chapter five

HOW PLANTS DEFEND THEMSELVES *against* PATHOGENS

Each plant species is affected by approximately 100 different kinds of fungi, bacteria, mollicutes, viruses, and nematodes. Frequently, a single plant is attacked by hundreds, thousands, and, in the leafspot diseases of large trees probably hundreds of thousands of individuals of a single kind of pathogen. Although such plants may suffer damage to a lesser or greater extent, many survive all these attacks and, not uncommonly, manage to grow well and to produce appreciable yields.

In general, plants defend themselves against pathogens by a combination of weapons from two arsenals: (1) structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant and (2) biochemical reactions that take place in the cells and tissues of the plant and produce substances which either are toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant. The combinations of structural characteristics and biochemical reactions employed in the defense of plants are different in different host–pathogen systems. In addition, even within the same host and pathogen, the combinations vary with the age of the plant, the kind of plant organ and tissue attacked, the nutritional condition of the plant, and the weather conditions.

PREEXISTING STRUCTURAL AND CHEMICAL DEFENSES

Preexisting Defense Structures

The first line of defense of a plant against pathogens i its surface, which the pathogen must penetrate if it is t cause infection. Some structural defenses are present i the plant even before the pathogen comes in contact wit the plant. Such structures include the amount and qua ity of wax and cuticle that cover the epidermal cells, th structure of the epidermal cell walls, the size, location and shapes of stomata and lenticels, and the presence o the plant of tissues made of thick-walled cells that hin der the advance of the pathogen.

Waxes on leaf and fruit surfaces form a water-repe lent surface and thereby prevent the formation of a film of water on which pathogens might be deposited and germinate (fungi) or multiply (bacteria). A thick mat o hairs on a plant surface may also exert a similar wate repelling effect and may reduce infection.

A thick cuticle may increase resistance to infection diseases in which the pathogen enters its host only throu direct penetration. Cuticle thickness, however, is not

ways correlated with resistance, and many plant varieties with cuticles of considerable thickness are easily invaded by directly penetrating pathogens.

The thickness and toughness of the outer wall of epidermal cells are apparently important factors in the resistance of some plants to certain pathogens. Thick, tough walls of epidermal cells make direct penetration by fungal pathogens difficult or impossible. Plants with such walls are often resistant, although if the pathogen is introduced beyond the epidermis of the same plants by means of a wound, the inner tissues of the plant are easily invaded by the pathogen.

Many pathogenic fungi and bacteria enter plants only through stomata. Although the majority of pathogens can force their way through closed stomata, some, like the stem rust of wheat, can enter only when stomata are open. Thus, some wheat varieties, in which the stomata open late in the day, are resistant because the germ tubes of spores germinating in the night dew desiccate owing to evaporation of the dew before the stomata begin to open. The structure of the stomata, for example, a very narrow entrance and broad, elevated guard cells, may also confer resistance to some varieties against certain of their bacterial pathogens.

The cell walls of the tissues being invaded vary in thickness and toughness and may sometimes inhibit the advance of the pathogen. The presence, in particular, of bundles or extended areas of sclerenchyma cells, such as are found in the stems of many cereal crops, may stop the further spread of pathogens like the stem rust fungi. Also, the xylem, bundle sheath, and sclerenchyma cells of the leaf veins effectively block the spread of some fungal, bacterial, and nematode pathogens that cause the various "angular" leaf spots because of their spread only into areas between, but not across, veins.

Preexisting Chemical Defenses

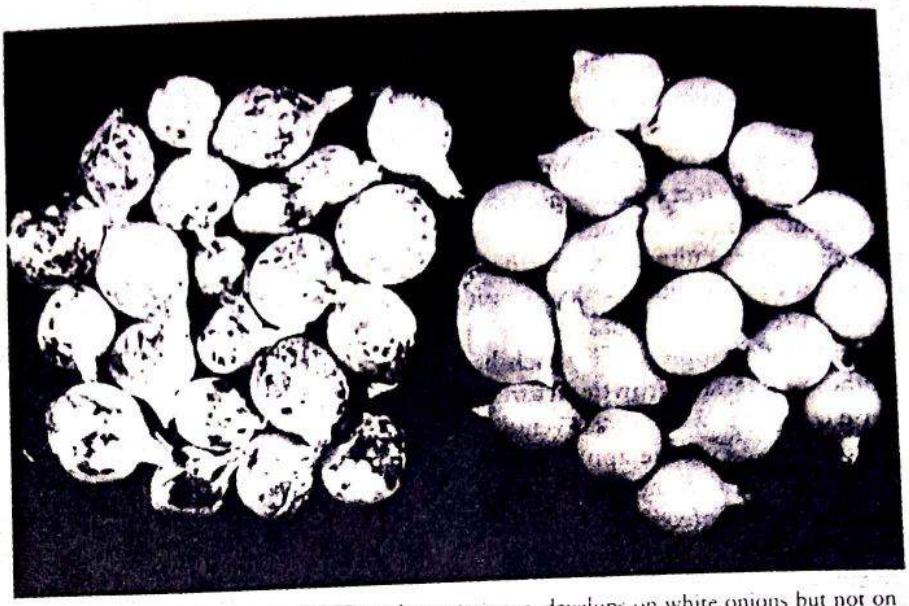
Although structural characteristics may provide a plant with various degrees of defense against attacking pathogens, it is clear that the resistance of a plant against pathogen attacks depends not so much on its structural barriers as on the substances produced in its cells before or after infection. This is apparent from the fact that a particular pathogen will not infect certain plant varieties even though no structural barriers of any kind seem to be present or to form in these varieties. Similarly, in resistant varieties, the rate of disease development soon slows down, and finally, in the absence of structural defenses, the disease is completely checked. Moreover, many pathogens that enter nonhost plants naturally, or that are introduced into nonhost plants artificially, fail to cause infection although no apparent visible host structures inhibit them from doing so. These examples suggest that defense mechanisms of a chemical rather than a structural nature are responsible for the resistance to infection exhibited by plants against certain pathogens.

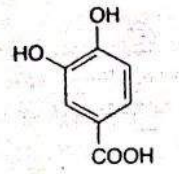
Inhibitors Released by the Plant in Its Environment

Plants exude a variety of substances through the surface of their aboveground parts as well as through the surface of their roots. Some of the compounds released by certain kinds of plants, however, seem to have an inhibitory action against certain pathogens. Fungitoxic exudates on the leaves of some plants, for example, tomato and sugar beet, seem to be present in sufficient concentrations to inhibit the germination of spores of the fungi *Botrytis* and *Cercospora*, respectively, that may be present in dew or rain droplets on these leaves. Similarly, in the case of onion smudge, caused by the fungus *Colletotrichum circinans*, resistant varieties generally have red scales and contain, in addition to the red pigments, the phenolic compounds protocatechuic acid and catechol. In the presence of water drops or soil moisture containing conidia of the onion smudge fungus on the surface of red onions, these two fungitoxic substances diffuse into the liquid, inhibit the germination of the conidia, and cause them to burst, thus protecting the plant from infection. Both the fungitoxic exudates and the inhibition of infection are missing in white-scaled, susceptible onion varieties (Figure 5-1).

Inhibitors Present in Plant Cells before Infection

It is becoming increasingly apparent that some plants are resistant to diseases caused by certain pathogens because of an inhibitory compound present in the cell before infection. Several phenolic compounds, tannins, and some fatty acid-like compounds such as dienes, which are present in high concentrations in cells of young fruits, leaves, or seeds, have been proposed as responsible for the resistance of young tissues to pathogenic microorganisms such as *Botrytis*. Many such compounds are potent inhibitors of many hydrolytic enzymes, including the pectolytic macerating enzymes of plant pathogens. As the young tissues grow older, their inhibitor content and their resistance to infection decrease steadily. Several other types of preformed compounds, such as the saponins (glycosylated steroidal or triterpenoid compounds) tomatine in tomato and avenacin in oats, not only have antifungal membranolytic activity, they actually exclude fungal pathogens, which lack enzymes (saponinases) that break down the saponin, from infecting the host. In this way, the presence or absence of saponin in a host and of saponinase in a fungus determines the host range of the fungus.





Protocatechuic acid

FIGURE 5-1 Onion smudge, caused by the fungus *Colletotrichum circinans*, develops on white onions but not on colored ones which, in addition to the red or yellow pigment, also contain the phenolics protocatechuic acid and catechol, both of which are toxic to the fungus. (Photo courtesy G. W. Simone.) See also Color Figures.

In addition to the simple molecule antifungal compounds listed above, several preformed plant proteins have been reported to act as inhibitors of pathogen proteinases or of hydrolytic enzymes involved in host cell wall degradation, to inactivate foreign ribosomes, or to increase the permeability of the plasma membranes of fungi. Similarly, **lectins**, which are proteins that bind specifically to certain sugars and occur in large concentrations in many types of seeds, cause lysis and growth inhibition of many fungi. On the other hand, plant surface cells also contain variable amounts of hydrolytic enzymes, some of which, such as glucanases and chitinases, may cause breakdown of pathogen cell wall components and thereby contribute to resistance to infection. The importance of either of these types of inhibitors to disease resistance is not currently known, but some of these substances are known to increase rapidly on infection and are considered to play an important role in the defense of plants to infection.

Defense through Lack of Essential Factors

Lack of Recognition between Host and Pathogen

A plant species either is a host for a particular pathogen, for example, wheat for the wheat stem rust fungus, or it is not a host for that pathogen, for example, tomato for wheat stem rust fungus. How does a pathogen recognize that the plant with which it comes in contact is a host or nonhost? Plants of a species or variety may not become infected by a pathogen if their surface cells lack specific recognition factors (specific molecules or structures) that can be recognized by the pathogen. If the pathogen does not recognize the plant as one of its host plants, it may not become attached to the plant or may not produce infection substances, such as enzymes, or structures, such as appressoria, penetration pegs, and haustoria, necessary for the establishment of infection. It is not known what types of molecules or structures are involved in the recognition of plants and pathogens, but it is thought that they probably include various types of oligosaccharides and polysaccharides, and proteins or glycoproteins. Also, it is not known to what extent these recognition phenomena are responsible for the success or failure of initiation of infection in any particular host–pathogen combination.

Lack of Host Receptors and Sensitive Sites for Toxins

In host–pathogen combinations in which the pathogen (usually a fungus) produces a host-specific toxin, the toxin, which is responsible for the symptoms, is thought to attach to and react with specific receptors or sensitive sites in the cell. Only plants that have such sensitive receptors or sites become diseased. Plants of other varieties or species that lack such receptors or sites remain resistant to the toxin and develop no symptoms.

Lack of Essential Substances for the Pathogen

Species or varieties of plants that for some reason do not produce one of the substances essential for the survival of an obligate parasite, or for development of in-

fection by any parasite, would be resistant to the pathogen that requires it. Thus, for *Rhizoctonia* to infect a plant, it needs to obtain from the plant a substance necessary for formation of a hyphal cushion from which the fungus sends into the plant its penetration hyphae. In plants in which this substance is apparently lacking, cushions do not form, infection does not occur, and the plants are resistant. The fungus does not normally form hyphal cushions in pure cultures but forms them when extracts from a susceptible but not a resistant plant are added to the culture. Also, certain mutants of *Venturia inaequalis*, the cause of apple scab, which had lost the ability to synthesize a certain growth factor, also lost the ability to cause infection. When, however, the particular growth factor is sprayed on the apple leaves during inoculation with the mutant, the mutant not only survives but it also causes infection. The advance of the infection, though, continues only as long as the growth factor is supplied externally to the mutant. In some host–pathogen combinations, disease develops but the amount of disease may be reduced by the fact that certain host substances are present in lower concentrations. For example, bacterial soft rot of potatoes, caused by *Erwinia carotovora var. atroseptica*, is less severe on potatoes with low reducing sugar content than on potatoes high in reducing sugars.

