Plant immunization

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Plant immunization is the process of activating natural defense system present in plant induced by biotic or abiotic factors. Plants are pre-treated with inducing agents stimulate plant defense responses that form chemical or physical barriers that are used against the pathogen invasion. Inducers used usually give the signals to rouse the plant defense genes ultimately resulting into induced systemic resistance. In many plant-pathogen interactions, R-Avr gene interactions results in localized acquired resistance or hypersensitive response and at distal ends of plant, a broad spectrum resistance is induced known as systemic acquired resistance (SAR). Various biotic or abiotic factors induce systemic resistance in plants that is phenotypically similar to pathogen-induced systemic acquired resistance (SAR). Some of the biotic or abiotic determinants induce systemic resistance in plants through salicylic acid (SA) dependent SAR pathway, other require jasmonic acid (JA) or ethylene. Host plant remains in induced condition for a period of time, and upon challenge inoculation, resistance responses are accelerated and enhanced. Induced systemic resistance (ISR) is effective under field conditions and offers a natural mechanism for biological control of plant disease.

**Keywords**: Hypersensitive response, Induced systemic resistance, Plant immunization, Systemic acquired resistance

Plants possess constitutive and inductive defense mechanisms against pathogen attack. These mechanisms fail when the plant is infected by a virulent pathogen because the pathogen avoids triggering or represses resistance reactions, or evades the effects of activated defenses. If defense mechanisms are triggered by a stimulus prior to infection by a plant pathogen, disease can be reduced. Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated. Induced resistance is not the conception of resistance where there is none, but the activation of dormant resistance mechanisms that are expressed upon subsequent, so-called challenge inoculation with a pathogen. Induced resistance occurs naturally as a result of limited infection by a pathogen, particularly when the plant develops a hypersensitive reaction. Although, tissue necrosis contributes to the level of induced resistance attained,activation of defense mechanisms that limit a primary infection appears sufficient to elicit induced resistance. 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 held up owing to environmental conditions. 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. Because of this systemic character, induced resistance is commonly referred to as systemic acquired resistance (SAR) . However, induced resistance is not always expressed systemically. Localized acquired resistance (LAR) occurs when only those tissues exposed to the primary invader become more resistant.

Activation of systemic acquired resistance (SAR) is a mechanism whereby plant cells can be stimulated to turn on their defenses to fight invasion by biotic / abiotic factors. This activation is essentially a form of 'plant immunization' and can be compared to immunization against disease in mammals. Plants are treated with activating agents to stimulate plant defense responses that form chemical or physical barriers that are used by the plant to ward off diseases. Inducing agents include pathogens, biocontrol agents, certain types of composts, and plant activating compounds. SAR and LAR are similar in that they are effective against various types of pathogens. A signal that promulgates the enhanced defensive capacity throughout the plant in SAR appears to be lacking in LAR. SAR is characterized by an accumulation of salicylic acid (SA) and pathogenesis-related proteins (PR). Accumulation of SA occurs both locally and, at lower levels, systemically, concomitant with the development of SAR. Exogenous application of SA also induces SAR in several plant species. SAR was originally described using weak pathogens as activators of plant defense responses. However, the utility of this form of plant inoculation is limited in the field because of the likelihood of pathogen spread.
following application. The discovery that certain biocontrol agents and specific plant activators could induce resistance has made the use of SAR as a disease control strategy an achievable goal. In nature, immunization can occur when plants come into contact with a necrotizing pathogen. Immunization takes place not only at the primary infection site, but also in distant (systemic) parts of the plant.

Necrotizing agents and chemicals are not the only way to immunize plants against pathogens. Plant-growth-promoting rhizobacteria have also been shown to induce systemic resistance in plants. For example, *Pseudomonas fluorescens* strains can induce resistance against viruses, bacteria and fungi. However, little is known about the underlying mechanisms and the molecular basis involved.

**Mechanism**

Plant immunization in plants is usually associated with the activation of a wide variety of defense responses that serve to prevent pathogen replication and/or movement. In some plant-pathogen interactions, the ability of the host plant to recognize the pathogen and activate these responses is regulated in a gene-for-gene specific manner by the direct or indirect interaction between the products of a plant resistance (R) gene and a pathogen avirulence (Avr) gene. When either the plant or the pathogen lacks its cognate gene, activation of the plant’s defense responses either fails or it is delayed sufficiently so that pathogen colonization ensues.

