying the susceptible potato cultivar Bintje with BABA (1 mM) 2 days before inoculation resulted in a phenocopy of the incompatible interaction shown by the resistant potato cultivar Matilda while BTH (1.5 mM) did not protect Bintje from severe infection. Thus, in both pathosystems, the mechanisms of induced resistance appeared to be similar, suggesting that the Arabidopsis - *P. brassicae* pathosystem is a promising.

Salicylic acid:

Salicylic acid is an important signal molecule that plays a critical role in plant defense against fungal, bacterial and viral pathogens. However, it has not been considered a practical solution to disease control,

because it does not translocate efficiently when applied exogenously. Exogenous SA becomes rapidly conjugated mostly into β- glucoside. These conjugates lack the phloem mobility of free salicylate.

Probenazole:

Probenazole (3-allyloxy-1,2-bezisothiazole-1,1-dioxide), was developed by Meiji Seika Kaisha Ltd. in Japan and it was approved by Japan ministry of Agriculture, Forestry and Fisheries. The compound is marketed as Oryzemate® for rice blast control and has been used by Japanese farmers in rice seedlings and paddy fields since 1975. After application to rice plants, probenazole is absorbed by the roots, then systemically transferred to the whole plant, almost completely controlling leaf blast for 40–70 days after application. Despite extensive use over many years no development of resistance in the target fungus has been observed.

Activation of the natural plant disease defense system:

Most plants have the ability to escape invasion of pathogens by using defense systems, even if they do not have a specific disease resistance gene. There is a delicate relationship between plant and pathogen. When environmental conditions such as temperature and humidity are favorable for the pathogen, the pathogen can easily invade the plant. When the defense system of the plant functions effectively, on the other hand, the plant can overcome pathogen attack. Probenazole activates the disease defense system of a plant – an unusual mode of action for a disease control chemical, previously unreported. By activating the plant defense system, probenazole alters the balance of the plant–pathogen relationship in favor of the plant.

Activation of defense-related phenylpropanoid pathway:

Activities of enzymes in the phenylpropanoid pathway, such as phenylalanine ammonia-lyase, peroxidase and polyphenoloxidase, are enhanced in rice plants treated with probenazole, especially in plants inoculated with the blast fungus after probenazole application. The phenylpropanoid pathway plays an important role in the plant defense system; when the plant is being infected lignin is synthesized and acts as a physical barrier against pathogen invasion, and a phytoalexin with antimicrobial activity is produced. These contribute to the limitation of pathogen invasion in the plant tissue. Probenazole activates the phenylpropanoid pathway and thereby enhances the defense response in the plant.

Accumulation of fungicidal substances:

Fungicidal substances accumulate within the tissue of the treated and inoculated rice leaf. Since probenazole and its metabolites do not have any fungicidal activity, these substances originated from the rice plant. They were identified as hydroxy unsaturated fatty acids derived from a-linolenic acid. A biosynthesis pathway of these hydroxyl unsaturated fatty acids is as follows: a-linolenic acid cut off by phospholipase A2 from phospholipid in cell plasma membrane is peroxidized into hydroperoxylinolenic acids by lipoxygenase; then the hydroperoxides are rapidly reduced to hydroxides. Activities of both enzymes in the rice leaf were enhanced when the plant was inoculated with a resistant-reaction-inducing, incompatible race of the blast fungus, suggesting participation of both enzymes in defense response. The

hydroperoxide synthesis forms part of the octadecanoid (18-carbon) pathway by which the plant hormone jasmonic acid, an endogenous elicitor of defense gene expression and phytoalexin biosynthesis is synthesized.

Amplification of superoxide production:

Superoxide production in a protoplast prepared from rice leaves treated with probenazole was amplified by treatment with an elicitor extracted from the blast fungus cell wall, showing that probenazole amplifies superoxide production in leaves attacked with the pathogen. Superoxide was released from the protoplast within several seconds after elicitor treatment, suggesting that superoxide production is one of the earliest defense responses in the rice plant. In many plants, production of reactive oxygen, including superoxide, is part of the hypersensitive response, which is a powerful defense mechanism against pathogen attack. Since the production of reactive oxygen proceeds with rapid oxygen consumption, this phenomenon is called an oxidative burst. Superoxide, after generation from the NADP(H) oxidase system in plant plasma membrane, is readily dismuted into hydrogen peroxide, which is the most stable form of reactive oxygen. It has been reported that hydrogen peroxide is implicated in the direct killing of invading pathogen, in the cross-linking of cell wall sugar proteins, in the plant cell death process as a cytotoxin, and in the induction of defense gene expression.

Activation of the signal transduction system:

Plants have intercellular and intracellular signal transduction systems which transfer information from cell to cell and from outside to inside a cell relating to stresses, pathogen attack, wounding etc. The defense system of the rice plant is activated through cell membrane and intracellular signal transduction pathways after treatment with a blast fungus elicitor. One of the metabolites of probenazole in the rice plant accelerated an activity of cell membrane GTPase, which plays an important role in membrane signal transduction from the receptor of the elicitor. Also the expression of protein kinase C to regulate the intracellular signal transduction is induced by treatment with probenazole. These observations suggest that cell membrane and intracellular signal transduction systems in the rice plant are activated by probenazole. The rice plant with an activated defense signal transduction pathway can more quickly respond to the attack of pathogen, and hence escape infection.