INDUCED STRUCTURAL AND BIOCHEMICAL DEFENSES

Recognition of the Pathogen by the Host Plant

Early recognition of the pathogen by the plant is very important if the plant is to mobilize the available biochemical and structural defenses to protect itself from the pathogen. The plant apparently begins to receive signal molecules, that is, molecules that indicate the presence of a pathogen, as soon as the pathogen establishes physical contact with the plant (Figure 5-2). Various pathogens, especially fungi and bacteria, release in their immediate environment a variety of substances such as glycoproteins, carbohydrates, fatty acids, and peptides. In various host–pathogen combinations, certain of these substances act as pathogen elicitors of recognition by the plant. In many cases, in which host enzymes break down a portion of the polysaccharides making up the pathogen surface, or pathogen enzymes break down a portion of the plant surface polysaccharides, the released oligomers or monomers of the polysaccarides act as recognition elicitors for the plant. The location of the host receptors that recognize the pathogen elicitors is not generally known, but several of those studied appear to exist outside or on the cell membrane whereas others apparently occur intracellularly. Once a particular plant molecule recognizes and reacts with a molecule (elicitor) derived from a pathogen, it is assumed that the plant "recognizes" the pathogen. Following such recognition, a series of biochemical reactions and structural changes are set in motion in the plant cell(s) in an effort to fend off the pathogen and its enzymes, toxins, etc. How quickly the plant recognizes the (presence of a) pathogen and how quickly it can send out its alarm message(s) and mobilize its defenses determine whether any infection will take place at all (as in the hypersensitive response) or how much the pathogen will develop, that is, how severe the symptoms (leaf spots, stem, fruit, or root lesions, etc.) will be, before the host defenses finally stop the further development of the pathogen.

Transmission of the Alarm Signal to Host Defense Providers: Signal Transduction

Once the pathogen-derived elicitors are recognized by the host, a series of alarm signals are sent out to host cell proteins and to nuclear genes causing them to become activated, to produce substances inhibitory to the pathogen, and to mobilize themselves or their products toward the point of cell attack by the pathogen. Some of the alarm substances and signal transductions are only intracellular, but in many cases the signal is also transmitted to several adjacent cells and, apparently, is often transmitted systemically to most or all of the plant.

The chemical nature of the transmitted signal molecules is not known with certainty in any host–pathogen combination. Several types of molecules have been implicated in intracellular signal transduction. The most common such signal transducers appear to be various protein kinases, calcium ions, phosphorylases and phospholipases, ATPases, hydrogen peroxide (H_2O_2), ethylene, and others. Systemic signal transduction, that leads to systemic acquired resistance, is thought to be carried out by salicylic acid, oligogalacturonides released from plant cell walls, jasmonic acid, systemin, fatty acids, ethylene, and others. Some synthetic chemicals, such as salicylic acid, and the synthetic dichloroisonicotinic acid, also activate the signaling pathway leading to systemic acquired resistance against several diverse types of plant pathogenic viruses, bacteria, and fungi.

Induced Structural Defenses

In spite of the preformed superficial or internal defense structures of host plants, most pathogens manage to penetrate their hosts and to produce various degrees of

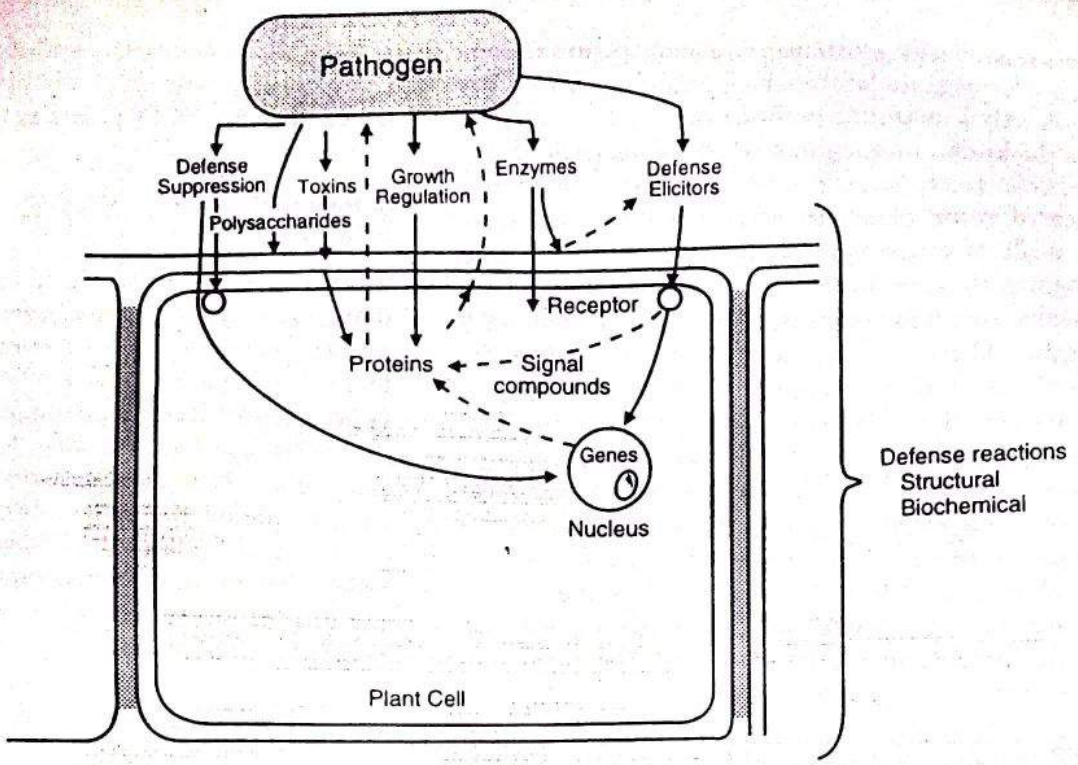


FIGURE 5-2 Schematic representation of pathogen interactions with host plant cells. Depending on its genetic makeup, the plant cell may react with numerous defenses that may include cell wall structural defenses (waxes, cutin, suberin, lignin, phenolics, cellulose, callose, cell wall proteins) or biochemical wall, membrane, cytoplasm, and nucleus defense reactions. The latter may involve bursts of oxidative reactions, production of elicitors, hypersensitive cell death, ethylene, phytoalexins, pathogenesis-related proteins (hydrolytic enzymes, β-1,3-glucanases, chitinases), inhibitors (thionins, proteinase inhibitors, thaumatinlike proteins), etc.

infection. Even after the pathogen has penetrated the preformed defense structures, however, plants usually respond by forming one or more types of structures that are more or less successful in defending the plant from further pathogen invasion. Some of the defense structures formed involve the cytoplasm of the cells under attack, and the process is called **cytoplasmic defense reaction**; others involve the walls of invaded cells and are called **cell wall defense structures**; still others involve tissues ahead of the pathogen (deeper into the plant) and are called **histological defense structures**. Finally, the death of the invaded cell may protect the plant from further invasion, and this is called the **necrotic or hypersensitive defense reaction.**

Cytoplasmic Defense Reaction

In a few cases of slowly growing, weakly pathogenic fungi, such as weakly pathogenic *Armillaria* strains and the mycorrhizal fungi, that induce chronic diseases or nearly symbiotic conditions, the plant cell cytoplasm surrounds the clump of hyphae, and the plant cell nucleus is stretched to the point where it breaks in two. In some cells, the cytoplasmic reaction is overcome and the protoplast disappears while fungal growth increases. In some of the invaded cells, however, the cytoplasm and nucleus enlarge. The cytoplasm becomes granular and dense, and various particles or structures appear in it. Finally, the mycelium of the pathogen disintegrates and the invasion stops.

Cell Wall Defense Structures

Cell wall defense structures involve morphological changes in the cell wall or changes derived from the cell wall of the cell being invaded by the pathogen. The effectiveness of these structures as defense mechanisms seems to be rather limited, however. Three main types of such structures have been observed in plant diseases. (1) The outer layer of the cell wall of parenchyma cells coming in contact with incompatible bacteria swells and produces an amorphous, fibrillar material that surrounds and traps the bacteria and prevents them from multiplying. (2) Cell walls thicken in response to several

pathogens by producing what appears to be a cellulosic material. This material, however, is often infused with phenolic substances that are cross-linked and further increase its resistance to penetration. (3) Callose papillae are deposited on the inner side of cell walls in response to invasion by fungal pathogens (see Figure 2-8C,D). Papillae seem to be produced by cells within minutes after wounding and within 2 to 3 hours after inoculation with microorganisms. Although the main function of papillae seems to be repair of cellular damage, sometimes—especially if papillae are present before inoculation—they also seem to prevent the pathogen from subsequently penetrating the cell. In some cases, hyphal tips of fungi penetrating a cell wall and growing into the cell lumen are enveloped by cellulosic (callose) materials that later become infused with phenolic substances and form a **sheath** or **lignituber** around the hypha (Figure 5-3).

Histological Defense Structures

Formation of Cork Layers

Infection by fungi or bacteria and even by some viruses and nematodes frequently induces plants to form several layers of cork cells beyond the point of infection (Figures 5-4 and 5-5), apparently as a result of stimulation of the host cells by substances secreted by the pathogen. The cork layers inhibit the further invasion by the pathogen beyond the initial lesion and also block the spread of any toxic substances that the pathogen may secrete. Furthermore, cork layers stop the flow of nutrients and water from the healthy to the infected area and deprive the pathogen of nourishment. The dead tissues, including the pathogen, are thus delimited by the cork layers and may remain in place, forming necrotic lesions (spots) that are remarkably uniform in size and shape for a particular host–pathogen combination (see Figures 11-41, 11-45, 11-48C, 11-50 to 11-55, 11-93, and 14-13). In some host–pathogen combinations the necrotic tissues are pushed outward by the underlying healthy tissues and form scabs that may be sloughed off, thus removing the pathogen from the host completely (see Figures 11-10, 11-70, 12-13, 12-23B, and 12-38).

Formation of Abscission Layers

Abscission layers are formed on young, active leaves of stone fruit trees after infection by any of several fungi, bacteria, or viruses (see Figure 12-15). An abscission layer consists of a gap formed between two circular layers of cells of a leaf surrounding the locus of infection. On infection, the middle lamella between these two layers of cells is dissolved throughout the thickness of the leaf, completely cutting off the central area from the rest of the leaf (Figure 5-6). Gradually this area shrivels, dies, and sloughs off, carrying with it the pathogen. Thus, the plant, by discarding the infected area along with a few yet uninfected cells, protects the rest of the leaf tissue from being invaded by the pathogen and from becoming affected by the toxic secretions of the pathogen.

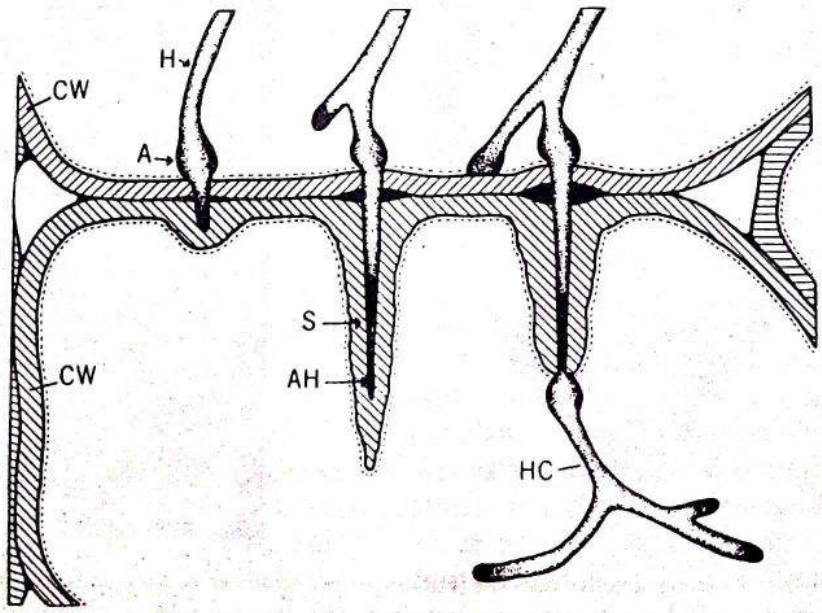


FIGURE 5-3 Formation of a sheath around a hypha (H) penetrating a cell wall (CW). A, Appressorium; AH, advancing hypha still enclosed in sheath; HC, hypha in cytoplasm; S, sheath.