After avirulent pathogen or some inducer compound has generated a defense-eliciting signal and the plant has perceived it, what are the downstream events that are stimulated? Gene-for-gene interaction can induce signaling responses, such as activation of protein-kinases, stimulation of an oxidative burst, and induction of ion fluxes across the cellular membrane. These and other events, in turn activate defense responses, such as cell wall cross-linking and the expression of defense-related genes. One downstream component of defense signal transduction that deserves particular emphasis is salicylic acid. Salicylic acid is a key signaling intermediary that triggers systemic acquired resistance (SAR). The earliest responses activated after host plant recognition of an Avr protein or a non-host specific elicitor is the oxidative burst, in which levels of reactive oxygen species (ROS) rapidly increase. These ROS are predominantly the superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) which arise from successive one electron reductions of molecular oxygen. ROS generation appears to occur irrespective of the type of eliciting pathogen (bacterial, fungal, or viral). ROS generation is localised to the point of infection and appears to occur primarily in the apoplast even if an intracellular pathogen is involved. A eicosionid pathway is particularly associated with the inflammatory response of the mammalian immune system. This pathway results in a series of inter- and intracellular signals derived from arachidonic acid (liberated from membrane phospholipids by activated phospholipase A$_2$). Two major branches of the pathway exist. One branch, leading to the synthesis of prostaglandins, prostacyclins and thromboxanes, is regulated by cyclooxygenase (more correctly prostaglandin endoperoxide synthase), whilst the other route, via a family of lipoxygenases, results in the formation of n-hydroxy-eicosa-5,8,10,14-tetraenoic acid (n-HETE), leukotrienes and lipoxins. A variety of anti-inflammatory drugs, including indomethacin and aspirin, inhibit cyclooxygenase. Aspirin exerts this effect via acetylation of an amino acid in the active site. A common feature of many plant-pathogen interactions is the rapid production of active oxygen species (AOS). Superoxide (O$_2^-$) and H$_2$O$_2$ are the species most commonly measured, but their accumulation undoubtedly results in a plethora of other species, some of which (e.g. the hydroxyl radical) are extremely damaging. While a rapid biphasic production of AOS has been observed in a number of systems, the source of this oxidative burst remains a subject of debate. One theory is that salicylate inhibits catalase, ascorbate peroxidase or other enzymes in the anti-oxidant armoury (thus allowing H$_2$O$_2$ to accumulate). This hypothesis suffers from a number of problems like non-physiologically high concentration of SA required to effect inhibition. The activation of lipoxygenase (yielding O$_2^-$), could also supply the burst, however, most of the available evidence suggests that AOS production is extracellular. AOS could be produced by an unusual pH-dependent reaction of cell wall peroxidases, and this may represent the principal source of AOS in some plants or during some interactions. Most attention has, however, focussed on a system similar to that which generates the oxidative burst in mammalian macrophages. In this model, an electron is transferred from an intracellular donor (NADH) across the plasma membrane (by the action of NADPH oxidase) to molecular oxygen, yielding O$_2^-$.
identified in plants, and, in some cases, the plant oxidative burst exhibits similar inhibitor sensitivity to the mammalian system.

