Biotic Stress

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In nature plants seldom grow in isolation. Their growth and development are greatly influenced by abiotic and biotic factors to which they are continuously exposed. Unlike animals they are rooted in the soil, and consequently they cannot escape adverse environmental conditions in their vicinity (Fig. 32.1). Rather they have to develop the strategies to adapt themselves to the hostile conditions in order to survive and grow. Biotic interactions of plants are not always harmful but they can be beneficial too. Plant-pollinator interactions, rhizobia-legume interactions, or mycorrhizal interactions are examples of **mutualism** in which both the partners of the association are benefitted. In rhizobia-legume interactions, host plant provides **ecological niche** and nutrients which are required for the growth of prokaryotes, while the prokaryotes provide nitrogen to the host plant. Some plants are not able to excrete protons or organic acids in the soil required for uptake of nutrients like phosphates. They do so through symbiotic associations with **mycorrhizal fungi.** Associations, in which one of the partners is benefitted and the other one is not affected, are called **commensalism**. In some cases, the association may become beneficial as the benefitting partner may stimulate defense mechanism in the other organisms. There may also be harmful biotic interactions among plants and other organisms. These include interactions of plants with **pathogens**, with the **plant pests** and **parasitic associations** between the plants. Plant pathogens are the organisms that spend a part of their life cycle or complete their life cycle inside the plant. These include the microbial pathogen such as viruses, bacteria, or fungi. On the contrary, plant pests include the **herbivores**, such as insects, nematodes, or mammals which cause damage to plants by eating their vegetative tissue, fruits, or seeds (Fig. 32.2). It is interesting to note that plants growing in the wild rarely develop disease. However, domestication of economically important species leads to the development of **monocultures**, thereby resulting in genetic uniformity which makes them more susceptible to infections. Understanding the interactions of plants with their biotic environment greatly helps in reducing the use of agrochemicals which, in turn, would facilitate reduction in pollution and also in

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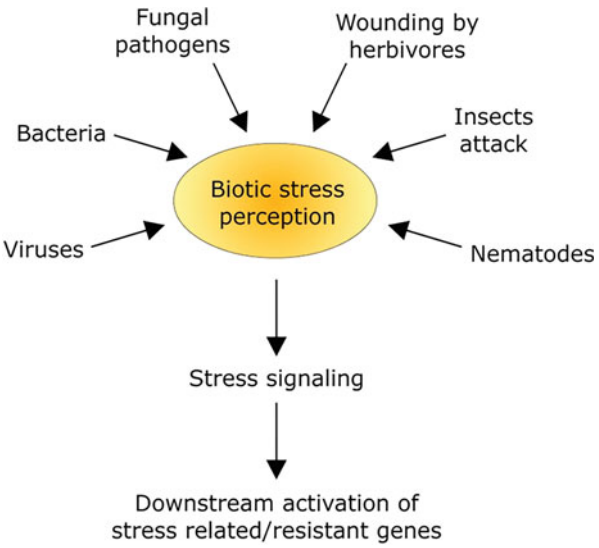


Fig. 32.1 Diversity of biotic stress factors which affect plants

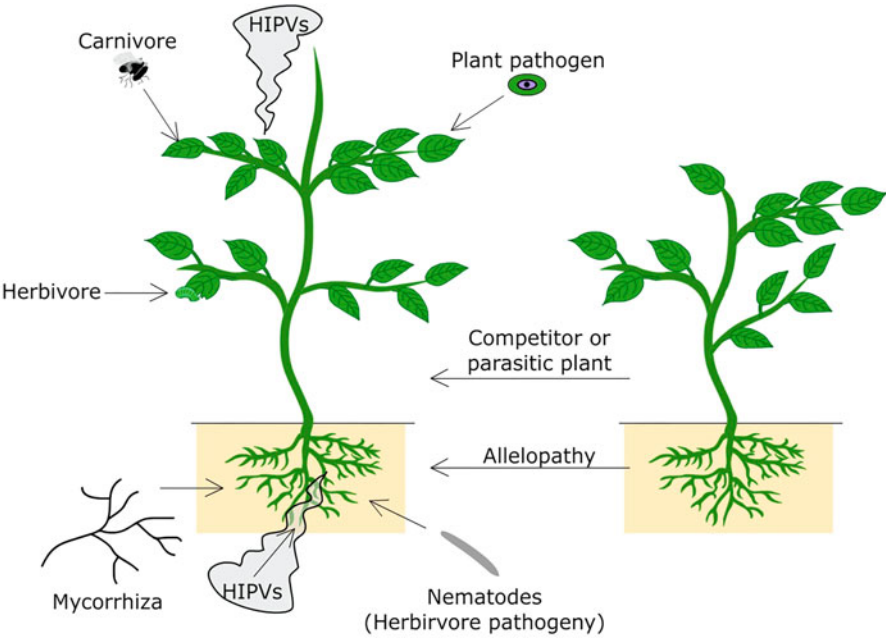


Fig. 32.2 HIPVs (herbivore-associated plant volatiles) release associated with different organisms (signal receivers) around a damaged plant

the cost of energy required for their production. **Allelopathic** interactions occur between plants growing nearby. Growth of some plants is inhibited because of chemicals produced by the adjacent plants which results in a sort of a **chemical warfare**. In this chapter plant's interactions with biotic factors will be dealt with at physiological and molecular level.

32.1 Interactions with Pathogens

A plant disease can be defined as an abnormal growth and/or dysfunction of a plant, and disease-causing microbes are called **pathogens**. Those microorganisms which are unable to induce disease in a host are termed as nonpathogenic with respect to host. There is a huge genetic polymorphism among the phytopathogenic agents. The climatic factors further influence this polymorphism, causing evolution of aggressive strains or biotypes that alter host-pathogen interactions (Fig. 32.3). A particular microbe is considered as a pathogen only when the suspected pathogen has the following characteristic features: (i) it should be consistently associated with the same symptoms, (ii) it should be possible to culture the pathogen in pure form away from the host, and (iii) it should be possible to reinoculate the pathogen into a healthy host (test host), and the symptoms then developed should be identical to those from where it had been originally isolated. Pathogens are responsible for about 15% losses in global food production and are a major challenge in breeding resistant crops. Pathogens include bacteria, fungi, oomycetes, and viruses. More than 1600 species of bacteria have been found to be responsible for plant diseases. These include gram-negative bacteria like *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Xylella*, and *Agrobacterium* and also gram-positive bacteria such as *Clavibacter* (*Corynebacterium*) and *Streptomyces* which commonly infect the plants. *Agrobacterium tumefaciens*, the cause of crown gall, is unique among plant pathogens as it transfers its DNA to the host. Mycoplasma-like organisms and spiroplasmas are cell wall-less prokaryotes known to cause about 200 diseases. More than 8000 species of fungi have also been found to infect plants. They have great diversity in their morphology and life cycle and develop complex relationship with the plants. Some of the microorganisms associated with plants are harmful though they may not have

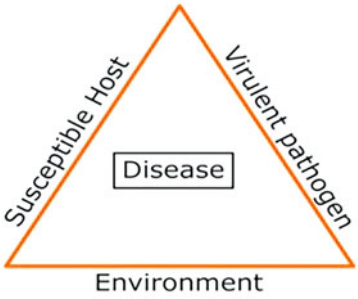


Fig. 32.3 Disease triangle of plants. For the development of disease, the pathogen should be virulent, host should be susceptible, and environment should be conducive for pathogen multiplication

pathogenic relationship, e.g., bacterium *Pseudomonas syringae* is found on the surface of the plant, where it lives as a saprophyte (Box 32.1). Under normal conditions, its presence does not affect the plant or its growth in any detectable way. But as the temperature drops below the freezing point of water, the bacteria act as nuclei for the formation of ice crystals. This ice crystal formation leads to frost damage in the plant. When bacteria are not present on the leaf surface, no ice crystals are formed, and water remains as liquid not only at 0 °C but even at several degrees below freezing point of water as well. It was discovered that "frost injury" is due to an *ice* **nucleation active (INA)** protein formed by bacteria and the mutant strain for this protein is not so harmful for the plant.