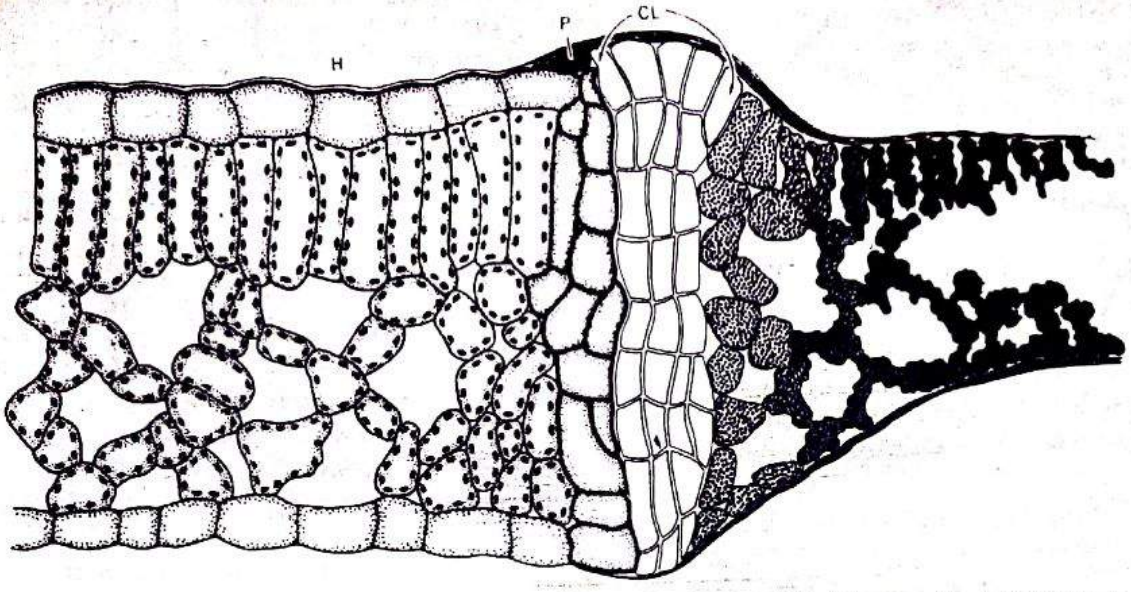


FIGURE 5-4 Formation of a cork layer (CL) between infected (I) and healthy (H) areas of leaf. P, Phellogen. [After Cunningham (1928), *Phytopathology* 18, 717–751.]

Formation of Tyloses

Tyloses form in xylem vessels of most plants under various conditions of stress and during invasion by most of the xylem-invading pathogens. Tyloses are overgrowths of the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through pits (Figure 5-7). Tyloses have cellulosic walls and may, by their size and numbers, clog the vessel completely. In some varieties of plants, tyloses form abundantly and quickly ahead of the pathogen, while the pathogen is still in the young roots, and block further advance of the pathogen. The plants of these varieties remain free of and therefore resistant to this pathogen. Varieties in which few, if any, tyloses form ahead of the pathogen are susceptible to disease.

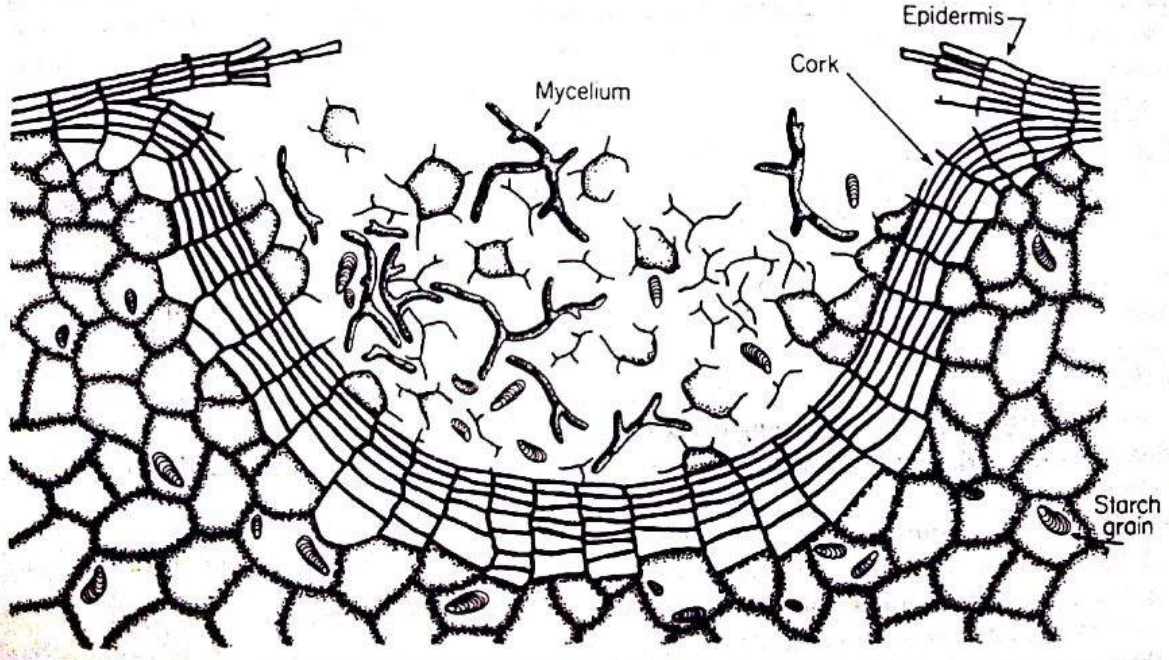


FIGURE 5-5 Formation of a cork layer on a potato tuber following infection with *Rhizoctonia*. [After G. E. Ramsey (1917), *J. Agric. Res.* 9, 421–426.]

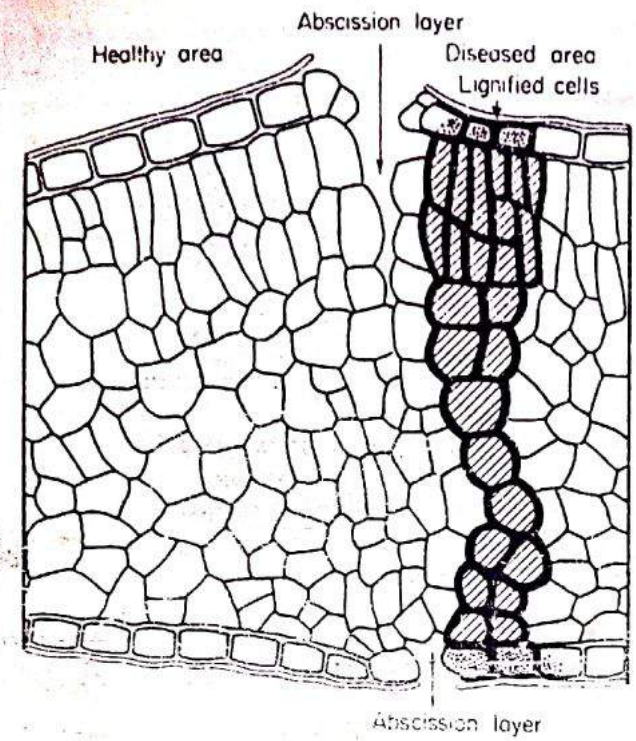


FIGURE 5-6 Formation of an abscission layer around a diseased spot of a *Prunus* leaf. [After Samuel, G. (1927), *Ann. Bot.* 41, 375–404.]

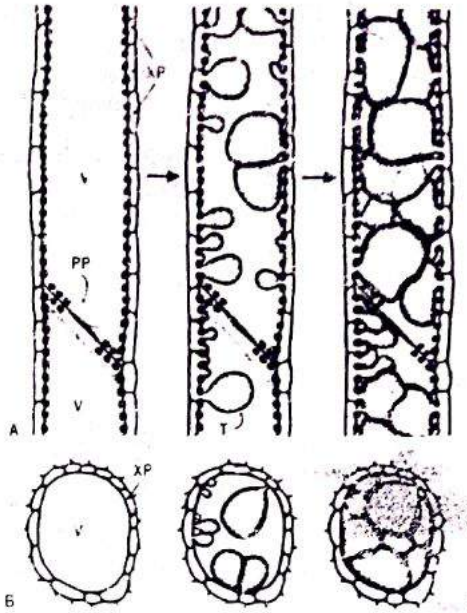


FIGURE 5-7 Development of tyloses in xylem vessels. Longitudinal (A) and cross-section (B) views of healthy vessels (left) and of vessels with tyloses. Vessels at right are completely clogged with tyloses. PP, Perforation plate; V, xylem vessel; XP, xylem parenchyma cell; T, tylosis.

Deposition of Gums

Various types of gums are produced by many plants around lesions after infection by pathogens or injury. Gum secretion is most common in stone fruit trees but occurs in most plants. The defensive role of gums stems from the fact that they are quickly deposited in the intercellular spaces and within the cells surrounding the locus of infection, thus forming an impenetrable barrier that completely encloses the pathogen. The pathogen then becomes isolated, starves, and sooner or later dies.

Necrotic Defense Reaction: Defense through the Hypersensitive Response

The hypersensitive response is considered a biochemical rather than a structural defense mechanism but is briefly described here because of the visible cellular responses that accompany it. In many host–pathogen combinations, as soon as the pathogen establishes contact with the cell, the nucleus moves toward the invading pathogen and soon disintegrates. At the same time, brown, resin-like granules form in the cytoplasm, first around the point of penetration of the pathogen and then throughout the cytoplasm. As the browning discoloration of the cytoplasm of the plant cell continues and death sets in, the invading hypha begins to degenerate (Figure 5-8). In most cases the hypha does not grow out of such cells, and further invasion is stopped. In bacterial infections of leaves, the hypersensitive response results in destruction of all cellular membranes of cells in contact with bacteria, and that is followed by desiccation and necrosis of the leaf tissues invaded by the bacteria.

The necrotic or hypersensitive type of defense is quite common, particularly in diseases caused by obligate fungal parasites and by viruses, bacteria, and nematodes. Apparently, the necrotic tissue not only isolates the parasite from the living substance on which it depends for its nutrition and, therefore, results in its starvation and death, but, more importantly, it signifies the concentration of numerous biochemical cell responses and antimicrobial substances that neutralize the pathogen. The faster the host cell dies after invasion, the more resistant to infection the plant seems to be.

Induced Biochemical Defenses

The Hypersensitive Response (H.R.)

The hypersensitive response, often referred to as HR, is a localized induced cell death in the host plant at the site of infection by a pathogen (Figure 5-9A). The hypersensitive response is thought to be responsible for limiting the growth of the pathogen and, in that way, is capable of providing resistance to the host plant against the pathogen. An effective hypersensitive response may not always be visible when a plant remains resistant to at-

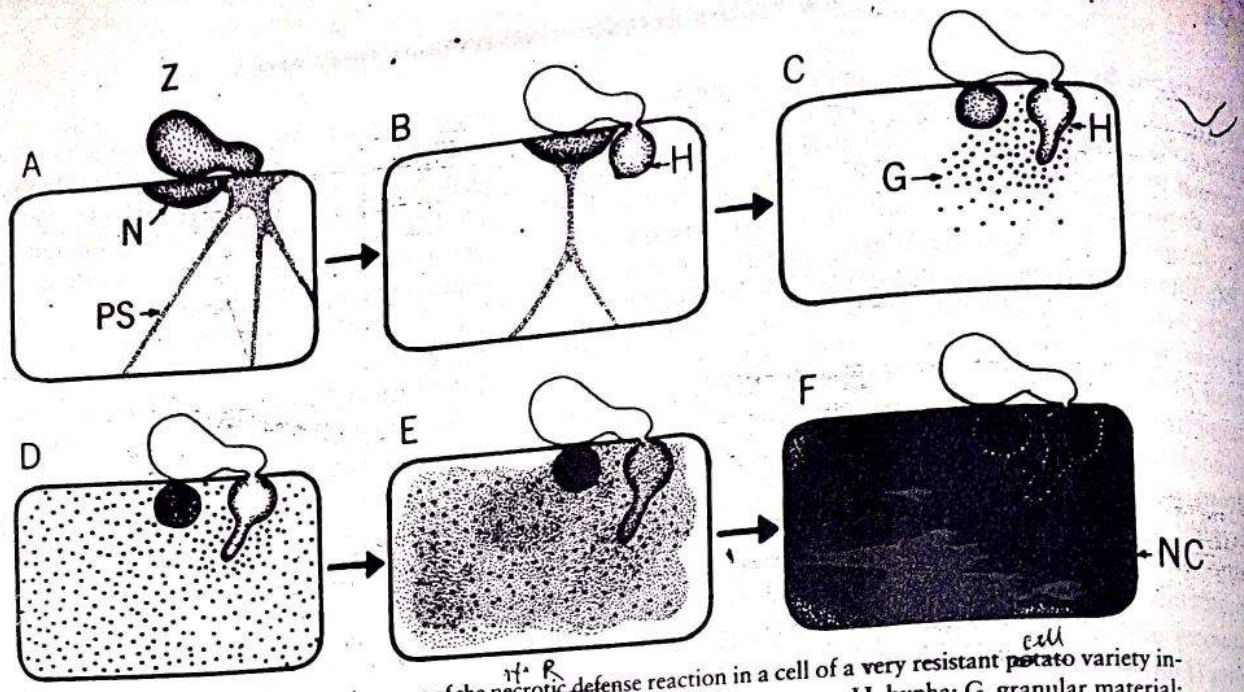


FIGURE 5-8 Stages in the development of the necrotic defense reaction in a cell of a very resistant potato variety infected by *Phytophthora infestans*. N, Nucleus; PS, protoplasmic strands; Z, zoospore; H, hypha; G, granular material; NC, necrotic cell. [After K. Tomiyama (1956), *Ann. Phytopathol. Soc. Jpn.* 21, 54–62.]

