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Concepts and direction of induced systemic resistance in plants and its application

Joseph Kuć
Professor Emeritus University of Kentucky, 700 Front Street #1202, San Diego, CA 92101-6061, USA (Phone/fax: +1 619 237 1224; E-mail: JoeKuc@Aol.com)

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Abstract

Resistance to plant disease is often specific and metabolites and receptors contributing to this specificity may have specific structures. However, simple, structurally-unrelated compounds induce systemic resistance in unrelated plants to diverse pathogens including fungi, bacteria and viruses. Both resistance and induced systemic resistance (ISR) are associated with the rapid accumulation of the same structurally unrelated putative defense compounds that have diverse functions. It has been suggested that cultivar (race)-specific resistance is initiated by the specific interaction of a pathogen product (or pathogen induced product) and a plant receptor. However, restricted infection by pathogens can result in ISR and many different compounds can cause ISR. It is thus evident that there are both specific and non-specific routes to the master switch for ISR and there may be more than one master switch. Are reactive oxygen species and free radicals regulating the master switch(es) via both routes? It is also evident there are many switches, other than the master switch. Adding to the complexity of resistance and ISR are the observations that different compounds and pathways may mediate different biochemical resistances. Activation of one of the pathways may antagonize or enhance the activation or effectiveness of another. The review will address these complexities and questions and propose directions of research which require high priority. Factors which encourage and suppress the application of ISR in agriculture will also be addressed.

Introduction

The observations of induced resistance which date back to the 19th century and those reported by Chester (1933), as well as the verification of the phenomenon by Kuć, Barnes et al. (1959); Loebenstein (1963) and Ross (1976) were largely ignored. This was even the case when induced resistance was demonstrated in the greenhouse and field with diseases caused by fungi, bacteria and viruses (for reviews see Kuć, 1982; 1993). The significance of induced resistance was ignored both from its potential for disease control and the fundamental insights into the molecular basis of disease resistance and induced resistance it provided.

When research from my laboratory with defense compounds started to be published in the late 1950s and early 1960s, and up to the late 1980s, it was greeted with curiosity and was often thought to be 'somehow mistaken'. After many years, 280 publications from my laboratory, including greenhouse and field tests, and many more publications from around the world, the stage has been reached where induced resistance is 'self evident and obvious'. It is being utilized for disease control on a commercial scale. What is it that is now 'obvious'?

Characterization of Induced Systemic Resistance

Induced Systemic Resistance (ISR) is a phenomenon whereby resistance to infectious disease is systemically

Table 1. Putative defense compounds/systems for disease resistance in plants

Passive and/or wound responses: Waxes, cutin, phenolic glycosides, phenols, quinones, steroid glycoalkaloids, suberin, terpenoids and proteins (thionins)
Increases: after infection: Phytoalexins, reactive oxygen species/free radicals, calcium, silicon/silicates, polyphenoloxidases, peroxidases, phenolic cross-linked cell wall polymers, hydroxyproline and glycine-rich glycoproteins, thionins, antimicrobial proteins and peptides, chitinases, β-1,3-glucanases, ribonucleases, proteases, callose, lignin, lipoxygenases and phospholipases

Table 2. Agents reported to elicit induced systemic resistance in plants

– fungi, bacteria, viruses, nematodes, insects
– fungal, bacterial and plant cell wall fractions, intercellular plant fluids and extracts of plants, fungi, yeasts, bacteria and insects
– potassium and sodium phosphates, ferric chloride and silica
– glycine, glutamic acid, α-aminobutyric acid, β-aminobutyric acid, α-aminoisobutyric acid, D-phenylalanine, D-alanine and DL tryptophan
– salicylic acid, *m*-hydroxybenzoic acid, *p*-phydroxybenzoic acid, phloroglucinol, gallic acid, isovanillic acid, vanillic acid, protocatecheic acid, syringic acid and 1,3,5 benzene tricarboxylic acid
– D-galacturonic acid, D-glucuroinic acid, glycollate, oxalic acid and polyacrylic acid
– oleic acid, linoleic acid, linolenic acid, arachidonic acid and eicosapentaenoic acid
– paraquat, acifluorfen, sodium chlorate, nitric oxide, reactive oxygen species
– 2,6-dichloroisonictonic acid, benzo(1,2,3) thiadiazolel-7-carbothioic acid s-methyl ester
– jasmonate and ethylene
– riboflavin
– probenazole and 2,2-dichloro-3,3-di-methyl cyclopropane carboxylic acid
– dodecyl DL-alanine and dodecyl L-valine
– penanthroline and phthalocyanine metal complexes (cobalt, iron and copper)