Resistant plants often develop a hypersensitive response (HR), in which a necrotic lesion forms at the site(s) of pathogen or elicitor activity\textsuperscript{32,33}. The localized cell death associated with HR resembles animal programmed cell death, and it may help to prevent the pathogen from spreading to uninfected tissues. Just before or concomitant with the appearance of a HR is the increased synthesis of several families of pathogenesis-related (PR) proteins in the inoculated leaves\textsuperscript{34}. Many of these proteins have been shown to exhibit antimicrobial activity either in vitro or in vivo. PR-proteins are subsequently expressed in the uninfected portions of the plant, concurrent with the development of a long lasting, broad spectrum resistance known as systemic acquired resistance (SAR)\textsuperscript{35}. Because of the correlation, increased PR gene expression is frequently used as a marker for SAR. The upstream regulatory region of the PR-1 gene has been characterized in details\textsuperscript{36}. Two positive regulatory elements within the region, both of which are required for induction of PR-1 by salicylic acid, manifest consensus sequences for recognition by transcription factors. The first of these elements corresponds to the cognate sequence of bZIP proteins, a class of transcription factors common to fungi, plants, and animals. bZIP factors are active as dimers, each protomer of which is defined by two domains, one is a basic domain that interacts with specific DNA sequences, and the second is characterized by a leucine zipper that is required for dimerization. One category of plant bZIP factor that deserves particular mention is the family of TGA proteins. In Arabidopsis, six distinct genes have been, therefore, allocated to TGA family\textsuperscript{37}. An ortholog of TGA1 has been first recognized in tobacco for its ability to bind with high specificity to the pentanucleotide element (TGACG) within 35S promoter of the cauliflower mosaic virus\textsuperscript{38}. Specific protein-DNA interactions that involve this consensus sequence are typical of all the TGA proteins. Significantly, this pentanucleotide exists within the first of the two elements that are necessary for salicylic acid induction of PR-1 gene\textsuperscript{35}. The second of the two elements within the PR-1 promoter appears to correlate not to the specificity of TGA protein, but rather to the recognition element of NF-kB, a vertebrate transcription factor of the Rel family\textsuperscript{38}. NF-kB has been extensively studied because it functions within various contexts of mammalian signal transduction pathways that lead to the transcription of genes involved in apoptosis, cell growth and differentiation, and immune functions. In addition, NF-kB represents an important model transcription factor because its molecular relationship to higher orders of regulatory control in transduction pathways has been elucidated. Specifically, the ability of NF-kB to activate transcription is controlled in that it remains sequestered in the cytoplasm by an inhibitory factor, I\textkappa B, to which it is bound. This inhibitory function, in turn, is regulated by a specific kinase that phosphorylates I\kappa B so as to tag it for ubiquitin-mediated degradation, thereby freeing NF-kB to enter the nucleus and activate the transcription of its target genes\textsuperscript{39}. One protein that proves to be crucial to SAR is the product of the NPR1 gene (for NONEXPRESSER OF PR-1; also known as NIM1 [for NONINDUCIBLE IMMUNITY])\textsuperscript{40,41}.

An extensive research indicates that salicylic acid (SA) is a critical signaling molecule in the pathway(s) leading to local and systemic disease resistance, as well as PR expression\textsuperscript{42,43}. In addition, recent studies have demonstrated that ethylene and jasmonic acid (JA) mediate the activation of various defense responses and resistance to certain pathogens\textsuperscript{44,45}. JA signaling can be induced by a range of abiotic stresses, including osmotic stress\textsuperscript{46,47}, wounding, drought, and exposure to "elicitors," which include chitin, oligosaccharides, oligogalacturonides\textsuperscript{48}, and extracts from yeast. A mitogen-activated protein kinases named WIPK is transcribed minutes after tobacco is wounded\textsuperscript{49}, and the WIPK protein product is activated\textsuperscript{50}. Jasmonic acid and its methyl ester accumulate in wounded tobacco plants, but do not accumulate in wounded transgenic plants, in which expression of WIPK is genetically suppressed. This indicates that expression of WIPK is required for wound-induced JA biosynthesis. However, the wounded transgenic plants accumulated SA and transcripts of the gene pathogenesis related protein 1 (PR1), indicating that suppression of JA pathway permits wound induction of the SA pathway\textsuperscript{51}. More significantly, transgenic tobacco plants overexpressing WIPK accumulate JA and proteinase inhibitor 2 (PIN2) transcripts\textsuperscript{50,51}. Apparently therefore, the wound-induced transcription of WIPK and activation of the protein product activates JA biosynthesis and suppresses SA-dependent signaling. Other than SA, JA and ethylene as signal molecules, nitric oxide (NO) has been implicated in the activation of plant defenses\textsuperscript{52}. This compound has previously been shown to serve as a key redox-active...
signal for the activation of various mammalian defense responses, including the inflammatory and innate immune responses\textsuperscript{53,54}. Till date, at least in three different plant-pathogen interaction, NO is documented as signal for activation of plant defense responses.