Box 32.1: Microbes in Rhizosphere and Phyllosphere

Plants grow along with enormous microorganisms, termed as plant microbiota, which play a very significant role in plant growth and provide protection against pathogens. The two most important zones where microbiota proliferates are **rhizosphere** and **phyllosphere**. The region in the vicinity of roots which is rich in microbes is known as rhizosphere, and a similar region around leaves is known as phyllosphere. Rhizosphere is modified by nutrient-rich mucigel secreted by the roots known as **rhizosheath**. Many of the microbes found in this zone are non-specific and feed saprophytically on the root exudates. In absence of any host, microbes survive in soil as spores and other resting structures. Pathogenic microbes are also present as resting spores but are stimulated to grow toward the root surface by the chemicals in the root exudates. For example, *Sclerotinia cepivorum* causes white rot in onion and is attracted by the compounds secreted by roots of the members of the onion family. Ethanol, which is produced under waterlogged conditions during anaerobic respiration of roots, stimulates production of infection structures in the spores of *Phytophthora infestans*. Plant root exudates also contain **siderophores** (or **phytosiderophores**), which are low molecular mass, iron-binding agents produced under iron-limiting conditions. Phytosiderophores limit the growth of microbes in the vicinity of roots by reducing iron availability. The beneficial effects of rhizosphere are manifold, viz., decomposition of plant residue and organic matter, increasing plant nutrient availability, symbiotic mycorrhizal association, production of organic chelates, mineralization of organic nitrogen, phosphorus solubilization, protection against root pathogens, enhancing drought tolerance, and biodegradation of synthetic pesticides or contaminates. In addition, several rhizobacteria trigger salicylic acid-dependent systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathways. In contrast to the beneficial effects, there are some harmful effects of microbes in the rhizosphere. Aerobic bacteria remove

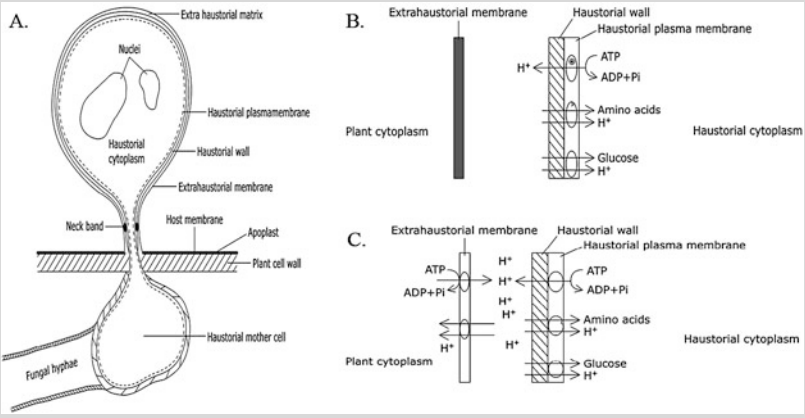
(continued)

Box 32.1 (continued)
oxygen or increase carbon dioxide, thereby reducing root elongation and development, as well as root hair formation. They also adversely affect the rate of nutrient and water uptake. In addition to nitrogen-fixing bacteria, other genera such as *Pseudomonas* and *Erwinia*, blue-green algae, fungi, and actinomycetes are commonly present on the leaf surface in the phyllosphere. These epiphytic microorganisms synthesize indole-3-acetic acid and help in nitrogen fixation, and their most important role is in inducing plant defense system to produce **phytoalexins**. Phytoalexins protect the plants from airborne microbes, e.g., infection of potato by *Phytophthora* sp. results in the production of many phytoalexins, such as caffeic acid, chlorogenic acid, scopolin, phytotuberin, etc.

A successful pathogen must enter the host plant to obtain nutrients after suppressing defenses of the host and finally reproduce to continue the life cycle (Box 32.2)**.** Once pathogen enters the plant, it employs different strategies for survival (Fig. 32.4). When the plant cells are killed prior to infection so as to facilitate the pathogen to colonize it, it is referred as **necrotrophy**. Necrotrophs make use of products of enzymatic digestion of cell walls, simultaneously releasing nutrients to survive and colonize. Some of the necrotrophic pathogens produce host-selective toxins which specifically inactivate particular plant enzymes. For example, the toxin **fusicoccin** produced by *Fusicoccum amygdali* (a fungus) inactivates the plasma membrane localized H^+-ATPase. As a result, an irreversible opening of stomata followed by plant wilting occurs, resulting in cell death followed by fungal colonization. *Sclerotinia sclerotiorum*, a necrotrophic fungus that infects ~400 plant species, secretes the phytotoxin oxalic acid which helps in early phases of infection. Oxalic acid alters the redox status of invaded plant tissues and suppresses several early plant defenses, including the oxidative burst and callose deposition. Once the plant is infected, it induces plant to produce reactive oxygen species (ROS) which leads to death of the host tissues followed by fungal colonization and growth. Many necrotrophs attack on broad range of plant species, e.g., *Botrytis cinerea*, a fungus, can attack 1000 plant species, and *Erwinia*, a bacterium, causes rot in many fruits and vegetables. The second strategy is **biotrophy**, when plant cells remain alive throughout the infection, e.g., in mutualism and parasitism (Box 32.2). Colonization by biotrophic pathogens leads to alterations in metabolism and development of the plant. Alteration in the levels of phytohormones can induce senescence or abnormal growth of the infected tissue and will reduce yield. Biotrophic fungal conidia germinate to produce germ tube on the leaves of susceptible plant. On sensing the contact of the appropriate host, the germ tube stops growing and hooks itself. It forms a specialized infection structure called **appressorium**, which acquires water from the dewdrop because of its water potential lowered due to accumulation of

Box 32.2: A Good Parasite Is a Poor Pathogen

When the invading microbes have some beneficial effects on the invaded partner, it is referred as symbiosis, but in case the balance shifts from mutualism to injurious effects on one partner, it is termed parasitism. Parasite is an organism living in intimate association with another living organism from which it derives some or all of its nutrients while giving no benefit in return. One of the most important differences between mutualism and parasitic association is the activity of H^+-ATPase on plasma membrane of the plant cells. In parasitic associations, H^+-ATPases are absent in the plasma membrane around the haustorium, while in mutualism the enzyme is present. This is mainly due to reciprocal movement of nutrients in mutualism, while there is unidirectional movement from the plants to the microbes in parasitism. When the parasitic interactions of host and microbes increase beyond nutrition, it results in disease. All parasites are potential pathogens, but not all pathogens are parasites. Any organism which is dependent upon another organism for its supply of nutrition might be expected to restrict its pathogenic effects to a minimum. Majority of parasites are nonpathogenic for their host but may be pathogenic to other organisms. However, there are parasites which become pathogenic to the same host due to changes in immediate environment. For example, most bacteria live as normal flora in the host, and with opportunity they become pathogens.