induced by localized infection or treatment with microbial components or products or by a diverse group of structurally unrelated organic and inorganic compounds. The activity of the inducing agents is not due to antimicrobial activity *per se* or their ability to be transformed into antimicrobial agents. However, antimicrobial agents can induce resistance, and they provide protection from the time of application until ISR is fully expressed. The process by which the plant becomes resistant is active. Events, are put in motion and compounds are synthesized and accumulated which may contribute to resistance (Table 1), and the plant is sensitized to further respond rapidly after infection (Hutcheson, 1998; Sticher et al., 1997; Kuć, 1993, 1995a,b, 1997, 1999; Kessman et al., 1994; van Loon et al., 1998). The compounds which accumulate are structurally unrelated and vary from biopolymers to inorganics (Table 1). Some of the compounds are directly antimicrobial whereas other restrict development of pathogens by forming barriers.

Many questions, however, remain unanswered. Which, if any, of the putative defense compounds reported in the literature actually contribute to resistance? In some cases of ISR, the classical defense compounds have not been found (van Loon et al., 1998). In other cases, some defense compounds may reach high levels and the plants are susceptible (Dalisay and Kuć, 1995). Are the timing of the appearance and quantity of individual defense compounds, as part of a multiple response, important? Are the synthesis of defense compounds and the timing of their individual appearance and accumulation regulated differently? Do plants respond to the infectious agent *per se* or to the stress (metabolic perturbation) caused by the pathogen, or both? What is the systemic signal(s) which induces resistance? What is the factor(s) which causes release of a signal(s) for resistance? Clearly, a compound which initiates ISR cannot be synthesized or released by a plant until an appropriate signal has been received for its synthesis or release.

Since so many unrelated agents can elicit ISR (Table 2), including some herbicides, it is clear that the agent used to elicit ISR is active largely because of what it does rather than what it is (Strobel and Kuć, 1995; Fought and Kuć, 1996). The structure of 2,6-dichloroisonicotinic acid (INA) is unrelated to benzo (1,2,3)thiadizole-7 carbothioic acid s-methylester (BTH), but both compounds are active inducers of ISR. The latter compound is being used commercially. However, the simple inorganic compounds, dipotassium phosphate and ferric chloride, also induce resistance and have been successfully tested in the field (Gottstein and Kuć, 1989; Mucharromoh and Kuć, 1991; Reuvini et al., 1996; Reuvini and Reuvini, 1998; Manandhar et al., 1998). A search for structural features in a compound as a key to its activity have been unsuccessful (Fought and Kuć, 1996).

A further observation which remains unexplained is the general non-specificity of ISR, both in regard to the inducing agent and to the spectrum of effectiveness

against different diseases. There are atleast six clearly defined types of resistance; parasite-specific, cultivar-specific, non-host (basic), organ-specific, age-related and induced (localized and sytemic) (Heath, 1996). Are the mechanisms/defense compounds for all types of resistance identical and the differences exist only in their regulation? Or, are the mechanisms/defense compounds for different types of resistance different? The plants responses in a susceptible plant–pathogen interaction generally appear qualitatively identical to a resistant one except for the timing of the responses. Resistance and ISR are generally associated with a rapid response, but the defense compounds are often the same. In susceptible interactions, the amounts of defense compounds produced are often higher than in resistance interactions late in the interactions.

Specificity and ISR

Though ISR has low specificity for effectiveness, some specificity is apparent. ISR is most effective against fungi, less effective against bacteria and least effective against systemic viruses. Some chemical agents which induce ISR are more effective against some diseases than others. This may be explained by their different effects on different components of a multi-component resistance response. Not all defense compounds are equally effective against all pathogens. For example, it is difficult to assign a role for chitinase as a defense compound against a fungus which lacks chitin in its cell wall, though such a fungus may elicit chitinase accumulation. Similarly, some viruses elicit accumulation of classical defense compounds, such as phytoalexins, chitinase and β-1,3-glucanase, though a role for these compounds in resistance to viruses is unlikely.