**Inducers**

SAR has been demonstrated to occur in many plants including cereals, vegetable, and nursery and tree crops, and can provide protection against pathogens for which there is no effective disease control measure currently available. There is a low risk of pathogen populations developing resistance to SAR since that would entail overcoming an array of plant defense mechanisms. Finally, the use of biocontrol agents as inducing agents can provide other advantages to the plant such as plant growth promotion, which is often associated with certain types of biocontrol agents. Growth enhancement has also been reported for a plant activator.

The necrosis-related immunization process seems to follow a salicylic acid (SA)-dependent pathway. Strong support for the implication of SA in this pathway comes from experiments with transgenic plants (expressing the NahG gene for salicylate hydroxylase) unable to accumulate SA. Such plants become susceptible to avirulent pathogens and cannot be immunized. Treatment of plants with SA or chemicals such as benzo (1,2,3) thiadiazole-7-carboxylic acid S-methyl ester (BTH) and 2,6-dichloroisonicotinic acid (INA) also leads to subsequent immunization. Benzothiadiazole, induces systemic resistance in wheat, which is one of the novel class of inducers of systemic acquired resistance\textsuperscript{55}. The expression analysis of genes induced in barley after chemical activation reveals distinct disease resistance pathways\textsuperscript{56}.

Two products are available to immunize plants from some fungal diseases. These two products are Messenger\textsuperscript{TM}, from Eden Bioscience\textsuperscript{57,58} and Elexa\textsuperscript{TM}, from Safe Science. Their main target is powdery mildew in vegetable crops and grapes, and they fall into the Environmental Protection Agency's Plant Defense Booster (PDB) category, which classifies them as "low risk biochemical pesticides". Messenger has an active ingredient called harpin Ea, which is a protein produced by bacteria. First, bacteria are filtered out of the protein product. The protein is harvested by culturing and applying heat to destroy the bacteria. When Messenger is applied to the foliage, the plant's defensive biochemical pathways are activated within 5-10 min\textsuperscript{59,60}. Besides the benefits of disease prevention, plant growth response is positively increasing plant biomass, flowering and early maturing, and crop yield and quality. Elexa's active ingredient, chitosan, is obtained from the shells of shrimp and crabs. The cell wall of fungal spores that attack plants is comprised of chitin. When chitosan is applied, the plant believes that fungal spores have arrived. The plant then triggers the production of a protein called chitinase, which attacks the fungal chitin skin, loosening its cell walls and causing potential cell wall disintegration. Messenger and Elexa represent the genesis of a new plant immunization technology that allows the plant to protect itself from disease.

One of the advantages of using biocontrol agents, composts, or plant activating compounds to stimulate SAR is that they will not cause disease. A key advantage to SAR is that the plant, once immunized, has its activity until flowering, thereby limiting the number of applications necessary for defense mechanisms turned on for an extended period of time, usual effective disease control\textsuperscript{61}. Immunization is generally broad spectrum, effective against an array of different pathogens and pathogen types (i.e. fungi, nematodes, viruses and bacteria). Studies on suppression of fusarium wilt of carnation and radish, caused by Fusarium oxysporum f.sp. dianthi (Fod) and F. oxysporum f.sp. raphani (For), respectively, established competition for iron as the mechanism of disease reduction by P. putida strain WCS358\textsuperscript{62-64}.