(**a**) A fungal hypha forming haustorium in the host cell. (**b**) Absence of H^+-ATPase pump in parasitic extrahaustorial membrane. (**c**) In mutualistic association H^+-ATPase pumps are present.

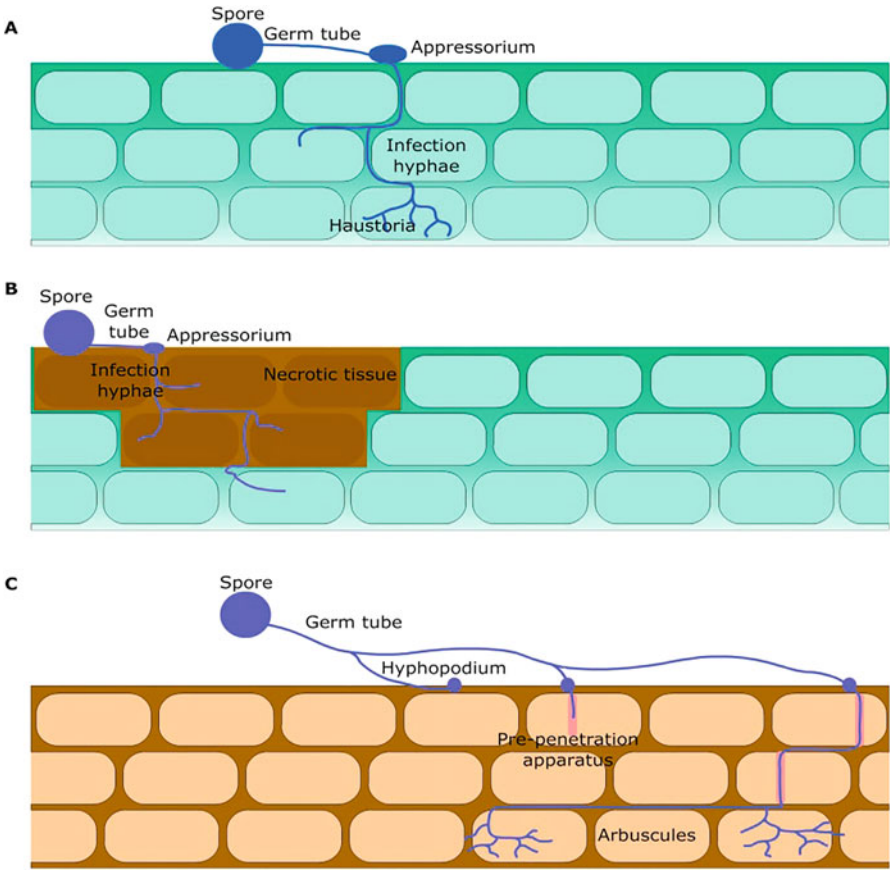


Fig. 32.4 Infection and colonization pattern in (**a**) biotrophic, (**b**) necrotrophic, and (**c**) biotrophic mutualist pathogens

glycerol and other solutes. As a result, extremely high turgor pressure is generated. The turgor pressure of the penetration plug and of secondary germ tube produced by appressorium is strong enough to push even through inert non-biological material such as Teflon and is sufficient enough to pierce through the cuticle into the rigid plant cell wall (Fig. 32.5). Haustorium, which develops as an invagination in plasma membrane of the host plant, is covered by extrahaustorial membrane (EHM). EHM helps in the absorption of nutrients and water favoring pathogen growth. In biotrophic associations, whether the microbes enter through cell wall of the host plant or not, the plasma membranes of the two (microbe and the host plant)

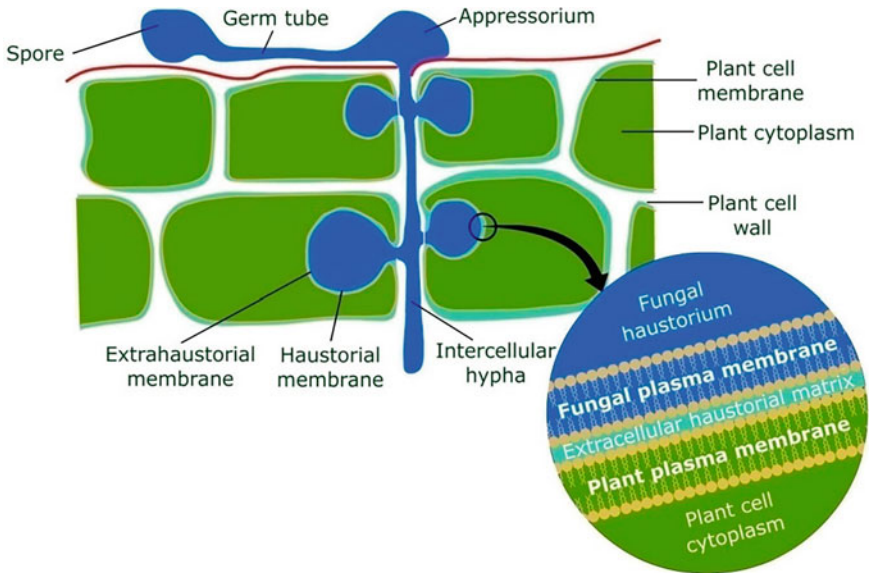


Fig. 32.5 Infection by fungal spore, forming germ tube attached to the host by an adhesion pad. An appressorium develops over the penetration hypha; the haustorium invades the host cell surrounded by the extrahaustorial membrane

remain intact and functional. Even though microbes remain outside the cell in the apoplastic space, levels of metabolites and phytohormones of the host plant are altered delaying senescence of the infected leaves besides causing stunting or abnormal growth patterns (Table 32.1). Biotrophic pathogens include mildew and rust fungi, viruses, and nematodes. The third strategy is **hemibiotrophy**, where the plant remains alive initially but is followed by death of the tissue. Hemibiotrophic pathogens use both modes of nutrition, first as a biotrophic and then as necrotrophic. Switching to necrotrophic mode of nutrition by the pathogen is due to high nutritional demand as there is increase in pathogen biomass and occurrence of asexual reproduction. For example, in potato late blight caused by *Phytophthora infestans*, the potato leaf remains alive during initial phases of infection, but as the infection becomes severe, the tissue is killed. **Oomycetes** and fungi can be biotrophic, necrotrophic, or hemibiotrophic plant pathogens. *Puccinia* shows both biotrophic and hemibiotrophic plant-pathogen interactions. Many bacteria of the genus *Pseudomonas* also show both biotrophic and hemibiotrophic interactions, i.e., it colonizes initially in the living tissues, and later on the leaf tissues show dead patches (lesions).