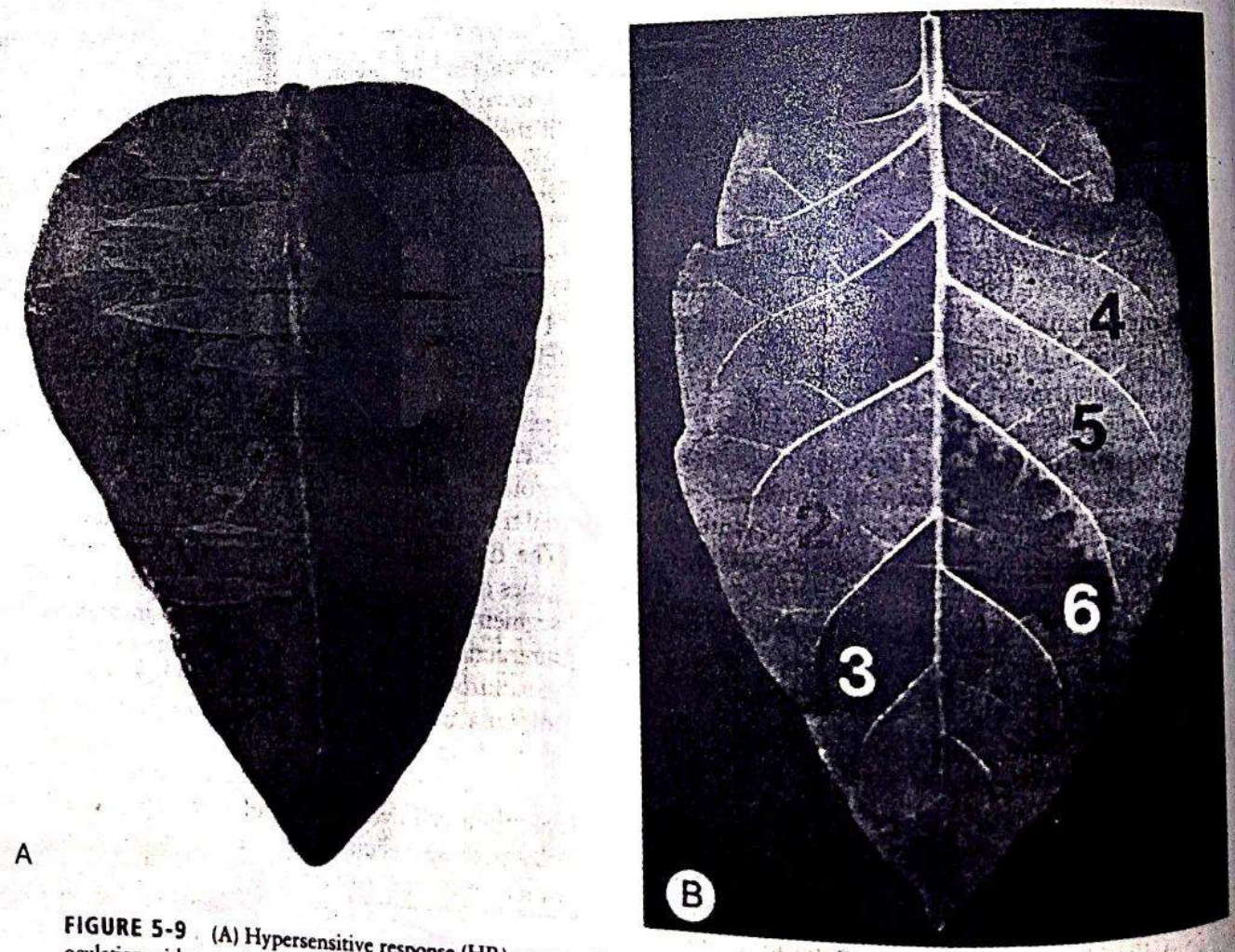


FIGURE 5-9 (A) Hypersensitive response (HR) expressed on leaves of a resistant cowpea variety following sap inoculation with a strain of a virus that causes local lesions (in this case, tobacco ringspot virus). The virus remains localized in the lesions. (B) Tobacco leaf showing typical hypersensitive responses (dark areas) 24 hours after injection with six preparations of soft-rotting bacteria. Three bacterial strains (1, 3, and 6) induced the hypersensitive response, but the others did not. [From Bauer *et al.* (1995), *Mol. Plant–Microbe Interact.* 8, 484–491.]

tack by a pathogen since it is possible for the hypersensitive response to involve only single cells or very few cells and thereby remain unnoticed. Under artificial conditions, however, injection of several genera of plant pathogenic bacteria into leaf tissues of nonhost plants results in the development of a hypersensitive response. The HR consists of large leaf sectors becoming watersoaked at first and, subsequently, necrotic and collapsed within 8 to 12 hours after inoculation (Figure 5-9B). The bacteria injected in the tissues are trapped in the necrotic lesions and generally are killed rapidly. The HR may occur whenever virulent strains of plant pathogenic bacteria are injected into nonhost plants or into resistant varieties, and when avirulent strains are injected into susceptible cultivars. Although not all cases of resistance are due to the hypersensitive response, HR-induced resistance has been described in numerous diseases involving obligate parasites (fungi, viruses, mollicutes, and nematodes) as well as nonobligate parasites (fungi and bacteria).

The hypersensitive response is the culmination of the plant defense responses initiated by the recognition by the plant of specific pathogen-produced signal molecules, known as elicitors. Recognition of the elicitors by the host plant activates a cascade of biochemical reactions in the attacked and surrounding plant cells and leads to new or altered cell functions and to new or greatly activated defense-related compounds. The most common new cell functions and compounds include a rapid burst of oxidative reactions; increased ion movement, especially of K^+ and H^+ through the cell membrane; disruption of membranes and loss of cellular compartmentalization (Figure 5-10); cross-linking of phenolics with cell wall components and strengthening of the plant cell wall; production of antimicrobial substances such as phytoalexins; and formation of antimicrobial so-called pathogenesis-related proteins such as chitinases.

The hypersensitive response occurs only in specific host–pathogen combinations in which the host and the pathogen are incompatible, that is, the pathogen fails to infect the host. It is thought that this happens because of the presence in the plant of a resistance gene (R) which recognizes and is triggered into action by the elicitor molecule released by the pathogen. The pathogen-produced elicitor is, presumably, the product of a pathogen gene which, because it triggers the development of resistance in the host that makes this pathogen avirulent, is called an avirulence gene. For several pathogens, primarily bacteria, avirulence genes have been isolated and the proteins coded by them have been identified. The first avirulence gene product to be identified was the protein of the avirulence gene D (*avrD*) of the bacterium *Pseudomonas syringae* pv. *glycinea*. This was shown to

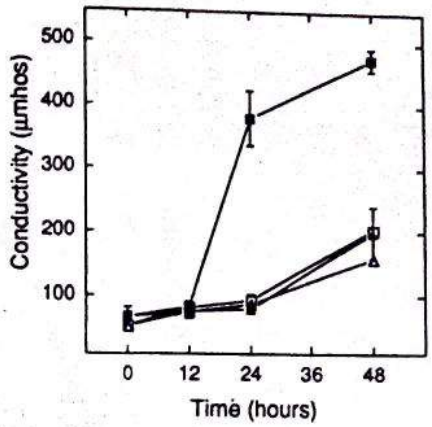


FIGURE 5-10 Disruption of cell membranes leads to a dramatic increase in cell electrolyte leakage, measured by increased current conductivity. This occurs when a resistant variety (■) containing an R gene is inoculated with pathogens containing an avirulence gene corresponding to the R gene. □, Same variety inoculated with a pathogen lacking the avirulence gene; △, ▲, another variety, susceptible to both pathogens. [From Whalen *et al.* (1993), *Mol. Plant–Microbe Interact.* 6, 616–627.]

be an enzyme involved in the synthesis of substances known as syringolides. The latter have the ability to elicit the hypersensitive response in soybean varieties which carry the resistance gene complementary to *avrD* of the bacterium.

Several resistance genes R have been isolated from plants such as corn, tomato, tobacco, flax, and *Arabidopsis*, a plant used mostly for experimental purposes. The corn R gene codes for an enzyme that inactivates the HC-toxin of the fungus *Cochliobolus carbonum*, the cause of northern leaf spot of corn, whereas the tomato gene that confers resistance to the tomato speck-causing bacterium *Pseudomonas syringae* pv. *tomato* codes for a protein kinase enzyme that most likely plays a role in signal transduction by triggering other enzymes into action. The functions of the proteins encoded by the other R genes are not known with certainty, but they all contain segments found in proteins involved in protein–protein interactions such as the protein kinases and the polygalacturonase-inhibiting proteins. The proteins coded by the tobacco R gene that protects against tobacco mosaic virus and of the *Arabidopsis* R gene that protects against a leaf-spotting bacterium appear to be present in the plant cell cytoplasm and therefore probably recognize pathogen elicitors that reach the cytoplasm. On the other hand, the protein encoded by the R gene of flax, which provides resistance against the flax rust fungus, seems to attach to the plant cell membrane by means of a signal anchor, and the protein of the *Cf9* R gene of tomato, which provides resistance against race 9 of the

leaf mold fungus *Cladosporium fulvum*, is primarily outside the plant cell plasma membrane and is attached to the membrane with a short anchor. The last two R gene products, therefore, apparently recognize pathogen-produced elicitors as they approach or come in contact with the plant cell membrane.

Active Oxygen Radicals, Lipoxygenases, and Disruption of Cell Membranes

The plant cell membrane consists of a phospholipid bilayer in which are embedded many kinds of protein and glycoprotein molecules. The protein molecules are often organized in groups, some of which form channels on the membrane and allow ions and metabolites to enter and exit the cell. The cell membrane in the form of endoplasmic reticulum and organelles compartmentalizes the cell into areas in which specific compounds are kept separated from others and certain biochemical reactions take place. In addition, the cell membrane is an active site for induction of defense mechanisms; for example, it serves as the anchor of R gene-coded proteins that recognize the elicitors released by the pathogen and subsequently trigger the hypersensitive response.

Attack of cells by pathogens, or exposure to pathogen toxins and enzymes, often results in structural and permeability changes of the cell membrane. These changes are generally thought to be an expression of susceptibility and disease development. In many host–pathogen combinations, however, particularly those involving the hypersensitive response, some membrane changes play a role in the defense against invasion by the pathogen. The most important membrane-associated defense responses include the following: (1) release of molecules important in signal transduction within and around the cell and, possibly, systemically through the plant; (2) release and accumulation of reactive oxygen "radicals" and of lipoxygenase enzymes; and (3) as a result of the loss of compartmentalization, activation of phenol oxidases and oxidation of phenolics.

