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Mini review

Activation of systemic acquired disease resistance in plants

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Introduction

Plants have evolved both preformed and inducible disease resistance mechanisms that together form an effective defense against pathogen infection, with disease resulting as the rare outcome in the spectrum of plant-microbe interactions. Infectious disease can result when a pathogen is able to overcome the plant defenses, e.g. by either actively suppressing or outcompeting them.

The ability of a plant to respond to an infection is determined by genetic traits in both the host and the pathogen. Some resistance mechanisms are specific for plant cultivars and certain strains of pathogens. In these cases, plant resistance genes recognize pathogen derived molecules resulting from expression of so-called avirulence genes which often triggers a signal cascade leading to rapid host cell death [hypersensitive response, HR, for review: Dixon and Lamb, 1990; Keen, 1990; Dangl, 1992; Pryor and Ellis 1993]. Such 'gene-for-gene' relationships are part of the vertical plant resistance that usually lead to highly efficient, but very specific, plant resistance. In contrast, horizontal plant resistance provides broad spectrum disease control. The mechanisms involved in horizontal resistance include preformed physical barriers (cell walls incl. lignin, waxes, accumulation of antimicrobial metabolites etc.) as well as inducible mechanisms. Induction of resistance mechanisms occurs locally at the site of attempted penetration (e.g. hypersensitive response, phytoalexins, cell wall strengthening, etc.) as well as in distant (systemic) parts of the plant. This short review will deal only with *systemic* acquired resistance (SAR; in contrast to local acquired resistance, LAR) and will specifically focus on two aspects: (a) the systemic signalling pathway including the role of salicylic acid and (b) the use of exogenously applied agents to induce SAR.

SAR: Biological phenomenon

An important milestone in the development of the current understanding of the SAR phenomenon was the publication of a classic group of experiments by Frank A. Ross in 1961 [Ross, 1961]. He demonstrated that resistance in tobacco (var. Xanthi nc) to tobacco mosaic virus (TMV) could be enhanced by a prior infection of a single leaf a few days before the subsequent challenge. Cruickshank and Mandryk [1960] showed that stem infection of tobacco with bluemold (*Peronospora tabacina*) can lead to an enhanced resistance to foliar pathogens. Later Cohen and Kuc [1981; Madamanchi and Kuc, 1991] described some carefully controlled experiments showing that SAR in tobacco induced by bluemold requires approximately 3 weeks to develop and that heat killed conidia are unable to induce SAR. Most interestingly, the effect of SAR induction on general plant health depends on the inoculation procedure. When conidia are injected into the cambium the SAR response was linked to severe dwarfing and premature senescence. On the other hand, infection external to the cambium leads to an increase in plant weight and leaf number [Madamanchi and Kuc, 1991; Tuzun and Kuc, 1985; Tuzun et al., 1992].

In addition to tobacco, cucumber has been developed as a biological model for SAR [for review see Madamanchi and Kuc, 1991]. SAR in cucumber can be induced by various microorganisms (e.g. TNV, *Pseudomonas lachrymans, P. syringae, Colletotrichum lagenarium*). After an incubation period of a few days, plants are protected against at least 13 diseases for up to 4–6 weeks.

Along with green bean, cotton, potato, soybean, tomato, alfalfa and other dicot species [for review see Madamanchi and Kuc, 1991; Kessmann et al., 1994], *Arabidopsis thaliana* has been recently established as another model for SAR. Turnip crinkle virus, certain *Pseudomonas syringae pv. tomato* (Pst) strains [Uknes et al., 1993; Ryals et al., 1994; Cameron et al., 1994], or *Fusarium oxysporum* [Mauch-Mani and Slusarenko, 1994] were used for SAR-induction in *Arabidopsis* and induced plants exhibited resistance towards different pathogens. The fact, that SAR could be induced in *Arabidopsis* by a certain *Pst* strain without a localized hypersensitive response [Cameron et al., 1994] indicates that HR may contribute, but is not essential for SAR development. Most interestingly, *Arabidopsis* mutants were isolated that spontaneously form necrotic lesions on leaves in the absence of a pathogen. In some of these mutants lesion formation correlates with expression of SAR and they will be a valuable tool to dissect the complex pathway leading to activation of the systemic resistance response [Dietrich et al., 1994; Greenberg et al., 1994].

In monocots, biologically induced SAR has been described in barley and rice. Smith and Metraux [1991] used *Pseudomonas syringae pv. syringae* to induce SAR in rice against *Pyricularia oryzae* whereas Hwang and

Hwang and Heitefuss [1982] used a spore suspension of *Erysiphe graminis* f. sp. *hordei* to induce SAR in barley against powdery mildew.