Table 32.1 Differences between necrotrophic and biotrophic pathogens

Features	Necrotrophic	Biotrophic
Biochemical	Host cells are not killed as few or no toxins/ enzymes are produced	Host cells are rapidly killed as more toxins/enzymes are produced
Morphological	Special parasitic structures, like haustoria, are formed Pathogen penetrates directly or through natural opening	No special structures are formed Pathogen penetrates through wounds or natural openings
Ecological	Host range is narrow The pathogen cannot grow without host Attack on healthy host	Host range is wide The pathogen can grow saprophytically without the host Attack at any stage of host

32.2 Susceptibility and Resistance

The terms "susceptible" and "resistance" are used with reference to host. If a particular pathogen can cause disease on a particular plant, that plant is susceptible, and if disease is not caused, that plant is resistant to that particular pathogen. A plant, if susceptible to one pathogen, is not necessarily susceptible to another pathogen. Pathogens which can invade the host but are unable to cause disease are known as **avirulent**, and those which drastically affect the host and cause disease are known as **virulent**. There is variation in the degree of susceptibility and in the strength of resistance of a host. **Pathogenicity,** which refers to the ability of a pathogen to cause disease, is an all-or-none disease situation, while virulence describes the gradation of pathogenicity. Features of a pathogen, like rapid growth and releasing cell wall-degrading enzymes, may or may not be related to virulence. An interaction between host and pathogen, where symptoms are expressed clearly, is known as **compatible** disease reaction, and when symptoms are not expressed clearly and the reaction has minimum effect on the host, it is known as **incompatible** disease reaction.

Specificity of a pathogen describes the extent to which a pathogen is restricted to particular host plants. Most pathogens are restricted to one or a few host species, and most host plants are susceptible to only a few pathogens. For example, *Puccinia* is pathogenic to cereals and never to soybeans, and *Phytophthora megasperma* is pathogenic to soybean and never to wheat. Some pathogens, such as oomycetes genus *Pythium* and Ascomycetes genus *Sclerotinia*, attack hundreds of different plant species. Protection of a host against particular pathogen strain is known as **vertical resistance**. On the other hand, protection of a host against wide range of pathogens is known as **horizontal resistance**. The process of infection, colonization, and reproduction of pathogen in the host is known as **pathogenesis.**

32.2.1 Entry of Pathogen

There are three main routes of entry for pathogens in plants, viz., direct penetration through intact surface since most pathogens produce a wide variety of cell wall-degrading enzymes, entry through natural openings (stomata and lenticels), and entry through wound sites. Fungi can enter through all the three routes depending on species. Invasion of fungi involves landing of spores on the leaves followed by host recognition and germ tube emergence. Hyphae grow through cell wall and press against the cell membrane. Hyphae differentiate into haustoria and nutrient transfer begins (Fig. 32.6). Some rust fungi invade through stomatal opening. Bacteria rarely enter by direct penetration. They either enter through natural openings or depend on

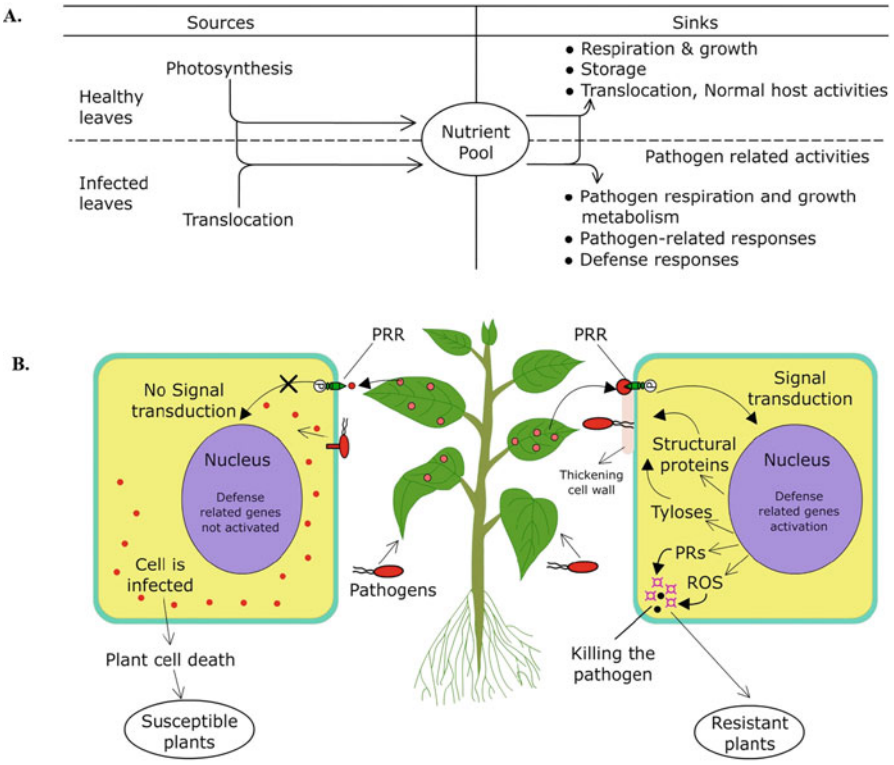


Fig. 32.6 (**a**) Carbon flow through a nutrient pool within mature tissues of healthy and biotroph infected leaves. (**b**) Diagrammatic view of plant-pathogen interactions and molecular mechanisms involved in susceptible and resistance disease reactions by plant cell on pathogen attack. If plant cell PRRs are unable to identify pathogen, then no signals are transduced, and hence defense-related genes are not activated, and plant becomes susceptible. On the other hand, if plant cell PRRs recognize pathogen, they show hypersensitive responses (thickening of cell wall, tyloses, etc.), or defense-related genes are activated resulting in formation of PR proteins and ROS, which kill the pathogenic spores and plant becomes resistant. *PRR* pattern recognition receptors, *PRs* pathogenesis related, *ROS* reactive oxygen species

insects for entry into the plants. When the mouth part of an insect penetrates phloem for feeding (phloem feeders), bacteria are taken along the phloem sap which are transmitted to the vascular cells. Phytopathogenic bacteria colonize the apoplast region of host plants causing rots, spots, vascular wilts, cankers, and blights. Most of pathogenic bacteria are rod-shaped and belong to genera *Agrobacterium*, *Xanthomonas*, *Pseudomonas*, *Erwiniam*, and *Dickeya*. In plants, most bacteria remain in extracellular spaces and use a specialized **Type III secretion system** (T3SS) to deliver bacterial proteins into the plant cell (discussed later in the chapter). The sequential events leading to successful entry of pathogen can be summarized as follows: 1. The microorganism must adhere to the plant surface to initiate infection. Some of the pathogens synthesize adhesive chemicals and secrete them when they come in contact with the host. It involves relatively non-specific molecular interactions between some components of the cell surface of both host and the pathogen. 2. The pathogen must penetrate the epidermal layer of the plant cell if it cannot enter through the wound or stomata. Some fungi secrete cutinase enzyme that disintegrates waxy cuticle covering the epidermis. 3. Once inside, the host tissue invading fungi may grow intercellularly without further invading the cytoplasm of the living cells. They secrete enzymes, such as cellulases and hemicellulases, which enable them to digest the cellulose and other cell wall polysaccharides for their (fungi) growth and multiplication. Phytopathogenic bacteria colonize the apoplast to cause spots, vascular wilts, and blights. During the parasitic relationship, the bacteria reside in the intercellular spaces of various plant organs or in the xylem. The bacteria get surrounded by an extracellular polysaccharide material (EPS) and proliferate in close contact with the plant cell wall. Bacteria cause huge damage to the plant tissue by secreting toxins or cell wall-degrading enzymes. 4. Most of the pathogens secrete toxins in the host tissues. For example, *Erwinia amylovora* (the bacterium that causes fire blight of apple, pear, and other members of Rosaceae family) secretes a 44 kDa glycine-rich hydrophobic toxic protein that kills plant cells by disrupting the ion flux across the membrane and induces hypersensitive response.