In many host–fungus interactions, one of the first events detected in attacked host cells or cells artificially treated with fungal elicitors is the rapid and transient generation of activated oxygen radicals, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$). These highly reactive oxygen radicals are thought to be released by the multisubunit NADPH oxidase enzyme complex of the host cell plasma membrane. They appear to be released in affected cells within seconds or minutes from contact of the cell with the fungus or its elicitors and reach a maximum activity within minutes to a few hours. The activated oxygen radicals trigger the hydroperoxidation of membrane phospholipids, producing mixtures of lipid hydroperoxides. The latter are toxic, their production disrupts the plant cell membranes, and they seem to be involved in normal or HR-induced cell collapse and death. Active oxygen radicals may also be involved in host defense reactions through the oxidation of phenolic compounds into more toxic quinones and into ligninlike compounds. The presence of active oxygen radicals, however, also affects the membranes and the cells of the advancing pathogen either directly or indirectly through the hypersensitive response of the host cell.

The oxygenation of membrane lipids seems to involve various lipoxygenases as well. These are enzymes that catalyze the hydroperoxidation of unsaturated fatty acids, such as linoleic acid and linolenic acid, which have been previously released from membranes by phospholipases. The lipoxygenase-generated hydroperoxides formed from such fatty acids, in addition to disrupting the cell membranes and leading to HR-induced cell collapse of host and pathogen, are also converted by the cell into several biologically active molecules, such as jasmonic acid, that play a role in the response of plants to wounding and other stresses. Jasmonic acid, for example, which is the precursor of the wound hormone traumatin, appears to induce numerous protein changes and acts as a signal transducer of the defense reaction in plant–pathogen interactions.

Reinforcement of Host Cell Walls with Strengthening Molecules

In several plant diseases caused by fungi, the walls of cells that come in contact with the fungus produce, modify, or accumulate several defense-related substances that reinforce the resistance of the wall to invasion by the pathogen. Among the defensive substances produced or deposited in plant cell walls being invaded by fungi are callose, glycoproteins such as extensin that are rich in the amino acid hydroxyproline, phenolic compounds of varying complexity including lignin and suberin, and mineral elements such as silicon and calcium. Some of these substances are also produced or deposited in defensive cell wall structures such as the papillae. Many of these substances form complex polymers and also react and cross-link with one another, thereby forming more or less insoluble cell wall structures that confine the invading fungus and prevent further development of disease. Of course, in cases in which the host lacks resistance or exhibits incomplete resistance, apparently the host, with or without interference by fungal secretions, fails to produce reinforcing compounds or produces them too slowly to be effective, and the fungus manages to invade the cell.

Production of Antimicrobial Substances in Attacked Host Cells

Pathogenesis-Related Proteins

Pathogenesis-related proteins, often called PR proteins, are a structurally diverse group of plant proteins that are toxic to invading fungal pathogens. They are widely distributed in plants in trace amounts but are produced in much greater concentration following pathogen attack or stress. The PR proteins exist in plant cells intracellularly and also in the intercellular spaces. Varying types of PR proteins have been isolated from each of several crop plants. Different plant organs, for example, leaves, seeds, and roots, may produce different sets of PR proteins.

The several groups of PR proteins have been classified according to their function, serological relationship, amino acid sequence, molecular weight, and certain other properties. The PR proteins are either extremely acidic or extremely basic and therefore are highly soluble and reactive. The better known PR proteins are PR1 proteins, β-1,3-glucanases (Figure 5-11), chitinases, lysozymes, PR4 proteins, thaumatinelike proteins, osmotinlike proteins, cysteine-rich proteins, glycine-rich proteins, proteinase inhibitors (Figure 5-12), proteinases, chitosanases, and peroxidases. There are often numerous isoforms of each PR protein in various host plants.

Although healthy plants may contain trace amounts of several PR proteins, attack by pathogens, treatment with elicitors, wounding, or stress induces transcription of a battery of genes that code for PR proteins. This occurs as part of a massive switch in the overall pattern of gene expression, during which normal protein production nearly ceases. The signal compounds responsible for induction of PR proteins include salicylic acid, ethylene, xylanase, the polypeptide systemin, jasmonic acid, and probably others.

The significance of PR proteins lies in the fact that they show strong antifungal and other antimicrobial activity. Some of them inhibit spore release and germination, whereas others are associated with strengthening of the host cell wall and its outgrowths and papillae. Some of the PR proteins, for example, β-1,3-glucanase and chitinase, diffuse toward and affect (break down) the chitin-supported structure of the cell walls of several but not all plant pathogenic fungi, whereas lysozymes degrade the glucosamine and muramic acid components of bacterial cell walls. Plants genetically engineered to express chitinase genes show good resistance against the soilborne fungus *Rhizoctonia solani*. Signal molecules that induce PR protein synthesis seem to be transported systemically to other parts of the plant and to reduce disease initiation and intensity in those parts for several days or even weeks.

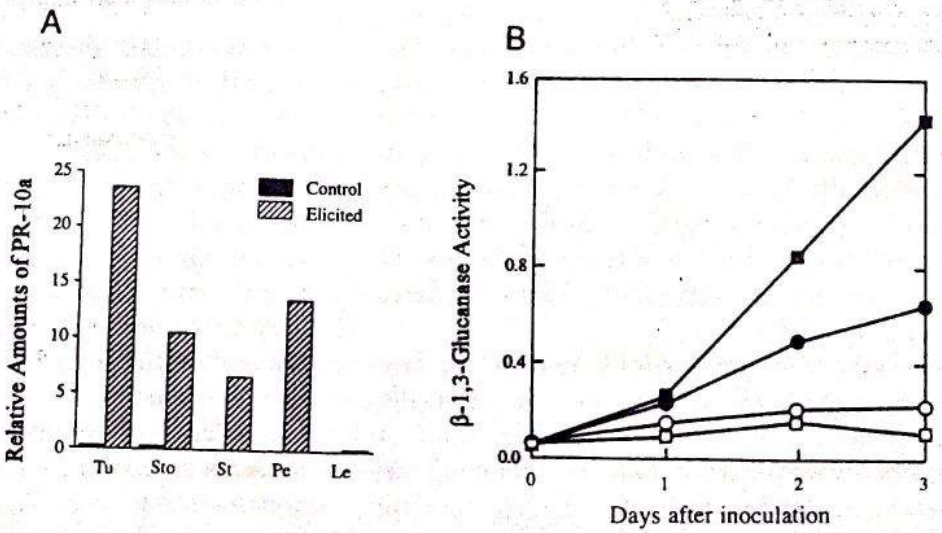


FIGURE 5-11 (A) Production and accumulation of a pathogenesis-related protein (PR10a) in potato tissues either untreated (control) or elicited by treating cut surfaces with a homogenate of the late blight fungus *Phytophthora infestans* and incubating for 4 days. Tu, Tuber; Sto, stolon; St, stem; Pe, petiole; Le, leaf. [From Constabel and Brisson (1995), *Mol. Plant–Microbe Interact.* 8, 104–113.] (B) Levels of activity of the antifungal protein β-1,3-glucanase in the intercellular fluid of barley leaves, either left uninoculated (□, ○) or inoculated with the powdery mildew fungus *Erysiphe graminis* f. sp. *hordei* (■, ●). The two barley varieties are nearly isogenic, except that one (□, ■) carries an additional resistance gene that makes it resistant whereas the other (○, ●) is susceptible. [From Jutidamrongphan *et al.* (1991), *Mol. Plant–Microbe Interact.* 4, 234–238.]

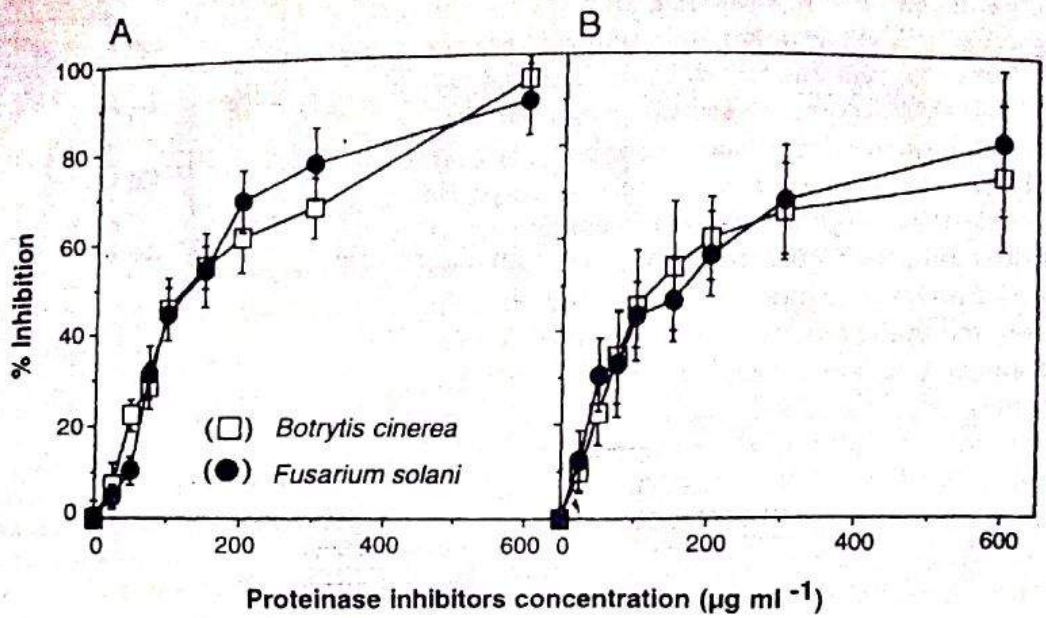


FIGURE 5-12 Inhibition of (A) spore germination and (B) germ tube elongation of the fungi *Botrytis cinerea* and *Fusarium solani*, that do not infect cabbage, by proteinase inhibitors obtained from young cabbage leaves. The inhibitors caused leakage of the cellular contents of these fungi. The cabbage fungal pathogen *Alternaria brassicicola* was not affected by these proteinase inhibitors. [From Lorito *et al.* (1994), *Mol. Plant–Microbe Interact.* 7, 525–527.]

Phytoalexins

Phytoalexins are toxic antimicrobial substances produced in appreciable amounts in plants only after stimulation by various types of phytopathogenic microorganisms or by chemical and mechanical injury. Phytoalexins are produced by healthy cells adjacent to localized damaged and necrotic cells in response to materials diffusing from the damaged cells. Phytoalexins are not produced during compatible biotrophic infections. Phytoalexins accumulate around both resistant and susceptible necrotic tissues. Resistance occurs when one or more phytoalexins reach a concentration sufficient to restrict pathogen development. Most known phytoalexins are toxic to and inhibit the growth of fungi pathogenic to plants, but some are also toxic to bacteria, nematodes, and other organisms. More than 300 chemicals with phytoalexinlike properties have been isolated from plants belonging to more than 30 families. The chemical structures of phytoalexins produced by plants of a family are usually quite similar; for example, in most legumes phytoalexins are isoflavonoids, and in the Solanaceae they are terpenoids. Most of the phytoalexins are produced in plants in response to infection by fungi, but a few bacteria, viruses, and nematodes have also been shown to induce production of phytoalexins. Some of the better studied phytoalexins include phaseollin in bean (Figure 5-13), pisatin in pea, glyceollin in soybean, alfalfa, and clover, rishitin in potato, gossypol in cotton, and capsidiol in pepper.

Phytoalexin production and accumulation occur in healthy plant cells surrounding wounded or infected cells and are stimulated by alarm substances produced and released by the damaged cells and diffusing into the adjacent healthy cells. Most phytoalexin elicitors are generally high molecular weight substances that are constituents of the fungal cell wall, such as glucans, chitosan, glycoproteins, and polysaccharides. The elicitor molecules are released from the fungal cell wall by host plant enzymes. Most such elicitors are nonspecific, that is, they are present in both compatible and incompatible races of the pathogen and induce phytoalexin accumulation irrespective of the plant cultivar. A few phytoalexin elicitors, however, are specific, since the accumulation of phytoalexin they cause on certain compatible and incompatible cultivars parallels the phytoalexin accumulation caused by the pathogen races themselves. Although most phytoalexin elicitors are thought to be of pathogen origin, some elicitors, for example, oligomers of galacturonic acid, are produced by plant cells in response to infection or are released from plant cell walls after their partial breakdown by cell-wall-degrading enzymes of the pathogen.