Mechanisms of SAR

Plants often respond at the site of attempted microbial infection with a localized cell death (hypersensitive response) followed by a wide range of additional defence responses, including phytoalexin and callose formation, lignification, and cell wall cross-linking [for recent reviews see Hahlbrock and Scheel, 1989; Dixon and Lamb, 1990; Keen, 1990; Dangl, 1992]. These mechanisms are strictly localized and are not induced during the maintenance state of SAR [Ryals et al., 1994; Uknes et al., 1993; Kessmann et al., 1994].

Van Loon and Van Kammen [1970] as well as Gianinazzi et al. [1970, for review see Bowles, 1990] showed that pathogen infection of tobacco with tobacco mosaic virus (TMV) leads to the accumulation of a set of so called 'pathogenesis-related' (PR) proteins. Acidic, extracellular forms of these PR-proteins accumulate during the onset of resistance indicating that they may play a role in SAR. Ward et al. [1991] showed that nine gene families are coordinately induced both in infected, as well as in distal, untreated leaves after local infection of tobacco with TMV. Some of these 'SAR genes' have been characterized as β-1,3 glucanases, chitinases and thaumatin-like proteins [Ward et al., 1991; Bowles, 1990; Ryals et al., 1994]. The role of the PR genes in the maintenance of SAR was further supported by results with transgenic plants in which some of the genes were constitutively expressed resulting in resistance towards certain pathogens [for review: Ryals et al., 1994].

During the SAR response in cucumber, chitinase activity is systemically induced [Metraux and Boller, 1986]. In *Arabidopsis*, a number of genes encoding PR-proteins are induced after biological or chemical induction of SAR, which show some homology to the PR genes described for tobacco [Uknes et al., 1993; Ryals et al., 1994].

Histological studies in cucumber have revealed that pathogen penetration, but not germination and appressoria formation is affected by SAR. Enhanced papilla formation, and lignification is believed to be causally involved in SAR [Xuei et al., 1988; Madamanchi and Kuc, 1991].

In rice, lipoxygenase is systemically induced after induction of SAR by infection with *Pseudomonas syringae* infection but well-known PR-proteins from dicot species, such as chitinase or β-1,3-glucanses, did not show increased activity [Smith and Metraux, 1991; Hofmann et al., 1994]. This supports the hypothesis that antimicrobial fatty acids derived from a lipoxygenase dependant pathway are involved in inducible as well as genetically derived resistance in rice to the leaf blast pathogen, *Pyricularia oryzae* [Kessmann et al., 1994, see also chapter on chemically induced

SAR]. The biologically induced SAR response in other monocots has not been studied in great detail mainly because efficient protocols for biological induction of SAR are still lacking. Therefore, SAR work in monocots has been mainly based on the application of chemical inducers (see below).

The role of salicylic acid in SAR

White showed in 1979 that exogenously applied salicylic acid (SA) and related benzoic acid derivatives result in the accumulation of PR-proteins and protection of tobacco against TMV. In independent studies on tobacco and cucumber it was recently shown that SA accumulates throughout the plant after induction of SAR by a local infection with pathogens [Malamy et al., 1990; Enyedi et al., 1992; Metraux et al., 1990]. Since earlier experiments by Kuc and coworkers showed that the signal for SAR is systemically transported in the phloem sap [Madamanchi and Kuc, 1991], and because exogenously applied SA can induce SAR and synthesis of PR-proteins [Ward et al., 1991; Malamy et al., 1990] it was thought that SA may be the systemic signal for the induction of SAR throughout the plant. Raskin and coworkers showed that in tobacco the endogenous levels of SA after the onset of SAR may be sufficient to induce resistance [Enyedi et al., 1992]. Furthermore, Ward et al., [1991] confirmed that exogenously applied SA results in the induction of transcription of the entire set of SAR gene families that are also activated through the biological induction of SAR.

Recently, tobacco was transformed with the *nahG* gene from *Pseudomonas putida* which encodes a salicylate hydroxylase, an enzyme that catalyzes the degradation of SA to the non-inducing metabolite catechol [Gaffney et al., 1993]. The *nahG*-transgenic plants were shown to express the nahG-gene and did not accumulate SA after the onset of SAR or exhibit the SAR response. These results confirm the role of SA in the signal transduction pathway that leads to the induction of SAR.