32.2.2 Hypersensitive Response

Hypersensitive response is a localized response of plant cells to the microorganisms so as to prevent further invasion of the adjacent cells. In most cases microorganisms induce hypersensitive response in the plant without causing any disease. Most microbial pathogen attacks result in ROS formation, changes in cell wall composition and membrane permeability, callose deposition, and induction of phytoalexin or phenolic production leading to programmed cell death.

32.2.2.1 Ion Fluxes

One of the earliest responses of a plant to any kind of invader is a rapid flux of ions. Penetration by pathogen leads to depolarization of the membrane in the cells of host plant followed by rapid movement of ions. Efflux of H^+ and an influx of extracellular Ca^{2+} into the cytoplasm of the cell through activated calcium channels are also

evident. As a result, intracellular cytosolic concentration of calcium increases due to its transport from the apoplast or from the stored Ca^{2+} in cell compartments. Increase in cytosolic Ca^{2+} stimulates formation of wall thickenings by cross-linking and callose deposition leading to hypersensitive cell death besides stimulating phytoalexin synthesis.

32.2.2.2 Oxidative Burst

Oxidative or **respiratory burst** is the rapid release of ROS from different types of cells. The synthesis of ROS during oxidative burst occurs through NADPH oxidase and extracellular peroxidases. An increase in rate of respiration and activation of a membrane-bound NADPH oxidase is observed. This leads to the rapid generation of superoxide (O_2^-) anions which are converted into hydrogen peroxide (H_2O_2) either spontaneously or through the action of **superoxide dismutase**. ROS act as antimicrobial agents and strong oxidants which cause massive cell damage (Fig. 32.7). Hydrogen peroxide stimulates the phenylpropanoid pathway and ion fluxes. In addition, it activates genes responsible for synthesizing the enzymes that protect the host cells from these effects. These enzymes include catalase which removes H_2O_2 and glutathione S-transferase (GST), which removes dangerous radicals.

32.2.2.3 Changes in Cell Wall Composition

Some plants alter composition of the cell wall as soon as pathogen tries to enter. Deposition of lignin or suberin in the primary cell wall may block the entry of pathogen. Activity of enzyme phenylalanine ammonia lyase (PAL), which plays a key role in the synthesis of lignin precursors, is elevated during pathogenesis. Peroxidases play an important role in lignin synthesis as well as other cross-linking reactions in the cell wall of the infected cell. Cross-linking gives strength to the cell wall making it resistant to microbial attack. Tannins and lignin also bind to proteins and deactivate the degrading enzymes released from the fungus. Callose deposition on the inner side of the cell wall helps to slow down the entry of the pathogen. It helps in gene activation and de novo synthesis of defensive agents. Minute papillae composed of callose and lignin are formed beneath the penetration site of the biotrophic fungi to block fungal penetration into the plant cell. There is blockage of plasmodesmatal connections by callose deposition, thereby blocking cell-to-cell movement of viruses. Plants may also modify cell walls by synthesizing and depositing **hydroxyproline-rich glycoproteins (HRGPs)**, such as extensins and arabinogalactan proteins, in walls of the cells in vicinity of invading pathogen. Extracellular HRGPs contribute to the thickening of cell wall in two ways. 1. HRGPs rapidly form covalently cross-linked network generating negatively charged cushions at the membrane-cell wall interface, thereby acting as barriers against the entry of pathogen. 2. De novo HRGP synthesis initiates additional lignin polymerization to further strengthen the cell wall. These thickenings make cell wall resistant to microbial penetration and enzymatic degradation. This may prevent penetration of haustorium into the cell. Another class of extracellular defense-related proteins secreted by plants are **polygalacturonase-inhibiting proteins (PGIPs)**, which have a leucine-rich repeat (LRR) motif. Fungal hyphae, mainly of

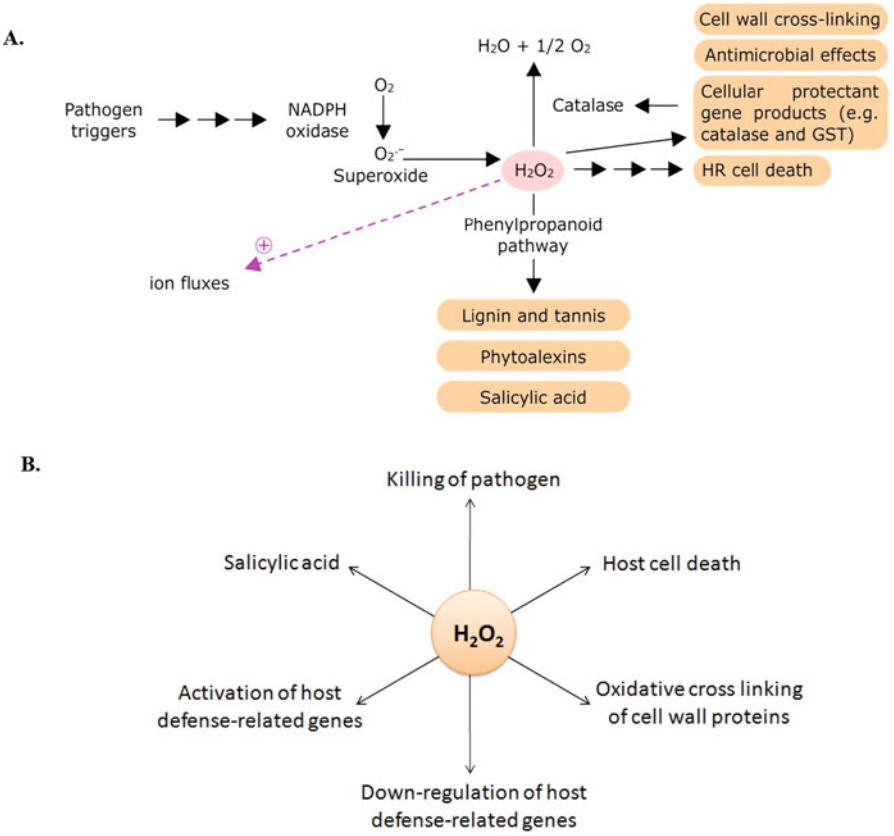


Fig. 32.7 (**a**) Steps involved in oxidative burst and its role in inducing plant defense. Superoxide ions are converted into hydrogen peroxide (H_2O_2), which acts as a signal promoter in a number of defense responses, viz., cell wall cross-linking, phytoalexin synthesis, and hypersensitive responses (if signal is strong). H_2O_2 stimulates ion fluxes and removes itself through enhanced catalase. (+) sign indicates positive feedback. (**b**) Central role of H_2O_2 in defense response of plants to microbial pathogen infections. Response to H_2O_2 may be different in different plant species

necrotrophic pathogen, release a specific type of cell wall-degrading enzymes called polygalacturonases (PGs). These PGs cause release of oligogalacturonides with chain length of >8 units which act as elicitors. These are sometimes referred as **damage-associated molecular patters (DAMPs)** which interact with the membrane-localized receptors resulting in signal transduction and expression of plant defense proteins, including PGIPs.