Rice genes expressed by probenazole:

The sensitization of the disease defense system in plants treated with probenazole would be brought about by a response involved with gene transcription. Rice plants were screened for expression induced by probenazole application and found a new rice gene *PBZ1*. The amino acid sequence estimated from the nucleic acid sequence of the *PBZ1* gene showed about 30% homology with PR (pathogenesis related) - 10 protein. This PR protein is induced after an infection of pathogen, and is thought to be an infection response and defense-participating protein. When rice plants untreated with probenazole were inoculated with the blast fungus, the *PBZ1* gene was also induced in the rice leaf tissue. Expression of the *PBZ1* gene

induced by inoculation with the incompatible fungus occurred earlier than with the compatible fungus. These results show that the PBZ1 gene product is a kind of PR protein, and that probenazole induces this PR protein. Expression of the *PBZ1* gene was highly induced in a lesion-mimic rice mutant in which defense responses were extremely expressed. Although the function of PBZ1 protein in disease defense is still unclear, the expression of the *PBZ1* gene is clearly correlated with expression of disease resistance. Sakamoto *et al.* (1999) isolated another rice gene *RPR1* (rice probenazole responsible gene) by a differential display technique. Transcription of the *RPR1* gene was detected 3 days after treatment of probenazole and reached its maximum level at 6–9 days. Mode of the *RPR1* expression in probenazole-treated rice plants correlated well with protection of the blast. The RPR1 protein deduced from the amino acid sequence contains a nucleotide binding site (NBS) and leucine-rich repeats (LRR). Interestingly, NBS and LRR are common characteristics in the proteins coded within disease resistance genes isolated from many plants including rice. These characteristics suggest that expression of the *RPR1* gene induced by probenazole leads to induction of a disease resistance response. Recently, researchers have reported that many defense related genes in the rice plant are induced by application of probenazole.

Riboflavin :

Riboflavin is involved in antioxidation and peroxidation resulting in the production of reactive oxygen intermediates (ROI) in oxidative burst and consequently hypersensitive response (Rommelt *et al.* 1999). Riboflavin induced defense response have been reported in rice against sheath blight (Taheri and Monica, 2007), in chick pea against Fusarium wilt (Saikia *et al.* 2006), in soybean against charcoal rot disease (Monaim, 2011).

The role of riboflavin as an elicitor of systemic resistance and an activator of a novel signaling process in plants was demonstrated by Dong and Beer (2000). Following treatment with riboflavin, Arabidopsis thaliana developed systemic resistance to *Peronospora parasitica* and *Pseudomonas syringae* pv. *tomato*, and tobacco developed systemic resistance to Tobacco mosaic virus (TMV) and *Alternaria alternata*. Riboflavin, at concentrations necessary for resistance induction, did not cause cell death in plants or directly affect growth of the culturable pathogens. Riboflavin induced expression of pathogenesis-related (PR) genes in the plants, suggesting its ability to trigger a signal transduction pathway that leads to systemic resistance. Both the protein kinase inhibitor K252a and mutation in the NIM1/NPR1 gene which controls transcription of defense genes, impaired responsiveness to riboflavin. In contrast, riboflavin induced resistance and PR gene expression in NahG plants, which fail to accumulate salicylic acid (SA). Thus, riboflavin-induced resistance requires protein kinase signaling mechanisms and a functional NIM1/NPR1 gene, but not accumulation of SA. Riboflavin is an elicitor of systemic resistance, and it triggers resistance signal transduction in a distinct manner.

Prohexadione – Ca:

The plant growth regulator prohexadione- Ca acts as plant activator also. It reduced the incidence of fire blight in apple by changing the flavanoid metabolism in the plant system. Costal *et al.* (2001) evaluated prohexadione-Ca (Apogee®) as a growth retardant and fire-blight control agent in the pear (*Pyrus communis* L.) on both bearing trees in the orchard and on 1-year-old scions under greenhouse conditions. Four sprays of 50 and 100 mg/l of the chemical were applied to trees in the orchard at 2-week intervals starting at petal fall, when terminal growth was 4 cm (mid-April). Scions received a single application (250 mg/l) and were transferred 2 weeks later to a greenhouse where the shoots were inoculated with a local, virulent strain of *Erwinia amylovora* (Burrill). In the orchard, the higher prohexadione-Ca concentration was more effective in reducing shoot growth, enhancing fruit weight and controlling fire blight incidence and severity. Similar effects on growth parameters and disease progression were observed under greenhouse conditions.

Miscellaneous plant activators:

Potassium phosphonate and Fosetyl- Al are systemic fungicides with good protective and curative activities mainly against diseases caused by oomycetes. Harpin is a 44-kDa protein encoded by hrp (hypersensitive reaction and pathogenecity) gene of *Erwinia amylovora* and it elicits protective response in plants and makes them resistant to a wide range of diseases. Jasmonic acid induces systemic resistance against many pathogens by strengthening the defense mechanisms in plants.

CONCLUSION:

The major drawback of the chemical induction of defense genes is that their effect is only transient and lasts only for a few days. They are not curative and cannot eliminate an already established infection. Moreover, the activation of induced resistance pathway requires a large energy input and thus compromises other metabolic processes. Therefore, successes of defense inducers for plant disease control depend on our ability to manage their phytotoxicity either by chemical modification of the compound or by modifying their formulation. Since, plant activators would never be able to provide complete protection; they could be more suited as a component of integrated disease management.

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