Rasmussen et al. [1991] have examined the hypothesis that SA is the systemic signal, by using elegant experiments with cucumber. They demonstrated that the removal of the primary leaf after SAR induction by localized *Pseudomonas syringae* infection several hours before significant accumulation of SA had no effect on SAR induction. Using the *nahG*-tobacco plants Vernooij et al. [1994] clearly showed that SA is not the systemic signal responsible for the induction of SAR. When scions from *nahG*-plants were grafted onto Xanthi nc rootstocks, there was no SAR induction in the *nahG* scion despite the presence of SA in the Xanthi nc rootstock after SAR induction. Conversely, the reciprocal grafts (Xanthi nc on *nahG* tobacco) showed that a local infection on the *nahG* plants led to a typical SAR response in the Xanthi scion even though no significant SA increase was detected in *nahG* rootstock. From these results it is clear

that SA is not the systemic signal for SAR but is most likely involved in transforming an as yet unidentified long distance signal into the resistance state in systemic tissue. The crucial role of SA in plant defense has been further studied by Klessig and coworkers [Chen and Klessig, 1991; Chen et al., 1994] who isolated a salicylic acid binding protein from tobacco which has catalase activity. However, although the role of oxygen radicals has frequently been shown to be involved in plant disease development [for review see Tzeng and DeVay, 1993], further experiments are required to define their precise role in the SAR response.

Induction of SAR by chemicals, biologicals and natural products

In principle, the SAR system presents some interesting opportunities for the control of plant diseases in agricultural practice and for enhancing our basic knowledge of disease resistance in plants. First, disease resistance that results from the SAR response is a natural defense phenomenon. Second, the biological models of SAR in cucumber and tobacco have demonstrated that SAR can lead to long lasting and broad spectrum disease control. Furthermore, there is ample evidence that SAR is based on multiple mechanisms which makes it less likely that pathogens can readily develop resistance to this control measure.

SAR can be induced by microorganisms, microbial extracts or defined chemicals. In this chapter we discuss the current knowledge as well as the opportunities offered by the different inducers.

The practical use of necrogenic microorganisms to induce SAR, similar to the 'biological model' of SAR (see above), seems to be feasible but is most likely restricted to selected crops grown on a small scale. However, no product has been introduced to the market with this mode of action. Some plant growth promoting rhizobacteria (PGPR) have recently been described which induce SAR against foliar diseases in tobacco and cucumber [Wei et al., 1991; Maurhofer et al., 1994], and to *Fusarium* wilt of carnation [Van Peer et al., 1991]. This strategy offers an exciting potential since disease control and increased plant health can be combined in a single seed treatment with naturally occurring microorganisms. At this time there is very little information on the biological mechanisms that are the basis for disease resistance induced by rhizobacteria. A recent report examined the induction of SAR by a *Pseudomonas fluorescens* biocontrol strain [Maurhofer et al., 1994]. It was demonstrated that in tobacco grown in soil inoculated with this bacterium the classical symptoms of SAR induction, including the appearance of PR-proteins and salicylic acid accumulation, were noted in the tobacco leaf tissue. This response was absent in tobacco grown in uninoculated soil, or soil inoculated with a different wild-type strain that was known not to induce SAR. Furthermore, induction of the SAR response was correlated with increased resistance to TNV.

Low molecular weight chemicals that are able to induce SAR would offer a great potential for disease control in economically important crops. Three criteria need to be fullfilled before an agent can be classified as SAR-inducing or 'plant activator':

- the treated plants are resistant to the same spectrum of diseases as those in which SAR is induced biologically
- lack of direct antimicrobial activity; no conversion of the compound *in vivo* into antimicrobial metabolites
- induction of the same preinfectional biochemical processes as seen in *systemic* plant tissues after biological induction of SAR.

It is possible that compounds will be discovered that induce SAR in addition to having a direct antimicrobial activity. A relatively simple set of experiments can be done to confirm this situation. First, pathogen strains resistant to the compound are selected *in vitro* (or *in vivo* in case of biotrophic pathogens). Second, dose-response studies using sensitive and fungicide-resistant pathogen-strains would indicate if the postulated resistance-induction actually plays a significant role in the disease control.

A number of well known fungicides were described to have additional, resistance inducing activity. Fosethyl-Al [a phosphonic acid derivative, Fettouche et al., 1981], metalaxyl [a phenylamide, Ward, 1984] and even triazoles are among this group [Hauthal, 1993]. However, all of these agents do not fullfill the criteria listed above for plant activators. As an example, fosethyl-Al shows weak *in vitro* activity only in phosphate enriched, but not in low-phosphate containing media [Farih et al., 1981; Fenn and Coffey, 1984]. On the other hand, metabolic inhibitors like glyphosate decreased the effectiveness of fosethyl-Al indicating that plant metabolism may contribute to the activity of this fungicide [Fettouche et al., 1981]. However, fungal strains selected for insensitivity to fosethyl-Al *in vitro* are also no longer controlled *in vivo* [Fenn and Coffey, 1985; Dolan and Coffey, 1988]. Furthermore, fosethyl-Al does not induce molecular markers of SAR such as chitinase in cucumber or PR1a in tobacco [H. Kessmann, unpublished]. The latter two points show that fosethyl-Al does not induce SAR in the plant which significantly contributes to its activity.