32.2.2.4 Phytoalexins

In addition to chitinases and glucanases, plants synthesize phytoalexins in response to pathogen attack because of expression of genes. These include sesquiterpenoids, flavonoids, isoflavonoids, and phenylpropanoids. Genes for the synthesis of

capsidiol and rishitin (sesquiterpenes) are expressed in response to pathogen attack in tobacco and potato. Disease resistance in plants has at times been correlated with accumulation of phytoalexins. Mutants of phytoalexins biosynthesis in plants have been found to be more susceptible to pathogen attack. Pathogens have different sensitivity to phytoalexins. Over 300 different types of phytoalexins have been identified in many families such as Brassicaceae, Fabaceae, Solanaceae, and Poaceae. Phytoalexins are antibiotics of low potency and specificity. They are low molecular mass lipophilic molecule (soluble in lipids) and hence can cross plasma membrane and exert their toxicity. Various phytoalexins, which are flavonoids and isoflavonoids, are synthesized from phenylalanine precursor via phenyl propanoid pathway. Phytoalexins are capable of forming free radicals, which can damage DNA and disrupt membranes. They can also disrupt mineral uptake and signal transduction pathways leading to inhibition of pathogen growth. **Phenylalanine ammonia lyase (PAL)** *and* **chalcone synthase (CHS)** are key enzymes in phytoalexins biosynthesis. Many fungi, mainly highly virulent strains, metabolize phytoalexins into less toxic derivatives. Some pathogens can synthesize suppressors that can effectively block phytoalexin accumulation. In pea, suppressor inhibits the accumulation of mRNA for PAL and CHS, therefore reducing synthesis of pisatin. Isoflavonoids are common phytoalexins in some leguminous plants, such as soybean and alfalfa. In solanaceous plants, such as potato and tomato, phytoalexins are sesquiterpenes. Phaseolin, a phytoalexin, is **fungistatic** at low concentration but is fungicidal at high concentrations. Large number of microorganisms, such as *Pseudomonas*, *Alternaria*, and *Botrytis*, induces synthesis of camalexin-a major phytoalexin in *Arabidopsis*. Enzymes required for phytoalexin biosynthesis are generally not stored prior to infection but rather are synthesized afterward.

32.3 Plant Defense Mechanisms

Within minutes of attack by pathogens or insects, plants exhibit a local response. An elaborate response is, however, activated within hours in plant tissues away from the initial site of infection or tissue damage. This induced systemic response is determined by the type of causal (attacking) organism. The induced systemic response is distinct in case of pathogens than the one induced by herbivores. In case of pathogens, this response is known as **systemic acquired resistance (SAR)**, which results in the synthesis of **pathogenesis-related proteins (PR proteins)** in plants. PR proteins include various enzymes, such as protease inhibitors (that inactivate the proteolytic enzymes secreted by the pathogens), or lytic enzymes such as β-1,3-glucanase or chitinase that degrade the invading pathogens, or enzymes which interfere with the growth of insects. Plants have innate (passive) immune system, unlike in animals where there are specialized cells which move to the infection site to kill the invading organisms, no such type of cells are reported in plants (Box 32.3). Rather, each cell needs to have its own system to fight the invading organism. Plant's immune responses include enabling other cells and the nearby plants for pathogenic infections in which salicylic acid plays an important role. Innate immunity is of greater efficiency and is the most common form of

Box 32.3: Immune System in Plants vs Animals

Plants, unlike mammals, lack mobile defensive cells and a somatic adaptive immune system. Instead, they rely on the innate immunity of each cell and on systemic signals emanating from the infection sites. Innate immunity system is always present in the cell and is readily mobilized to fight microbes at the site of infection. In other words, innate immunity refers to the immunity that occurs naturally because of genetic factors or physiology and is not caused by infection or vaccination. **Adaptive immune system** (AIS) involves action against pathogens that are able to evade or overcome *innate* **immune system (IIS).** However, when activated, the components "adapt" to the presence of infection agents by activating, proliferating, and creating potent mechanisms for neutralizing or eliminating the microbes. Although plants neither have such a complicated AIS nor antibodies, they have the capacity to differentiate between self and nonself molecules. Plants do have cell surface receptors to identify certain patterns characteristic of pathogens. Such receptors, when activated, trigger the production of chemical substances, such as methyl jasmonates, that may induce both local as well as systemic defense responses. Humans and other vertebrates have a complex immune system, i.e., adaptive immune system. The main components of this system are the organism's ability to biochemically distinguish between its own and foreign cells and remember the specific features of the foreign cell. AIS uses foreign macromolecules as antigens, and using the characteristic features of these molecules, it generates specific antibodies. These antibodies tag the foreign molecules for destruction with the specialized blood cells, called lymphocytes. These specific antibodies then rapidly detect subsequent infection by a particular pathogen and show quick defense responses. Plants have unique and sensitive *chemoperception system* for substances derived from pathogens. It resembles olfactory (smell) perception in animals. Plants as well as animals are able to recognize common patterns on the surface of pathogens using **pattern recognition receptors (PRRs).** Similar to vertebrates, phytohormones play significant roles as signaling molecules as well as immunomodulators (altering the sensitivity of immune system) in the regulation of plant immune response.

resistance to microbes in plants which depends on the ability of plant to distinguish between self and nonself molecules. Plants remain disease-free or suffer from a disease depending on the balance between the pathogen's ability to cause disease and the host's ability to defend itself against it. Plant possesses a wide array of defense mechanisms to counter the undesirable invasion of pathogenic microbes. Some of the defense mechanisms are **constitutive**, which include the structural barriers (cell walls, epidermis layer, trichomes, thorns, etc.) and accumulation of chemical compounds (such as phenolics, nitrogen compounds, saponins, terpenoids, steroids, and glucosinolates) and proteins and enzymes with inherent antimicrobial properties. These compounds confer tolerance or resistance to biotic stresses by not

only protecting the plants from invasion, but also by providing them strength and rigidity. The constitutive defense mechanisms are always present whether the plant is attacked or not. Plants may exhibit **qualitative defense** by producing low amount of highly toxic substances, or **quantitative defense**, which refers to production of less toxic compounds in much greater amount. Though qualitative defense requires less investment by plants, it is a more vulnerable strategy since chance for the pathogen to cope up is more in comparison to quantitative defense. On the contrary inducible defense mechanisms are apparent only when infection is underway. It includes the ability of the plant to detect the signal, transduction of the signal followed by the biochemical changes in the plants, which may respond by developing mechanical barriers, such as by triggering callose deposition or by producing metabolites which are toxic to invading pathogens. For example, a pathogen may be prevented to attack a nonhost plant part not only due to constitutive defense mechanisms but also because new cell wall material is synthesized and deposited at the site of infection as the induced response of plants to pathogenic infection. A fungal pathogen, *Gaeumannomyces graminis* var. *tritici*, is not able to attack oat because of synthesis of a toxic triterpene, avenacin, by the roots of oat plant, but it can infect wheat. However, a different form of fungus can infect oats because it has a mechanism to detoxify avenacin produced by the plant. A range of antibiotic compounds produced by plants inhibit or deter pathogens.