Formation of phytoalexins in a susceptible (compatible) host following infection by a pathogen seems, in some cases, to be prevented by suppressor molecules produced by the pathogen. The suppressors seem to also be glucans or glycoproteins, or one of the toxins produced by the pathogen.

The mechanisms by which phytoalexin elicitors, phy-

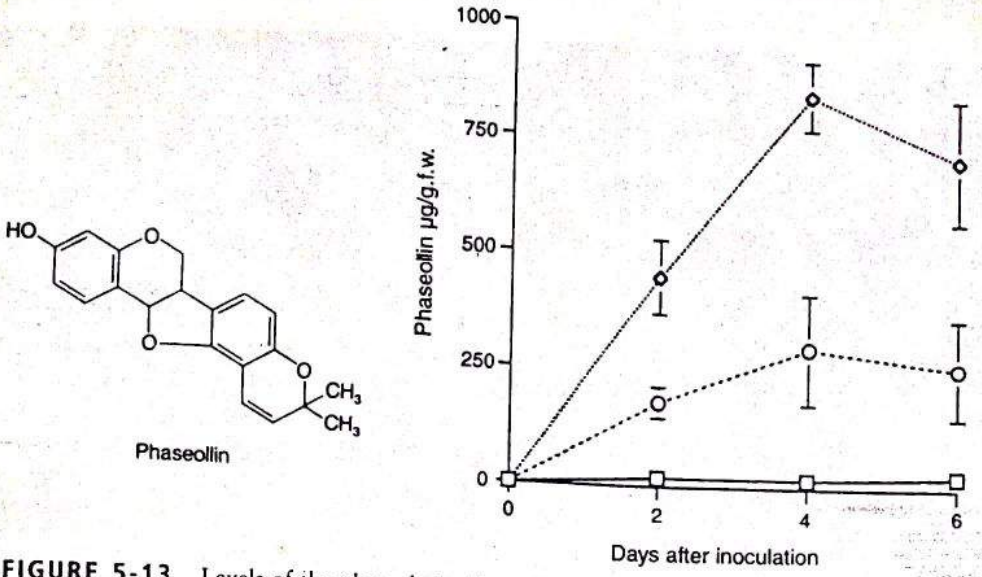


FIGURE 5-13 Levels of the phytoalexin phaseollin produced at infection sites in bean pods following inoculation with three races of the halo blight bacterium *Pseudomonas syringae* pv. *phaseolicola*. Virulent race 6 (□) infects without causing a defense response nor production of the phytoalexin. The same race 6 was transformed with an avirulence gene corresponding to resistance gene R2 (○) and with an avirulence gene to R3 (◇), and the transformants induced visibly different hypersensitive responses and also different levels of phytoalexin. [From Mansfield *et al.* (1994). *Mol. Plant–Microbe Interact.* 6, 726–739.]

toalexin production, phytoalexin suppressors, genes for resistance or susceptibility, and the expression of resistance or susceptibility are connected are still not well understood. Several hypotheses have been proposed to explain the interconnection of these factors, but much more work is needed before a satisfactory explanation can be obtained.

Species or races of fungi pathogenic to a particular plant species seem to stimulate production of generally lower concentrations of phytoalexins that nonpathogens. For example, in the case of pisatin production by pea pods inoculated with the pathogen *Ascochyta pisi*, pea varieties produce concentrations of pisatin that are approximately proportional to the resistance of the variety to the pathogen. When the same pea variety is inoculated with different strains of the fungus, the concentration of pisatin produced is approximately inversely proportional to the virulence of each particular fungal strain inoculated on the pea variety. Also, in soybean plants infected with the fungus *Phytophthora megasperma* f. sp. *glycinea*, inoculations of fungal races on incompatible host cultivars resulted in earlier accumulations and higher concentrations of the phytoalexin glyceollin than inoculations of fungal races on compatible cultivars. It has been suggested that the higher concentrations of glyceollin in incompatible host–pathogen combinations are the result of reduced biodegradation rather than increased biosynthesis of the phytoalexin. In some host–pathogen systems, however, for example, in the bean–*Colletotrichum lindemuthianum* and the potato–*Phytophthora infestans* systems, the respective phytoalexins such as phaseollin and rishitin reach equal or higher concentrations in compatible (susceptible) hosts compared to incompatible (resistant) ones.

On the other hand, pathogenic races or species of fungi seem to be less sensitive to the toxicity of the phytoalexin(s) produced by their host plant than are nonpathogenic fungi. It has been suggested that pathogens may have an adoptive tolerance mechanism that enables them to withstand higher concentrations of the host phytoalexin after earlier exposures to lower concentrations of the phytoalexin. It is known, however, that many pathogenic fungi can metabolize the host phytoalexin into a nontoxic compound and thereby decrease the toxicity of the phytoalexin to the pathogen. It is also known that numerous pathogenic fungi are successful in causing disease although they are sensitive to or unable to metabolize the host phytoalexins. Furthermore, some fungi that can either degrade or tolerate certain phytoalexins are unable to infect the plants that produce them.

In general, it appears that phytoalexins may play a decisive or an auxiliary role in the defense of some hosts against certain pathogens, but their significance, if any, as factors of disease resistance in most host–pathogen combinations is still unknown.

Simple Phenolic Compounds

It has often been observed that certain common phenolic compounds that are toxic to pathogens are pro-

duced and accumulate at a faster rate after infection, especially in a resistant variety of plant relative to a susceptible variety. Chlorogenic acid, caffeic acid, and ferulic acid are examples of such phenolic compounds (Figure 5-14). Although some of the common phenolics may each reach concentrations that could be toxic to the pathogen, it should be noted that several of them appear concurrently in the same diseased tissue, and it is possible that the combined effect of all fungitoxic phenolics present, rather than that of each one separately, is responsible for the inhibition of infection in resistant varieties.

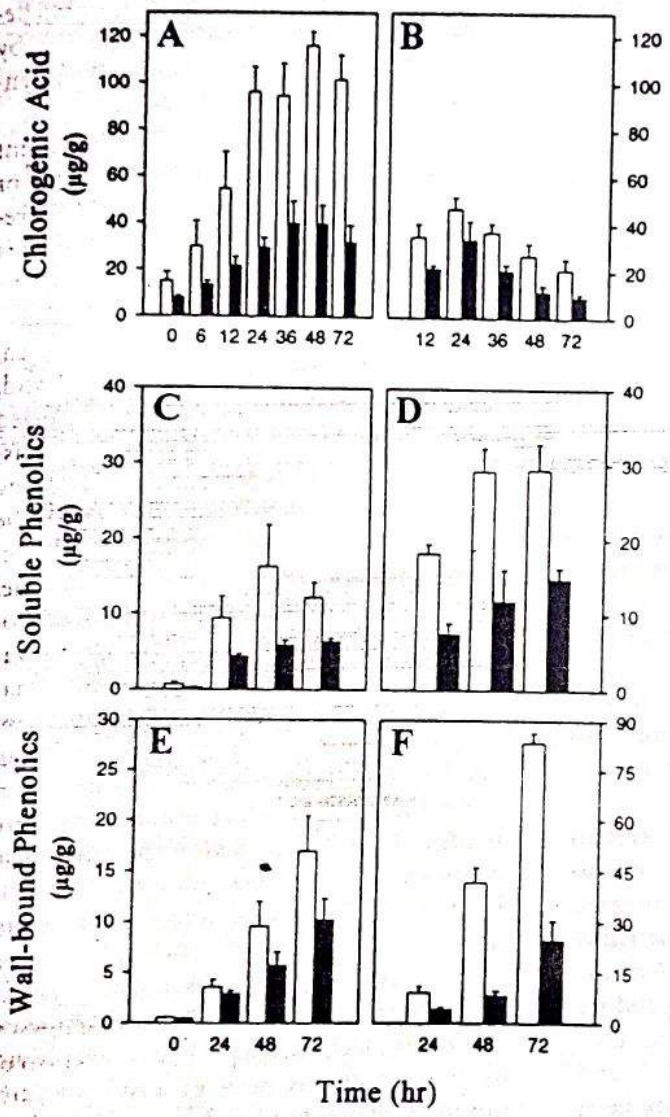


FIGURE 5-14 Production of chlorogenic acid and other soluble and wall-bound phenolics in normal (white bars) and transgenic (dark bars) potato tubers after wounding (A, C, and E) and after spraying with arachidonic acid, an elicitor of the hypersensitive defense response (B, D, and F). Transgenic plants produced an enzyme that inactivates tryptophan, a precursor of phenolics and lignin. Chlorogenic acid was increased by wounding but not by elicitation. Soluble and wall-bound phenolics increased after wounding and even more following treatment with the elicitor, but the increase was smaller in the transgenic tubers (dark bars) than in the normal tubers. Accordingly, the transgenic tubers in these treatments were more susceptible to infection when inoculated with zoospores of *Phytophthora infestans* than were the treated normal plants. [From Yao, DeLuca, and Brison (1995), *Plant Cell* 7, 1787–1799.]

Toxic Phenolics from Nontoxic Phenolic Glycosides

Many plants contain nontoxic glycosides, that is, compounds consisting of a sugar (such as glucose) joined to another, often phenolic, molecule. Several fungi and bacteria are known to produce or to liberate from plant tissues the enzyme glycosidase that can hydrolyze such complex molecules and release the phenolic compound from the complex. Some of the released phenolics are quite toxic to the pathogen and appear to play a role in the defense of the plant against infection.

Role of Phenol-Oxidizing Enzymes in Disease Resistance

The activity of many phenol-oxidizing enzymes (polyphenol oxidases) is generally higher in the infected tissue of resistant varieties than in infected susceptible ones or in uninfected healthy plants. The importance of polyphenol oxidase activity in disease resistance probably stems from its property to oxidize phenolic compounds to quinones, which are often more toxic to microorganisms than the original phenols. It is reasonable to assume that an increased activity of polyphenol oxidases will result in higher concentrations of toxic products of oxidation and therefore in greater degrees of resistance to infection. A complex interaction occurs during fruit ripening in which levels of lipoxygenases increase and break down diene, a compound that is present in young, immature fruit and is toxic to fungi. These events normally result in infection (loss of resistance) of the ripening fruit. In some fruit, however, elicitors from nonpathogenic fungi stimulate production of the phenolic compound epicatechin that inhibits the activity of lipoxygenases. As a result, epicatechin decreases degradation of the antifungal diene and thereby prevents decay of the ripening fruit by anthracnose fungi.

Another phenol oxidase enzyme, peroxidase, both oxidizes phenolics to quinones and generates hydrogen peroxide. The latter not only is antimicrobial in itself, but it also releases highly reactive free radicals and in that way further increases the rate of polymerization of phenolic compounds into ligninlike substances. These substances are then deposited in cell walls and papillae and interfere with the further growth and development of the pathogen.

Detoxification of Pathogen Toxins

In at least some of the diseases in which the pathogen produces a toxin, resistance to disease is apparently the same as resistance to the toxin. Detoxification of at least some toxins, for example, HC-toxin and pyricularin, produced by the fungi *Cochliobolus carbonum* and *Magnaporthe grisea,* respectively, is known to occur in plants, and it may play a role in disease resistance. Some of these toxins appear to be metabolized more rapidly by resistant varieties or are combined with other substances and form less toxic or nontoxic compounds. The amount of the nontoxic compound formed is often proportional to the disease resistance of the variety.

Resistant plants and nonhosts are not affected by the specific toxins produced by *Cochliobolus*, *Periconia*, and *Alternaria*, but it is not yet known whether the selective action of these toxins depends on the presence of receptor sites in susceptible but not in resistant varieties, on detoxification of the toxins in resistant plants, or on some other mechanism.