Probenazol is widely used to protect rice against *Pyricularia oryzae* and, to a lesser extent, *Xanthomonas oryzae*. However, this compound does not show significant direct activity towards these two pathogens. In treated rice, accumulation of fungitoxic fatty acids was described, including an increase in the activity of enzymes involved in the biosynthesis of these compounds [Iwata et al., 1980; Kato et al., 1984; Shimura et al., 1983]. Probenazol treatment led to an increased 'respiratory burst' and accumulation of superoxide anion radicals after *Pyricularia* infection when compared to untreated plants [Sekizawa et al., 1985].

2,6-dichloroisonicotinic acid (INA) was discovered as a plant activator since it demonstrated the criteria of SAR-induction described above [Metraux et al., 1991, for review see Kessmann et al., 1994]. INA does not exhibit significant direct *in vitro* activity, but it protects cucumber against the same spectrum of diseases as does biological SAR-induction. It induces class III chitinase, a molecular marker for SAR in cucumber [Metraux et al., 1991]. INA effectively induced resistance in the field against major fungal and bacterial pathogens on various crops [Metraux et al., 1991; Staub et al., 1993; Kessmann et al., 1994]. Molecular studies with tobacco showed that INA induces the same set of genes as a local infection with TMV and corresponding studies with cucumber and Arabidopsis confirmed that INA is indeed able to mimic the biological induction of SAR [Uknes et al., 1993; Ryals et al., 1994; Ward et al., 1991; Kessmann et al., 1994, authors unpublished data]. On barley, INA treatment led to an increased resistance against powdery mildew which looks histologically like a phenocopy of the mlg-mediated resistance in genetically caused powdery mildew resistance [K. Kogel, pers. comm.]. INA induces the accumulation of thionin, a 6 kD peptide with antimicrobial properties [Wasternack et al., 1994]. Thionin is a member of the 'jasmonic acid induced proteins' (JIP) but other members of the JIP family are not induced, indicating a complex regulation of thionin gene expression. In rice, INA induces lipoxygenase activity [Hofmann et al., 1994]. This enzyme activity is also systemically induced after induction of SAR by a local infection with *Pseudomonas syringae* which confirms that INA can mimic the biological induction of SAR.

Histological studies with *Arabidopsis* showed that INA treated plants respond to infection with downy mildew (*Peronospora parasitica*) with a single cell necrosis at the site of attempted penetration [Uknes et al., 1993]. When lower INA concentrations were used some hyphae successfully invaded the leaves but infection also finally stops at later stages. As shown by Kauss and coworkers [Kauss et al., 1992], INA is able to somehow sensitize plant tissue to respond faster to microbial attack. They showed that INA treated parsley cell cultures accumulate certain phenylpropanoids more rapidly after elicitor treatment than control cultures without INA application. Seguchi et al. [1992] described a similar sensitizing effect for rice using a closely related INA analogue (N-cyanomethyl-2-chloroisonicotinamide). Chemically treated and *Pyricularia oryzae*-infected rice plants showed an higher increase in lipoxygenase and peroxidase activity compared to non-treated but infected controls. The molecular basis of such a sensitizing effect is unknown but may play an important role in the SAR response.

In addition to the agents described above, a wide range of chemicals and natural products have been described to have resistance-inducing properties. This list includes jasmonic acid, ethylene, β-amino acids, unsaturated fatty acids, silicon, oxalate, phosphate, and DL-dodecylester HCl [see Kessmann

et al., 1994 for ref.]. Some of these agents may induce a SAR response by causing a localized necrosis, while in other cases direct antimicrobial activity is likely. None of the compounds *specifically* induce only the systemic disease resistance response (authors unpublished data). However, the fact that an agent does not specifically activate the biological SAR response regarding biochemical changes and spectrum of protection does not rule out the possibility that other, yet unknown, resistance mechanisms are activated.

Conclusion and outlook

Utilization of SAR as an inducible, broad spectrum, and long-lasting disease control mechanism that is most likely present in all plant species, would offer a valuable new option for practical disease control as well as plant health management in general.

However, our understanding of SAR is still primitive in both applied and basic aspects. For practical application, agents have to be identified and developed which induce SAR with good crop tolerance in economically important crops. These agents should not only be seen as disease control agents but also as compounds which affect plant health in general.

For basic research, the molecular bases of SAR, including the signalling pathway, are most interesting questions. Although *Arabidopsis thaliana* is an excellent model system, work on a variety of plant species is highly desirable.

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