Inducible defense responses in the plants include **basal defense mechanisms** and **R gene-mediated defense mechanisms** which are also known as **microbial-associated molecular-pattern-triggered immunity** (MTI) or **pattern-triggered immunity (PTI)** and effector-induced resistance, often referred as **effector-triggered immunity (ETI)**, respectively. PTI is first-level immunity response triggered by plant **pattern recognition receptors (PRRs)** that sense molecular patterns of the pathogen. PTI is in response to the molecular patterns of the pathogen which are common among them, i.e., it is triggered in response to most of the pathogenic bacteria. ETI is the immune response of plant to specific pathogens and occurs in response to specific molecules secreted by the pathogenic bacteria which are known as effectors. ETI is the most successful means of controlling pathogens that are able to evade pattern-triggered immunity (PTI) (Table 32.2). ETI is much faster and quantitatively stronger than PTI. ETI is often associated with a localized cell death or hypersensitive response (HR) to restrict further spread of microbial attack. Cell death occurs through apoptosis and necrosis. The important feature of ETI is the plant's ability to sense microbe-mediated modifications of defense mechanism in the host, whereas PTI is able to sense infectious-self and nonself by guarding against pathogen. In other words, ETI is an efficient defense system for more progressed infections, whereas PTI is important for nonhost resistance and for basal immunity in susceptible host plant cultivars (Fig. 32.8a).

Inducible defense mechanisms include the following phases:

1. Most microorganisms have molecules at their surfaces that are recognized by receptors present on plant cell membrane, and once pathogen is identified, the activation of defense mechanism in the host plant is triggered. These are also

Table 32.2 Differences between pattern-triggered immunity (PTI) and effector-triggered immunity (ETI)

Pattern-triggered immunity (PTI)	Effector-triggered immunity (ETI)
General type of defense response common among all pathogens	Highly specific type of defense response and is different among different pathogens
Plant can detect molecular signatures of all pathogens	It is gene-for-gene defense response. Plant can detect molecular signature of specific pathogen
The molecular signature of pathogen is recognized by pattern recognition receptors (PRRs)	It is initiated by the secretion of effector molecule called Avr (avirulence) protein and is detected by plant resistance gene R
Pattern recognition leads to mitogen-activated protein cascade, a signal transduction pathway common to all organism	The R gene encodes Ser/Thr protein kinases which interacts with nucleotide-binding leucine rich proteins (NB-LRRs)
The signal cascade results in the activation of several transcription factors which control defense genes	The signaling complex interacts with kinase cascade and results in the activation of hypersensitive response and rapid cell death around the site of infection

referred as PAMP-triggered immunity (PAMP-pathogen-associated molecular patterns) or pattern-triggered immunity PTI. These defenses are also referred as basal defense mechanisms.

2. The basal defense mechanism puts a selective pressure on potential pathogens to produce effector molecules which interfere with basal defense mechanisms, further enabling pathogen to attack the plant. This is referred as **effector-triggered susceptibility (ETS)**.
3. Successful colonization of pathogen following ETS triggers further defense mechanisms in the host plant, which is known as R gene-mediated defense or effector-triggered immunity (ETI). This defense mechanism of the host plant is stronger than PTI. This defense mechanism is known as R gene-mediated defense, or effector-triggered immunity (ETI). This defense mechanism of the host plant is stronger than PTI. Receptors for these effector molecules are coded by resistance (R) genes of the host plant.
4. R proteins provide selective pressure on the pathogens to further produce effector molecules that are not recognized by the host plant. If a pathogen is able to produce such effector molecules not recognized by R gene-mediated defense mechanism, it will be able to attack and exploit the plant for its use, causing disease (Fig. 32.8b).

32.3.1 Pathogen- or Microbial-Associated Molecular-Pattern (PAMP/MAMP)-Triggered Immunity (PTI)

PTI (formerly called basal or horizontal disease resistance) can be considered as the primary driving force of plant-microbe interactions. The basal defense mechanisms are elicited by the molecules that are common in most of pathogenic species. This

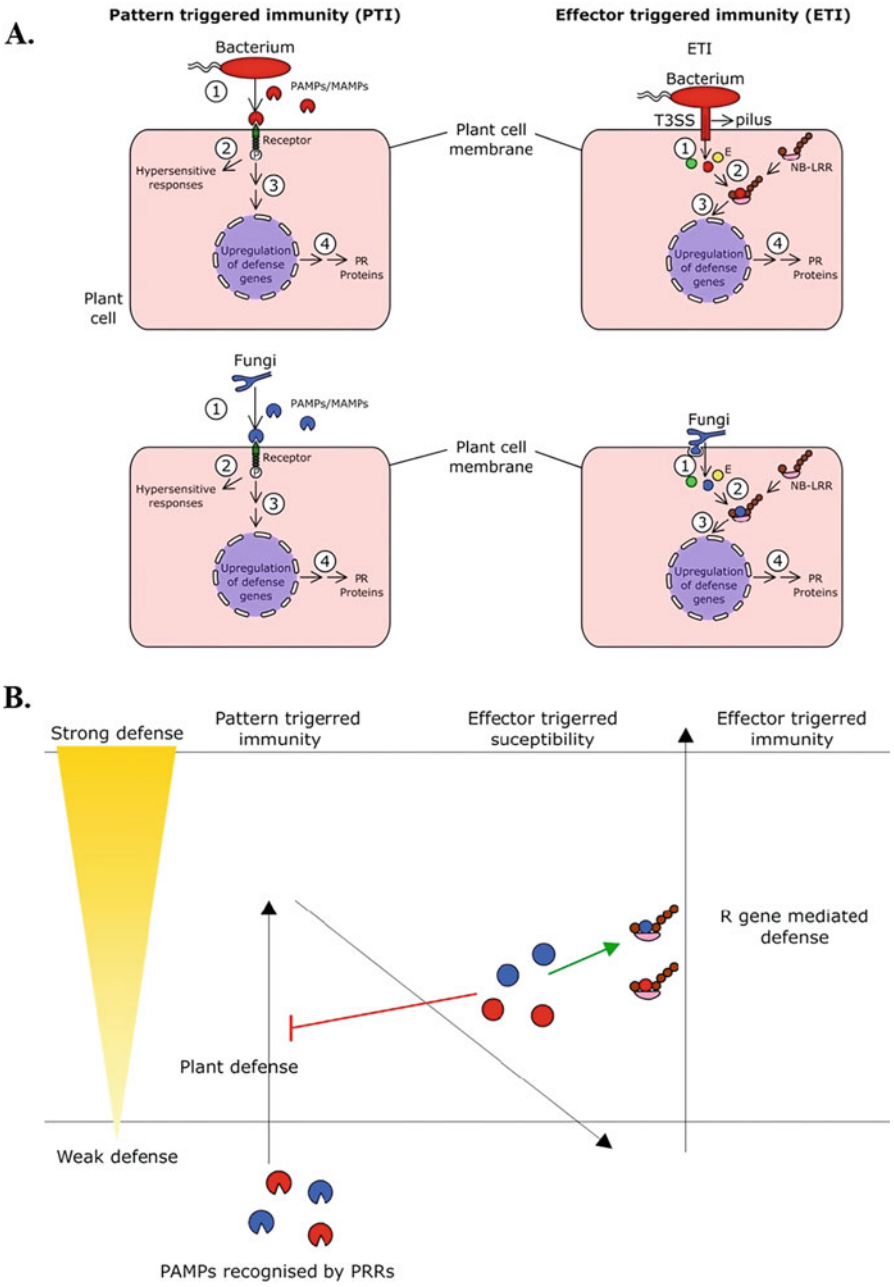


Fig. 32.8 (**a**) Diagrammatic representation of steps involved in pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), by bacterial and fungal pathogens, respectively. (**b**) Phases of inducible defense mechanism