Immunization of Plants against Pathogens

Defense through Plantibodies

In humans and animals, defenses against pathogens are often activated by natural or artificial immunization, that is, by a subminimal natural infection with the pathogen or by an artificial injection of pathogen proteins and other antigenic substances. Both events result in the production of antibodies against the pathogen and, thereby, in subsequent prolonged protection (immunity) of the human or animal from infection by any later attacks of the pathogen.

Plants, of course, do not have an immune system like that of humans and animals, that is, they do not produce antibodies. In the early 1990s, however, transgenic plants have been produced that have been genetically engineered to incorporate in their genome, and to express, foreign genes, such as mouse genes that produce antibodies against certain plant pathogens. Such antibodies, encoded by animal genes but produced in and by the plant, are called plantibodies. It has already been shown that transgenic plants producing plantibodies against coat proteins of viruses, for example, artichoke mottle crinkle virus, to which they are susceptible, can defend themselves and show some resistance to infection by these viruses. It is expected that, in the future, this type of plant immunization will yield great dividends by expressing in plants animal antibody genes that will produce antibodies directed against specific essential proteins of the pathogen, such as viral replicases or movement proteins and fungal and bacterial enzymes of attack.

Local and 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** (Figure 5-15A). Subsequently, resistance spreads systemically and develops in distal, untreated parts of the plant, and it is called systemic acquired resistance (SAR) (Figure 5-15B). It is known now that several chemical compounds, for example, salicylic acid, arachidonic acid, and 2,6-dichloroisonicotinic acid, may induce localized and systemic resistance in plants at levels not causing tissue necrosis. Such chemicals may be effective in inducing resistance in plants when they are applied through the roots, as a foliar spray (Figure 5-16), or by stem injection. Local acquired resistance is induced, for example, in a 1 to 2 mm zone around local lesions caused by tobacco mosaic virus (TMV) on hypersensitive tobacco varieties, and probably in other host–pathogen combinations. Local acquired 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 nonspecifically throughout the plant and reduces the severity of disease caused by all classes of pathogens including normally virulent ones. It has been observed in several 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 (Figure 5-17). Localized infections of young plants, for example, cucumber with either 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 pro-

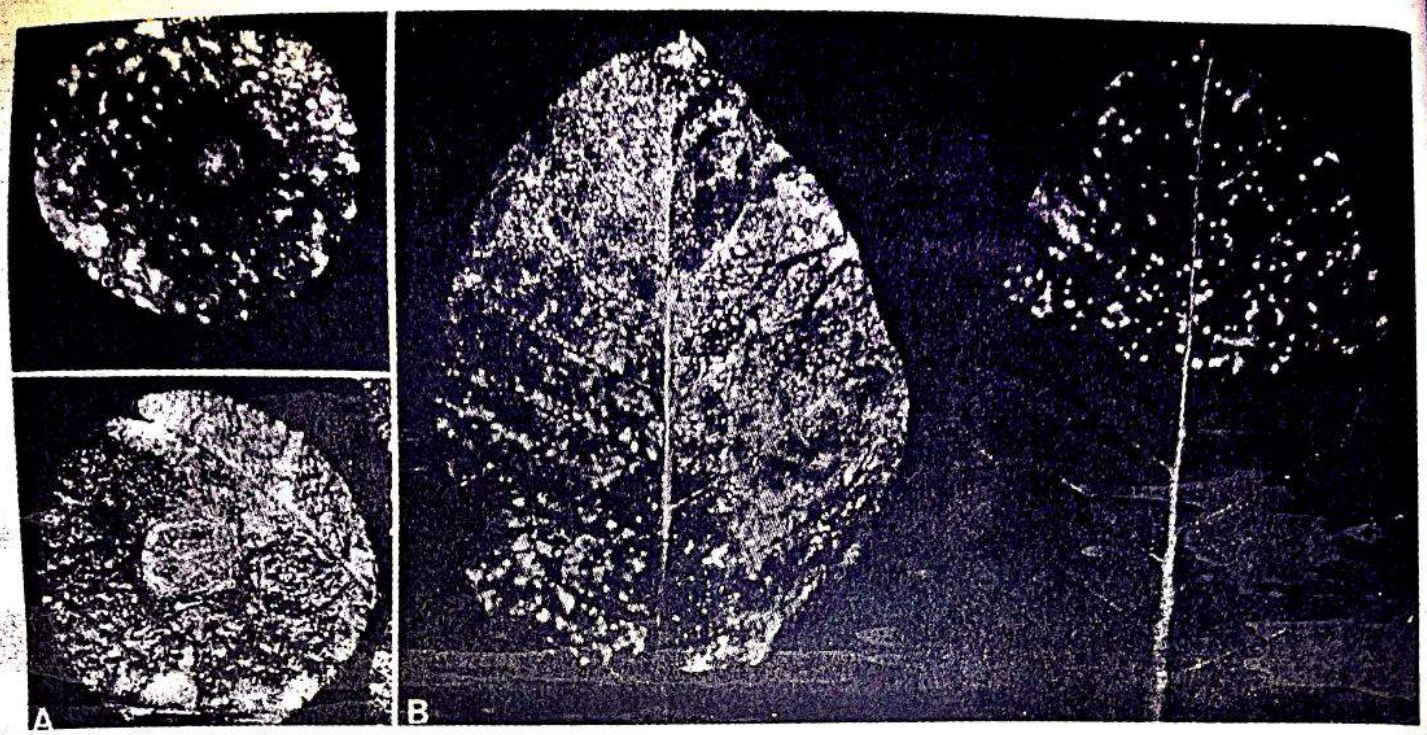


FIGURE 5-15 (A) Development of local acquired resistance to tobacco mosaic virus (TMV) around a local lesion caused by the same virus on a resistant tobacco variety. When the same leaves were reinoculated with TMV 7 days later, no new lesions formed near the original one because of local acquired resistance (top), but when they were reinoculated with a different virus, no zone free of lesions remained (bottom). (B) The upper (tip) half of the leaf at right was inoculated with TMV, and 7 days later both leaves were inoculated with the same virus over their entire surface. The leaf at left developed numerous local lesions throughout, whereas the previously half-inoculated leaf at right developed almost no additional lesions because of acquired local and systemic resistance. [From A. F. Ross (1961), *Virology* 14, 329–339 and 340–358.]

tects 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 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, for example, 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 drastically reduced in systemically induced resistant tissue, probably as a result of formation beneath the appressoria of papillalike material that quickly becomes impregnated with lignin and silicon. In some host–pathogen systems, systemic acquired resistance is characterized by 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 (Figure 5-18) 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 (Figure 5-19). 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, and 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 systemic expression of systemic acquired resistance, but sal-

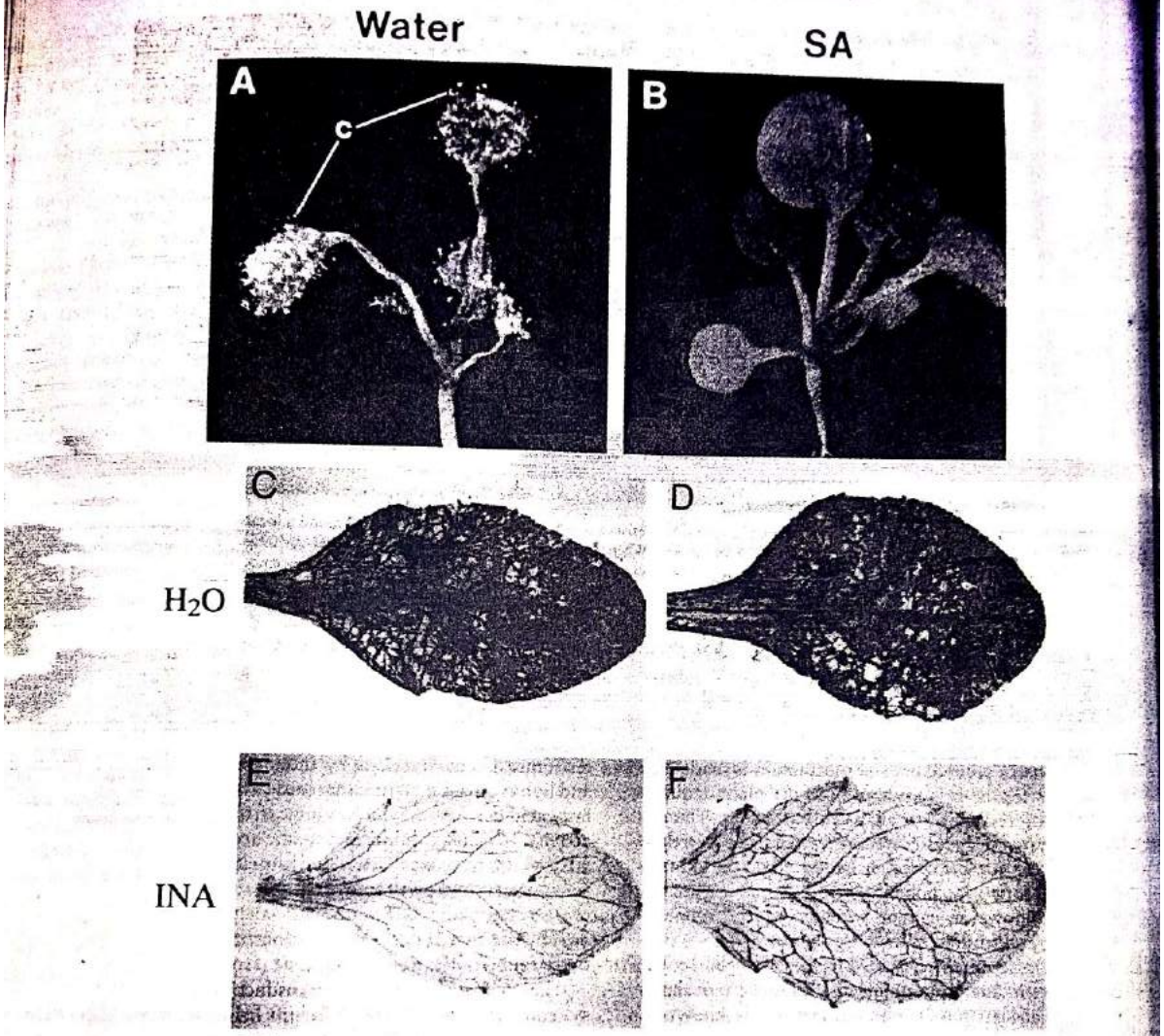


FIGURE 5-16 Induced resistance in *Arabidopsis* plants sprayed with water (A, C, D), salicylic acid (B) or INA (2,6-dichloroisonicotinic acid) and inoculated with spores of *Peronospora parasitica* 5 days (A, B) or 4 days (C–F) later. At 6 (A, B) or 10 days (C–F) after inoculation, individual leaves revealed numerous fungal structures in heavily infected H_2O-treated leaves and almost no fungal structures in INA-treated leaves. Plants in A–C and E are normal, whereas those in D and F were transformed with a gene that blocks accumulation of salicylic acid, indicating that INA can induce resistance in the absence of salicylic acid accumulation. c = Conidiophores. [Photos courtesy J. A. Ryals, Ciba Agric. Biotechnology. A and B from Uknes *et al.*, *Mol. Plant–Microbe Interact.* 6, 692–698; C–F from Vernooij *et al.* (1995), *Mol. Plant–Microbe Interact.* 8, 228–234.] See also Color Figures.

icylic acid must be present for the real signal to be transduced into gene expression and acquired resistance. It has been reported that salicylic acid reacts with an oxidative enzyme (catalase) and generates reactive oxygen radicals, and this has been suggested as a mechanism by which the plant cell reacts to salicylic acid signaling and induces systemic acquired resistance (Figure 5-20).