Under iron-limiting conditions in the rhizosphere, WCS358 secretes a pyoverdin-type siderophore (pseudobactin 358) that chelates the scarcely available ferric ion as a ferric-siderophore complex that can be transported specifically into the bacterial cell. Siderophores released by Fod or For under these circumstances are less efficient iron-chelators than pseudobactin 358, so iron available to the pathogens can become limiting in the presence of WCS358. Due to iron deficiency, fungal spore germination is inhibited and hyphal growth restrained, effectively lowering the chance that the plants become infected, and disease incidence and severity are reduced. The plant, in contrast, does not appear to suffer from iron shortage\textsuperscript{65-66}. A bacterial mutant generated by Tn5 transposon mutagenesis and unable to produce pseudobactin 358 (WCS358 Sid\textsuperscript{d}) did not reduce disease incidence\textsuperscript{67}. A different bacterial strain, Pseudomonas fluorescens WCS417, was about twice as effective as WCS358 in suppressing fusarium wilt in carnation. However, a Sid\textsuperscript{d} mutant of this strain was...
as effective as the wild type in suppressing the disease\textsuperscript{65}. Clearly, a mechanism other than common procedures to accomplish induced resistance are pouring a suspension of the bacteria on, or mixing it with, autoclaved soil; dipping the roots of seedlings in a bacterial suspension at transplanting; or coating seeds with high numbers of bacteria before sowing\textsuperscript{68}. Subsequently, seedlings are challenged with a pathogen. Because rhizobacteria are present on the roots, systemic protection against root pathogens must be demonstrated by applying the inducing bacteria to one part of the root system and the challenging pathogen to another part, for instance by making use of split-root systems. Testing for protection against foliar pathogens is easier, because the pathogens are naturally separated from the rhizobacteria. However, rhizobacteria applied to seeds, or to soil into which seeds are sown or seedlings are transplanted, can move into the interior of aerial plant tissues and maintain themselves to some extent on the exterior of aerial surfaces\textsuperscript{69}. The stems, petioles, cotyledons and/or leaf extracts of tested plant were free from, or aerial surfaces \textsuperscript{69}. The stems, petioles, cotyledons and/or leaf extracts of tested plant were free from, or

Salicylic acid is known to activate plant defense responses and is thought to function as a signal molecule within the plant after the onset of resistance. However, bacterial salicylic acid production is not the only determinant of SAR since there are examples of biocontrol agents that can activate resistance but do not produce salicylic acid. It is thought that some biocontrol agents activate resistance at low iron concentrations through the production of iron-scavenging compounds called siderophores. Additionally, certain plant growth-promoting biocontrol agents have demonstrated altered insect feeding patterns in cucumber, making treated plants less susceptible to predation than non-treated plants and limiting the spread of pathogens that are vectored by the insects. Field experiments with several of these biocontrol agents have demonstrated that SAR is a viable disease control strategy\textsuperscript{76}.

\textit{P. syringae} pathovars syringae and phaseolicola elicit a non-host hypersensitive response (HR) when injected into the leaves of tobacco\textsuperscript{77,78}. This HR is dependent upon the ability of the bacteria to assemble an \textit{hrp} (for HR and pathogenesis) pilus (a kind of biological microinjection system) utilised to introduce \textit{avr} gene products into the plant cells\textsuperscript{58}. Assembly of the pilus and transfer of the \textit{avr} gene products is required to elicit HR. Mutant bacteria (such as the \textit{hrpL} strain used in this study) fail to produce the pilus and do not induce HR. Neither \textit{syringae} nor phaseolicola are pathogens of tobacco (hence 'non-host' HR), and in this respect are not as ideal as tobacco mosaic virus (TMV) which is a pathogen of tobacco and which causes disease or elicits HR depending upon absence or presence of a single gene (the \textit{N} gene) in a given tobacco cultivar's genotype.

Expression and introduction of \textit{avr} gene products into a plant cell would seem to be uniquely counterproductive as far as a pathogen is concerned, since it is these very products that elicit defense responses such as HR\textsuperscript{78}. This is true of the response of resistant plants (incompatible interaction) that have the necessary gene or genes required to recognize the \textit{Avr} gene product. However, \textit{Avr} genes are actually part of the pathogen's colonisation mechanism when in contact with a susceptible host (compatible interaction), and are, thus, necessary for pathogen survival in a suitable host plant. One good example of this involves the interaction between tobacco mosaic virus (TMV) and tobacco plants. In susceptible tobacco plants (those lacking \textit{N} resistance gene) the virally-encoded replicase is an integral part of the virus's replication machinery. However, it is the same replicase that is recognised in resistant plants (by \textit{N} gene product), and that results in the characteristic hypersensitive response. However, TMV strains in which replicase has been mutated to produce an enzyme that is still active, but has lost the recognition sequence, will be able to avoid the defense response even in tobacco plants possessing \textit{N} gene. This is the basis for the so-called 'evolutionary arms race' between plants and pathogens.
In recent years the majority of pathogenesis research has focussed on defense responses dependent upon Avr/R gene interactions. This is quite natural that such systems are highly tractable to a genetic approach, offer the opportunity for an enter into the molecular biology of defense signal transduction, and Avr/R interactions are often a feature of the most aggressive and economically important diseases. In fact, the aggressive nature of such pathogens is probably the driving force of the ‘evolutionary arms race’ that underlies avr/R-dependent responses (Avr gene products are actually agents of pathogenesis in susceptible plants; resistant plants have acquired the ability to recognize these products and turn the pathogen’s own weapon against it).

