

SYSTEMIC ACQUIRED RESISTANCE

Induction of Plant Defenses by Artificial Inoculation with Microbes or by Treatment with Chemicals

As discussed earlier, plants do not naturally produce antibodies against their pathogens, and most of their biochemical defenses are inactive until they are mobilized by some signal transmitted from an attacking pathogen. It has been known for many years, however, that plants develop a generalized resistance in response to infection by a pathogen or to treatment with certain natural or synthetic chemical compounds. Induced resistance is at first localized around the point of plant necrosis caused by infection by the pathogen or by the chemical, and it is then called local acquired resistance (Fig.). Subsequently, resistance spreads systemically and develops in distal, untreated parts of the plant and is called systemic acquired resistance (Fig.). It is known now that several chemical compounds, e.g., salicylic acid, arachidonic acid, and 2,6-dichloroisonicotinic acid, may induce localized and systemic resistance in plants at levels not causing tissue necrosis. Jasmonic acid is another type of compound, derived primarily from oxidation of fatty acids, which leads to systemic acquired resistance, often in cooperation with salicylic acid and ethylene, leading to the production

of defenses. Probenazole, a synthetic chemical used in Asia for the control of rice blast disease caused by the fungus *Magnaporthe grisea*, has been shown to act upstream from the salicylic acid transcribing gene and, thereby, causing accumulation of salicylic acid. Probenazole induces systemic acquired resistance in rice against rice blast, in tomato against the bacterial pathogen *P. syringae* pv. *tabaci*, and in tobacco against the *tobacco mosaic virus*. Similarly, riboflavin was shown to induce systemic acquired resistance but it activates it in a distinct manner not involving salicylic acid. Such chemicals may be effective in inducing resistance in plants when they are applied through the roots, as a foliar spray (Fig.), or by stem injection. Local acquired resistance is induced, for example, in a 1 to 2mm zone around local lesions caused by tobacco mosaic virus on hypersensitive tobacco varieties and probably in other host–pathogen combinations. Local acquired

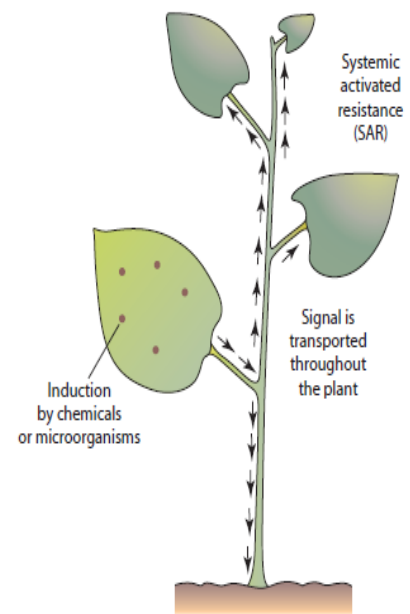


FIGURE 6-24 Principle of systemic activated (or acquired) resistance. A leaf treated with certain chemicals or with pathogens causing necrotic lesions produces a signal compound(s) that is transported systemically throughout the plant and activates its defense mechanisms, making the entire plant resistant to subsequent infections.

resistance results in near absence of lesions immediately next to the existing lesion and in smaller and fewer local lesions developing farther out from the existing local lesions when inoculations are made at least 2–3 days after the primary infection. Local acquired resistance may play a role in natural infections by limiting the number and size of lesions per leaf unit area. Systemic acquired resistance acts non-specifically throughout the plant and reduces the severity of disease caused by all classes of pathogens, including normally virulent ones. It has been observed in many dicot and monocot plants, but has been studied most in cucurbits, solanaceous plants, legumes, and gramineous plants following infection with appropriate fungi, bacteria, and viruses. Systemic acquired resistance is certainly produced in plants following expression of the hypersensitive response (Fig.). Localized infections of young plants, e.g., cucumber with a fungus (*Colletotrichum lagenarium*), a bacterium (*Pseudomonas lachrymans*), or a virus (*tobacco necrosis virus*), lead within a few days' time to broad-spectrum, systemic acquired resistance to at least 13 diseases caused by fungi, bacteria, and viruses. A single inducing infection protects cucumber from all pathogens tested for 4 to 6 weeks; when a second, booster inoculation is made 2 to 3 weeks after the primary infection, the plant acquires season-long resistance to all tested pathogens. The degree of systemic acquired resistance seems to correlate well with the number of lesions produced on the induced leaf until a saturation point is reached. Systemic acquired resistance, however, cannot be induced after the onset of flowering and fruiting in the host plant.

Systemic acquired resistance is characterized by the coordinate induction in uninfected leaves of inoculated plants of at least nine families of genes now known as systemic acquired resistance genes. Products of several SAR genes, e.g., β -1,3-glucanases, chitinases, cysteine rich proteins related to thaumatin, and PR-1 proteins, have direct antimicrobial activity or are closely related to classes of antimicrobial proteins. The set of SAR genes that are induced in a plant may vary with the plant species. Although systemic acquired resistance does not affect spore germination and appressorium formation, penetration is reduced drastically in systemically induced resistant tissue, probably as a result of formation beneath the appressoria of papilla-like material that becomes impregnated quickly with lignin and silicon. In some host–pathogen systems, systemic acquired resistance is characterized by the induction of peroxidase and lipoxygenase activities that lead to the production of fatty acid derivatives, which exhibit strong antimicrobial activity. In plants exhibiting systemic acquired resistance in response to plant

defense activators such as salicylic acid, bacterial growth and multiplication are reduced drastically (Fig. 6-25), although salicylic acid is tolerated by the bacteria at concentrations much higher than those found in the treated plant. The mechanism of signal transduction in triggering systemic acquired resistance is still being studied. Salicylic acid seems to be involved in both the hypersensitive response and the systemic acquired resistance but may not be the signal that induces systemic acquired resistance (Fig. 6-26).