Further evidence for non-specificity of ISR is provided by Strobel and Kuć (1995). Pro-oxidant chemicals, including the herbicide paraquat, induced systemic resistance in cucumber and tobacco to pathogens and the herbicide. They further reported that induction of resistance with pathogens or INA also included systemic protection against damage caused by paraquat, cupric chloride, t-butylhydroperoxide and other oxidants. An oxidative burst appears important for induction of ISR, but it also helps the plants cope with oxidative agents that would cause extensive damage. It is likely that ISR protects plants by inhibiting development of pathogens as well as protecting against damage (oxidative, hydrolytic) caused by pathogens and damage caused by oxidants, some herbicides and heavy metals. The products arising from an oxidative burst can also be antifungal (Peng and Kuć, 1992). The significance and relation of an oxidative burst and reactive oxygen species to ISR have been reviewed (Kiraly, 1998; Lamb and Dixon, 1997).

Cultivar (race)-specific resistance may be initiated by the specific interaction of a pathogen product or pathogen-induced product and a plant receptor. This interaction (recognition?) regulates a master metabolic switch and the turning on of this switch turns on the synthesis/accumulation of the putative defense compounds. How all of this happens has not been reported. It is important to remember, however, that restricted infection by pathogens can result in ISR and many different compounds can cause ISR. It is evident to me that there are both specific and non-specific routes to the proposed master switch. Are reactive oxygen species and free radicals regulating the master switch via both routes? It is also possible that there may be more than one master switch and it is likely that there are many switches for regulation past the master switch. Thus, in a home's electrical supply there is a master switch as well as circuit breakers or fuses, on and off controls on appliances and regulators for the extent the appliance is turned on. Compartments in a cell may house different switches.

Added to the complexity of ISR is that it includes both herbivores and pathogens. Different compounds and pathways may mediate different biochemcial resistances. These mediators include salicylic acid, jasmonic acid, abscisic acid, ethylene and nitric oxide (Delledonne et al., 1998; Lusso and Kuć, 1999; Wasternach and Parthier, 1997). Activation of one of the pathways may antagonize the activation or effectiveness of another, e.g., salicylic acid and jasmonic acid. In other cases, two pathways may result in an enhanced response (Karban and Kuć, 1999). Choi et al. (1994) demonstrated that lipid-derived signals can discriminate wound- and pathogen-responsive isoprenoid pathways in plants. Methyl jasmonate and the fungal elicitor arachidonic acid induced different 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes and antimicrobial isoprenoids in potato.

Application of ISR

One question that often arises when ISR is discussed is why is ISR not more widely commercialized? There are

factors favorable and unfavorable for the development and use of ISR.

Favorable factors include:

(1) Problems with the resistance of pathogens to classical pesticides.
(2) The necessity to remove some pesticides from the market, the increased testing and cost of testing to meet requirements of regulatory agencies and the lack of substitutes for removed compounds.
(3) Health and environmental problems, real and perceived, associated with pesticides and the increased popularity of 'organic crops' and 'sustainable agriculture.'
(4) The inability of pesticides to effectively control some pathogens, e.g., virus and soilborne pathogens.
(5) Classical pesticides may not be economically feasible for farmers in developing countries. In these countries the level of awareness for the safe and effective application of classical pesticides is low, thus creating dangers to human health and the environment.
(6) Resistance of the public to genetically modified plants. In ISR, foreign genes are not introduced. The 'traditional' genes for resistance in the plant are those that are expressed.
(7) ISR has a broad spectrum and is effective for a long time.
(8) Since many defenses are activated, ISR is less likely to develop resistance in pathogens.

Unfavorable factors include:

(1) Some plant pathologists still scoff at the applicability of ISR.
(2) Only high profit, patented and complex inducers make the major markets. Who champions the simple non-patented compounds?
(3) Lack of sufficient information exchange and financial support for non mega-agribusness-oriented scientists and a lack of adequate information flow to farmers and the public.
(4) Unlike classical pesticides which directly kill or inhibit development of a pathogen, ISR depends upon the expression of genes for resistance in the plant. Therefore, ISR is more subject to physiological and environmental influences for effectiveness.
(5) Public and farmer apprehension of new technologies.

Priorities for research

Priorities for research include investigations that should have and could have been completed years ago as well as those that require new information and technology for their initiation.

Which of the putative defense compounds contribute to resistance? Is the timing of their appearance important? Is the synthesis of the compounds and timing of their appearance regulated differently? More attention should be given to individual plant–pathogen interactions, to determine which inducers and their doses, as well as which putative defense compounds and the timing of their appearance, are important. This is more important for ISR than for classical pesticides.

Do plants respond to the pathogen *per se* or to the stress (metabolic perturbation) caused by the pathogen or both? What is the translocated signal(s)? What causes synthesis or release of the signal(s).