Induction of systemic acquired resistance through ex-

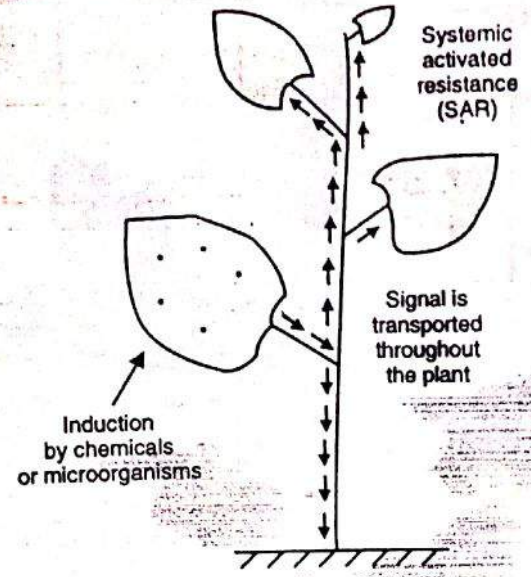


FIGURE 5-17 Principle of systemic activated (or acquired) resistance. A leaf treated with certain chemicals or with pathogens causing necrotic lesions produces a signal compound(s) that is transported systemically throughout the plant and activates its defense mechanisms, making the entire plant resistant to subsequent infections.

ternal 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 salicyclic 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 CGA245704 is being market-tested (in 1996) for commercial release. These 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 does 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 do 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

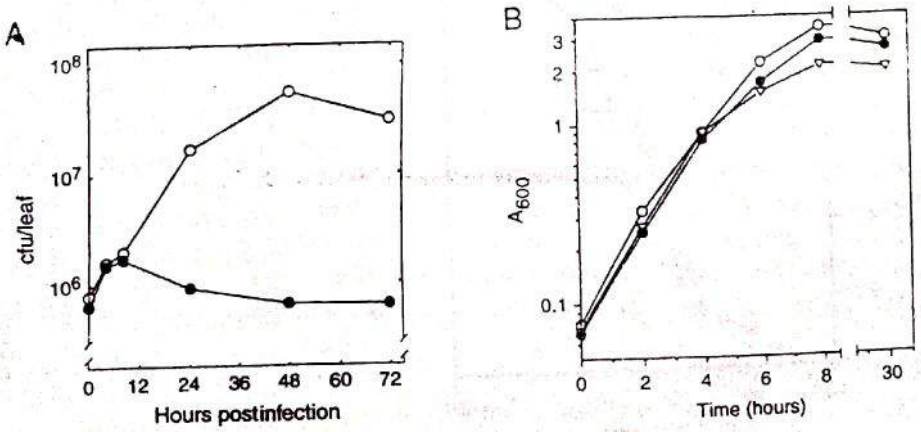


FIGURE 5-18 (A) Inhibition of growth and multiplication of *Erwinia carotovora* bacteria in inoculated leaves of tobacco seedlings growing in a medium containing 1 mM salicylic acid (●) or without salicyclic acid (○). cfu = Colony-forming units (bacteria). The control leaves were nearly macerated 12 hours after inoculation, whereas the salicylic acid-treated leaves had one small local lesion at the point of inoculation. (B) Lack of inhibition of growth and multiplication of the same bacteria in culture by various concentrations (0, 1, and 5 mM) of salicylic acid, indicating that the effect in (A) is caused by the plant defenses activated by salicylic acid and not by the salicylic acid itself. [From Palva *et al.* (1994), *Mol. Plant–Microbe Interact.* 7, 356–363.]

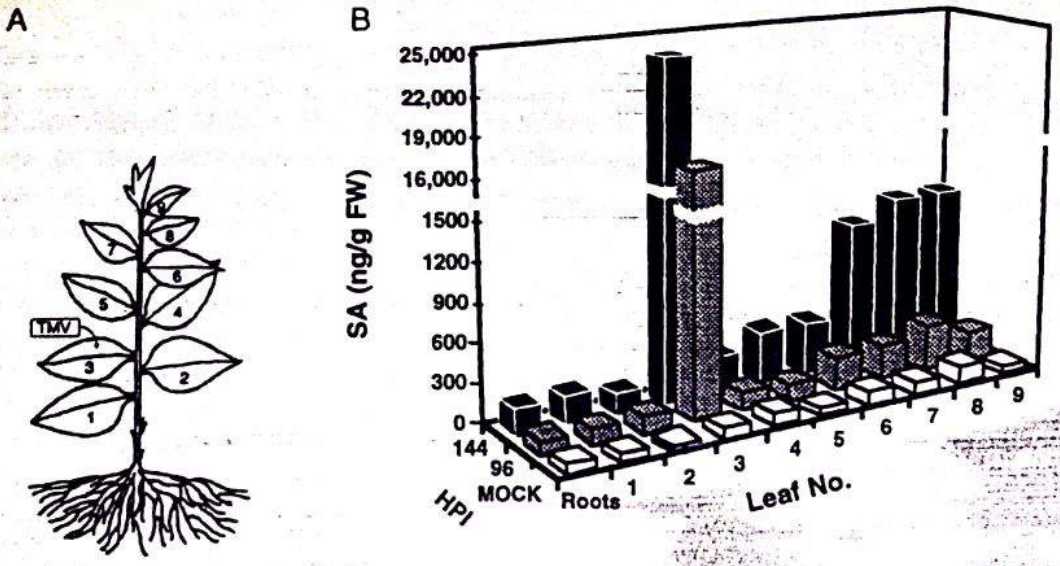


FIGURE 5-19 Salicylic acid accumulation throughout a 6-week-old tobacco plant after inoculation of a single leaf with a strain of tobacco mosaic virus that causes local lesions only and no systemic infection. (A) Inoculated leaf 3 in relation to other leaves and roots of the plant. (B) Concentrations of total salicylic acid (SA, in nanograms per gram fresh weight) in the inoculated leaf (leaf 3) and in uninoculated roots and leaves at 96 and 144 hours postinoculation (HPI). MOCK, Inoculation without virus. [From Shulaev *et al.* (1995), *Plant Cell* 7, 1691–1701.]

tested for their ability to induce systemic acquired resistance in plants, but so far none have 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.

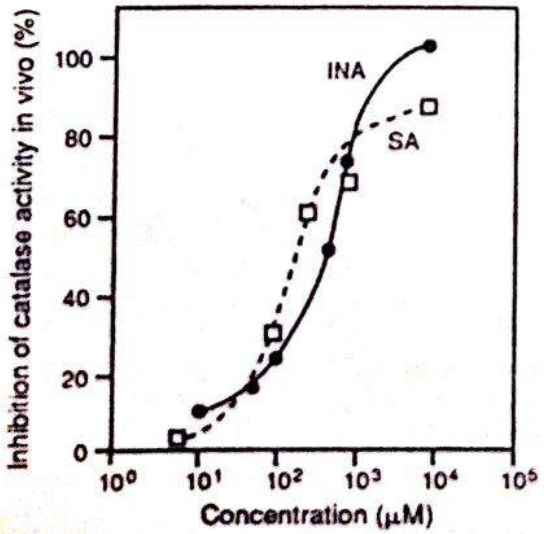


FIGURE 5-20 Inhibition of catalase activity by the plant defense-promoting compounds salicylic acid (SA) and the *in vivo* produced active form of isonicotinic acid (INA). Such inhibition in resistant plants results in accumulation of active oxygen radicals and in the hypersensitive defense response. [From Conrath *et al.* (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92, 7143–7147.]

Defense through Genetically Engineering Disease-Resistant Plants

With Plant-Derived Genes

The number of plant genes for resistance (R genes) that have been isolated is increasing rapidly. The first plant gene for resistance to be isolated was the *Hml* gene of corn in 1992, which codes for an enzyme that inactivates the HC-toxin produced by the leaf-spot fungus *Cochliobolus carbonum*. In 1993, the PTO gene of tomato was isolated; this gene encodes a protein kinase involved in signal transduction and confers resistance to strains of the bacterium *Pseudomonas syringae* pv. *tomato* that carry the avirulence gene *avrPto*. In 1994, four additional plant genes for resistance were isolated: the *Arabidopsis* RPS2 gene which confers resistance to the strains of *Ps. syringae* pv. *tomato* and *Ps. syringae* pv. *maculicola* that carry the avirulence gene *avrRpt2*; the tobacco N gene which confers resistance to tobacco mosaic virus; the tomato *Cf9* gene which confers resistance to the races of the fungus *Cladosporium fulvum* that carry the avirulence gene *avr9*; and the flax L^6 gene which confers resistance to certain rust fungus races of *Melampsora lini* carrying the avirulence gene *avr6*. The last five plant resistance genes are triggered into action by the corresponding avirulence genes of the pathogen, the products of which serve as signals that elicit the hypersensitive response (HR) in the host plant. Several more plant resistance genes have since been isolated. Some of these genes appear to provide plant resistance to

pathogens expressing one or the other of two unrelated *avr* genes of the pathogen. It is expected that these and many other R genes, which are likely to be isolated in the years to come, will be used extensively in genetically engineering transgenic plants that will be resistant to many of the races of the pathogens that affect these plants.

In addition to these specific plant genes, several other plant genes encoding enzymes or other proteins (PR proteins) found widely among plants have been shown to confer resistance to transgenic plants in which they are expressed. For example, tobacco plants transformed with a chitinase gene from bean became resistant to infection by the soilborne fungus *Rhizoctonia solani*. Similarly, transgenic tobacco plants expressing a PR1 protein gene were resistant to the blue mold fungus *Peronospora tabacina*, and plants expressing the systemic acquired resistance gene SAR8.2 were resistant to the black shank fungus *Phytophthora parasitica*. Moreover, transgenic potato plants expressing the gene for the antibacterial enzyme T4 lysozyme exhibited resistance to the soft rot and black leg caused by the bacterium *Erwinia carotovora* pv. *atroseptica*. Also, transgenic tobacco and potato plants expressing a gene from pokeweed (*Phytolacca* sp.) that codes for an antiviral, ribosome-inactivating protein exhibited resistance against several potato and other viruses. To these must also be added the induction of resistance in potato and tobacco transgenic plants transformed, respectively, with a mouse gene coding for an enzyme involved in the synthesis of an interferonlike compound and with a mouse gene coding for an antibody (plantibody) against the coat protein of a plant virus (artichoke mottle crinkle virus).

With Pathogen-Derived Genes

In 1986, it was shown for the first time that tobacco plants transformed (genetically engineered) to express the coat protein gene of tobacco mosaic virus (TMV) showed various degrees of resistance to subsequent inoculation with the same virus. Once the TMV coat protein gene was integrated in the tobacco genome, it was carried through the seed and behaved like any other tobacco gene. Since then, numerous other crop plants, especially solanaceous ones such as tobacco, tomato, and pepper, legumes such as alfalfa, grains such as barley, corn, oats, and rice, cucurbits such as cucumber, cantaloupe, and squash, and several other plants (papaya, impatiens, etc.), have been transformed with the coat protein gene of one or more of the viruses that infect them. The viruses from which the coat protein genes were obtained represent most of the virus groups.

In the vast majority of cases, transgenic plants show quite high levels of resistance to the virus from which the coat protein gene was derived and, in many cases, to other more or less related viruses. In some cases the transgenic plants were resistant to the virus if they were inoculated mechanically but not if inoculated by the specific vector of the virus, whereas in others the plants remained resistant even when inoculated by their aphid or fungus vector. In some cases, plants were transformed concurrently with as many as three viruses, the coat protein genes of which had been introduced in tandem into one location of the plant genome; such transgenic plants exhibited resistance to all three viruses.

Transgenic plants transformed with viral genes other than the coat protein gene often exhibit even higher levels of resistance to the virus providing the gene(s) and to, perhaps, additional viruses. Quite often the transferred genes either are portions of genes or are artificially mutated and thereby inactivated genes, so that they can be reproduced and expressed by the plant but do not produce a functional gene product that might aid a virus on infection. For example, highly resistant transgenic tobacco plants have been produced by transformation with modified virus replicase-coding genes of several viruses. Also, tobacco plants transformed with the TMV gene coding for the movement protein or for a dysfunctional movement protein are resistant to TMV and to several other viruses. Resistance to viruses has also been induced in plants transformed with viral genes coding for proteases needed for processing the viral nucleic acid, in plants transformed with small defective or satellite nucleic acids, and even in plants transformed with untranslatable or antisense segments of the viral nucleic acid.

There is every expectation that the area of inducing plant resistance to pathogens through genetic transformation with pathogen-derived genes will grow and improve rapidly. Such genetic engineering strategies will provide an excellent additional tool for plant disease control.

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