Salicylic acid is present in the phloem of plants after the primary inoculation but before the onset of acquired resistance; its concentration levels correlate with the induction of PR proteins. External application of salicylic acid activates the same sets of SAR genes that are expressed after SAR induction by pathogens. Nevertheless, other evidence suggests that a signal other than salicylic acid is responsible for the systemic expression of systemic acquired resistance, but salicylic acid must be present for the real signal to be transduced into gene expression and acquired resistance. It had been reported earlier that salicylic acid reacts with an oxidative enzyme (catalase) and generates reactive oxygen radicals. This had been suggested as a mechanism by which the plant cell reacts to salicylic acid signalling and induces systemic acquired resistance (Fig). This notion, however, is no longer accepted. The onset of systemic acquired resistance in *Arabidopsis* is controlled by a single gene, NPR1, which also affects local acquired resistance, i.e., the ability of plants to restrict the spread of virulent pathogen infections. Disruption of the gene produces mutant plants that fail to respond to a variety of SAR-inducing treatments, they display minimum expression of pathogenesis-related genes, and they exhibit increased susceptibility to infections by allowing lesions to grow and spread much more than in non-mutant plants. The NPR1 gene encodes a novel protein that contains ankyrin repeats and these repeats are needed for NPR1 to function. Also, when the NPR1 gene was inserted into a mutant that had lost the NPR1 gene, the mutant not only reacquired the responsiveness to SAR induction in terms of expression of PR genes and resistance to infection, the mutant transgenic plants actually became more resistant to infection by the bacterium *P. syringae* even in the absence of SAR induction. It was further shown that induction of NPR1 leads to overexpression of the NPR1-coded protein and this, in turn, induces the expression of numerous downstream pathogenesis related genes. NPR1 seems to confer resistance to some bacterial and oomycete diseases in a dosage-dependent manner. The increased resistance provided by the overexpression of NPR1 seems to occur without any detrimental effects on the plants. The induction of systemic acquired resistance through external application of salicylic acid

raised the very important question of whether salicylic acid or other chemical compounds could be used to artificially induce systemic acquired resistance in plants against their numerous pathogens. Unfortunately, externally applied salicylic acid is not translocated efficiently in the plant and, in addition, salicylic acid is strongly phytotoxic when applied at even slightly higher levels above the level required for efficacy. Therefore, salicylic acid per se has not been considered for use as a practical solution for disease control. So far, in addition to salicylic acid, derivatives of isonicotinic acid and benzothiazoles have been shown to induce systemic acquired resistance in plants against a variety of pathogens. As a matter of fact, the benzothiazole (BTH) is being used commercially. When the three compounds were used separately to protect barley against the powdery mildew fungus, they did so by inducing differential expression of a number of newly identified defense response genes, including genes encoding a lipoxygenase, a thionin, an acid phosphatase, a Ca^{2+} -binding protein, a serine proteinase inhibitor, a fatty acid desaturase, and several other proteins whose function had not been determined. Of the three chemicals, INA and BTH were more potent inducers of both gene expression and resistance. In experiments in which cowpea seeds were treated with BTH and were then inoculated with the anthracnose fungus *Colletotrichum destructivum*, the young cowpea plants were effectively protected from infection through a hypersensitive response of cells coming in contact with the pathogen. In addition, the plants showed a rapid transient increase of the phenoloxidizing enzymes phenylalanine ammonia lyase and chalcone isomerase while there was an early, accelerated accumulation of the phytoalexins kievitone and phaseollidin and of several other proteins. It was concluded that BTH protects cowpea seedlings by potentiating an early defense response rather than by altering the constitutive resistance of the tissues. The SAR-activating compounds induce expression of the same set of SAR genes that are induced either by salicylic acid or by various infectious agents and, in addition, seem to prime or sensitize plants to respond faster and with additional defense reactions than those characteristic of SAR genes. Isonicotinic acid, however, functions even in transgenic plants that are unable to accumulate salicylic acid. Apparently, therefore, isonicotinic acid triggers the signal transduction pathway that leads to SAR by acting either at the same site as salicylic acid or downstream from it. Salicylic acid and isonicotinic acid are true SAR activators because not only do they induce resistance to the same spectrum of pathogens and induce expression of the same genes as pathogens, but these chemicals have no antimicrobial activity. Several other chemical compounds, such as the fungicides fosethyl-

Al, metalaxyl, and triazoles, appear to have some resistance-inducing activity. The fungicide–bactericide probenazole is only slightly toxic *in vitro*, but induces various defense responses in rice plants, including an oxidative burst and appearance of reactive oxygen radicals, as well as significant accumulation of antimicrobial factors such as fungitoxic unsaturated fatty acids. A large number of other compounds, and also many microorganisms, have been tested for their ability to induce systemic acquired resistance in plants, but so far none has proved effective. This area of research, however, has a tremendous commercial potential, and therefore the search for SAR inducing compounds is likely to continue and, actually, to increase.