Is it possible to develop plants with enhanced ISR through plant breeding? When breeding for resistance, are we also often breeding for enhanced ISR? What are the genetic and metabolic bases of the cascade of events associated with defense compounds, ISR and sensitization (priming)? The phenomenon of enhanced defense response to infection elicited by prior infection or treatment with an inducer is key to our understanding and the utilization of ISR. It is not the continual high expression of all putative defense compounds that explains most ISR. The similarity to memory is evident and an explanation may be found in the production of latent components in the mechanisms of protein synthesis which require a common infection-initiated substance for activation and/or enhancement.

What are the molecular and practical significances of the non-specificity of the agents which elicit ISR?

Are the mechanisms for the different types of resistance (non-host, age-related, organ specific) the same or different and do they have components common to ISR? Can the genes for the different types of resistance be selectively expressed without detrimentally influencing plant development, e.g., express genes for age-related resistance without prematurely aging the plant?

What are the roles of oxidative stress, reactive oxygen species (ROS) and nitric oxide as defenses against disease and initiators of defense mechanisms? In mammals hydrogen peroxide and superoxide anion are the major microbiocides produced by circulating phagocytic leukocytes. However, hydrogen peroxide and ROS may function alone or together with NO to

enhance death of pathogens, as well as triggering transcriptional activation of plant defense genes and the hypersensitive response (Delledonne et al., 1998). Elevated levels of Ca^{2+} can enhance NO synthase activity and perhaps this partially explains the frequent association of calcium and calcium channels with resistance. In this meeting Averyanov and colleagues (2000) reported that phenanthroline and phthalocyanine metal complexes induced systemic resistance to rice blast when applied to foliage or the soil. Both compounds produced ROS. In addition, metal complexes of phthalocyanine were effective when applied to rice seeds before sowing and the ISR lasted for at least one month in seedlings. The authors suggest that increased ROS generated by the inducer result in ISR, sensitization and the hypersensitive response.

Can defensins and protegrins be utilized effectively for ISR? Defensins and protegrins are antimicrobial peptides found in plants and animals ranging from insects to humans. They are part of an innate immune system which evolved before antibodies and lymphocytes. Two French companies, RhoBio and EntoMed, will characterize highly active peptides from insects and use gene coding for the peptides to produce plants that resist diseases caused by bacteria and fungi. Since antimicrobial peptides are reported in plants, ISR may provide a mechanism to enhance production of the peptides in plants without the introduction of foreign genes.

Do DNA-binding proteins (zinc fingers) and cell-permeable polyamides have a role as agents for the selective expression of genes for ISR? Synthetic transcription factors have been developed which are designed proteins containing DNA-binding elements called zinc fingers (Borman, 2000). Similar structures are found in some natural transcription factors. Zinc fingers are independently folding domains of about 30 amino acid residues centered on a zinc ion. These proteins and synthetic polyamides can turn endogenous genes on and off in living cells in a very specific manner. Novartis has taken a license for the zinc finger protein technology to control genes in plants.

Does the progress made with Harpin indicate the presence of many similar proteins for ISR? The protein Harpin, produced by the pathogenic bacterium responsible for fire blight, *Erwinia amylovora*, induces systemic resistance in plants against many diseases caused by fungi, bacteria and viruses as well as some insects (Brasher, 2000). It also promotes root growth, reducing the need for water. The protein can be sprayed on plants before they are attacked by pathogens and it degrades so quickly that it cannot be detected within two hours of application. It is likely that Harpin is not the only protein with such a capability for ISR.

Conclusions

Though resistance and susceptibility to pathogens are often specific and biochemicals determining this specificity have specific structures and receptors (Hahn, 1996; Hutcheson, 1998), non-specific agents and multiple signals and pathways for their transduction can also induce resistance to unrelated pathogens. This makes the possibility of finding additional effective agents for ISR and disease control highly promising. The agents need not be patented, expensive or complex. Much more research is needed on the use of ISR agents to reduce dependence on chemical pesticides and enhance utilization of high-yielding plants that presently have a level of resistance that is inadequate for disease control under high pathogen pressure. ISR does not depend upon introducing genes into the plants, and it would not meet the resistance from the public engendered by genetically modified plants. ISR should be increasingly incorporated into IPM. Increased funding and information exchange is needed to better utilize and direct the rapidly emerging information concerning signals, receptors, signal transduction and gene expression for the control of plant disease.

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