Observations made in rice emphasize the complexity of interactions between signals. 2,6-dichloroisonicotinic acid (INA), an inducer of systemic acquired resistance (SAR) and putative SA mimic\(^1\), protects rice against the rice blast pathogen (Magnaporthe grisea). Wasternack et al.\(^7\) have also reported induction of thionin, a antifungal protein in barley by INA. In tobacco; INA induces expression of a set of genes associated with SA-dependent SAR\(^9\) and homologues of these are expressed in rice following INA application. However, contrary to the standard antagonism model, not only does INA induce endogenous JA accumulation in rice, but exogenous JA application enhances INA-mediated resistance and potentiates INA-induced defense gene expression\(^1\).

Pathologists describe two different types of disease suppression in compost and soil. General suppression is due to many different organisms that either compete with pathogens for nutrients and/or produce general antibiotics that reduce pathogen survival and growth. This type of suppression is effective on those pathogens that have small propagule size, resulting in small nutrient reserves and the need to rely on external carbon sources. Thus, an active microflora in the soil or compost will often prevent disease since the pathogens are outcompeted. Examples of this mechanism are suppression of damping off and root rot diseases caused by Pythium species and Phytophthora species\(^1\). Specific suppression, on the other hand, is usually explained by one or a few organisms. They exert hyperparasitism on the pathogen or induce systemic resistance in the plant to specific pathogens, much like a vaccination. With specific suppression, the causal agent can be clearly transferred from one soil to another. Pathogens such as Rhizoctonia solani and Sclerotium rolfsii are examples where specific suppression may work but general suppression does not work. This is because these organisms have large propagules that are less reliant on external energy and nutrients and thus less susceptible to microbial competition. Specific hyperparasites such as Trichoderma species will colonize the propagules and reduce disease potential. Methyl bromide is found to be harmful chemical for animals and human beings. In substitution to methyl bromide, composts and biocontrol agents are used in biological control of plant diseases\(^8\). To control the disease incidence and increase production of tomato, compost amendments were exploited by conventional and organic production systems\(^9\). Some species of rhizobacteria are known to induce systemic resistance in plants. Screening assay for this type of bacteria has been developed by Han et al.\(^8\). Trichoderma hamatum 382 and Flavobacterium balustinum 299 have been identified as effective biocontrol agents in compost-amended substrates\(^10\). They consistently induce suppression of diseases caused by a broad spectrum of soilborne plant pathogens if inoculated into compost after peak heating but before substantial recolonization with mesophilic microorganisms has occurred\(^8\). The presence of significant populations of T. hamatum 382 and F. balustinum 299 in fortified commercial mixes is one of several factors crucial to efficacy\(^8\). Compost and compost-water extract were also used to induce systemic resistance in cucumber and Arabidopsis\(^9\). Suppression of plant diseases caused by Pythium and Phytophthora species were controlled to an extent by using compost\(^8\). Foliar diseases of ornamental plants were also suppressed by use of compost and T. hamatum 382\(^9\).

**Concluding remarks**

Understanding the evolution of induced defense will be advanced by applying our knowledge of quantitative genetic variation in induced resistance to fitness consequences for the plant. Such experiments should be conducted in environments with and without plant attackers. More researches on signal transducers and their downstream components are in progress for elucidation of biochemical aspect of resistance in plants. With isolation of some of the genes like Nim 1, NPR 1 or PIN 2, immense opportunities have been unfolded for protein biochemists, biologists, physiologists and geneticists alike to elucidate how these gene products function and the gene families evolve. The concept of this induced resistance has been tried on plants like arabidopsis, tobacco, tomato, cereal, pearl millet,
barley, but there are yet many economically important crops left which are destroyed due to devastating pathogens. Biotic factors like *P. syringae* or some strains of PGPR and abiotic factors like salicylic acid, acetylsalicylate, benzothiadazole should be marked as low risk factor like MESSENGER and EDEN. Field trials with such inducers should be encouraged and should be provided to the farmers.

**References**


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