includes 1. **microbial- or pathogen-associated molecular patterns (MAMPs/PAMPs)**; these molecules are highly conserved across larger groups of pathogens and are indispensable for their survival. These molecules do not exist in the host, such as fungal chitin, xylanase or bacterial flagellin, lipopolysaccharides, and peptidoglycans, and 2. the other correspond to a compromised "self," also called **damage-associated molecular patterns (DAMPs).** Both PAMPs and DAMPs stimulate the plant defense responses and are known as **elicitors.** An elicitor is a molecule that triggers defense responses in the host cells. Many molecules which are released either by the plants or pathogens act as elicitors. The elicitors released by plants are known as **endogenous elicitors**, while those released by pathogen are **exogenous elicitors**. Fungal wall fragments which are formed when plant enzyme (chitinase) degrades the fungal cell wall act as exogenous elicitors. Plant cell wall fragments which are formed by the enzymes (cutinase) secreted by pathogen act as endogenous elicitors. A large proportion of both kinds of elicitors are common to many plant-pathogen interactions. Elicitors can also be categorized in two different classes on the basis of their specificity, viz., **general or non-specific elicitors**, which provide general resistance and are not different in their effect on different plant species. Others are **specific elicitors**, which are formed by specialized pathogens and function only in plants carrying disease resistance genes. It is only when the plants show defense responses to such elicitors plant-pathogen interactions begin (Fig. 32.9). Elicitors are active at very low concentrations (in nanomolar range); they bind to receptors localized on plant cell membrane and specificity of the host-pathogen interaction lies in the chemical nature of the elicitor. Elicitor-receptor binding triggers the signal cascade which is responsible for expression of plant defense genes responsible for basal defense**.**

Basal defense response in plants is the first defense against pathogens triggered by conserved elicitor molecules (PAMPs and DAMPs). PAMPs include molecular patterns of the bacterial cells such as bacterial **flagellin** (flg22) and **EF-Tu** (elf18), **fungal chitin** (CEBiP), and oomycetes heptaglucan (Fig. 32.10). These microbial elicitors are recognized by receptor proteins, located on the plasma membrane of the plant cell, which are referred as pattern recognition receptors (PPRs). PRRs belong to either the receptor-like kinase (RLK) or receptor-like protein (RLP) families, which are membrane-bound extracellular receptors. RLPs resemble the extracellular domains of RLKs but lack the cytosolic signaling domain, whereas RLKs have both extracellular and intracellular kinase domains. PPRs, such as **CERK1** (chitin elicitor receptor kinase 1), FLS2, and EFR protein, have been identified in plants (rice, wheat, and *Arabidopsis*) for fungal chitin, flagellin (flg22), and elongation factor (EF-Tu), respectively (Fig. 32.10).

Chitin, a homopolymer of β-(1,4)-linked N-acetylglucosamine (GlcNAc) units, is a major constituent of fungal cell walls and is a classical PAMP. Chitin is an ideal point of attack during plant defense responses since glucosamine polymers are not found in plants. Upon pathogen contact, chitinases (hydrolytic enzymes) are released by the host plant which breaks down the fungal cell wall chitin polymers. These fungal chitin fragments are recognized by CEBiP (chitin elicitor-binding protein) and CERK1 (chitin elicitor receptor kinase 1) where both are located on the plasma

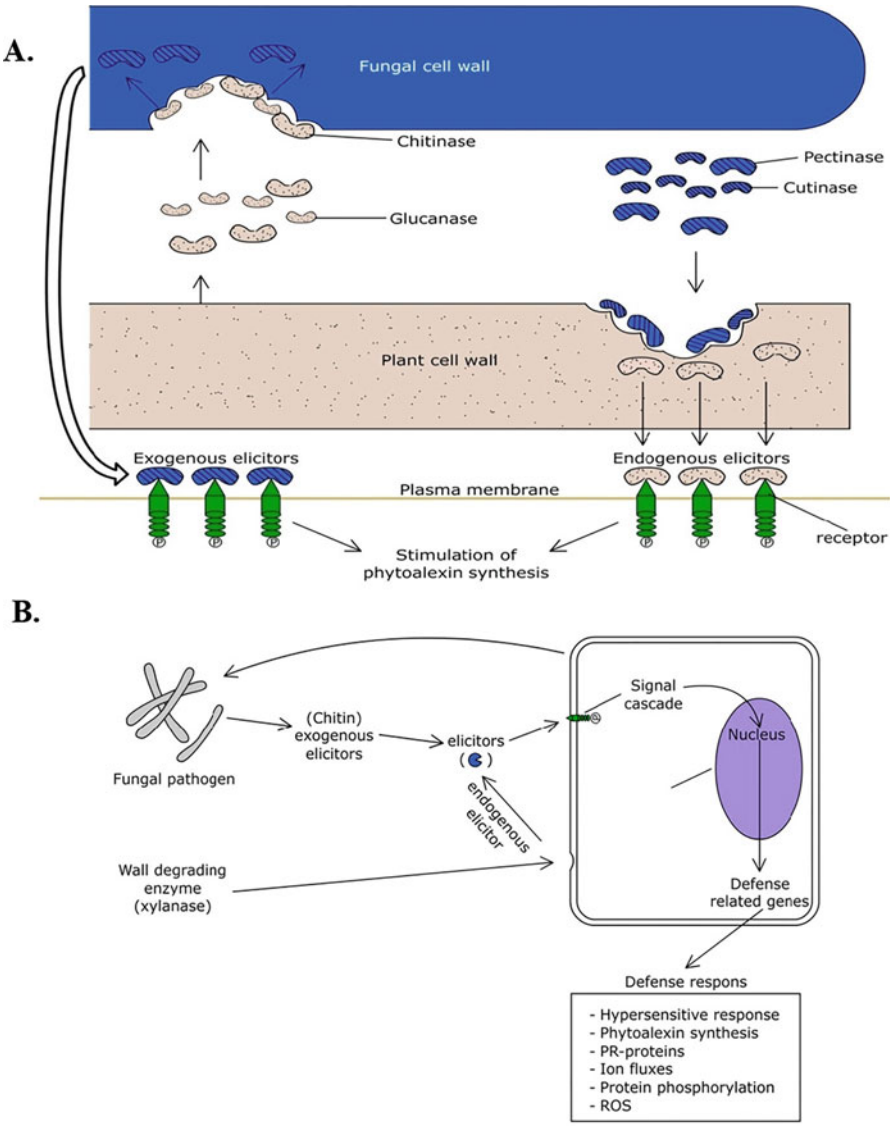


Fig. 32.9 (**a**) Changes in the metabolism during plant-fungal interaction leading to production of elicitors and stimulation of phytoalexin synthesis. Plant cell releases glucanase and chitinase which lead to degradation of fungal cell wall. The fungal wall fragments act as elicitors (exogenous elicitors) and trigger phytoalexin synthesis (hypersensitive response). At the same time, fungi also release cutinase and pectinase which degrade plant cell wall resulting in the formation of elicitors (endogenous elicitors) and triggering hypersensitive response. (**b**) Diagram depicting elicitor-receptor binding which triggers signal transduction cascade and expression of plant defense genes responsible for basal defense

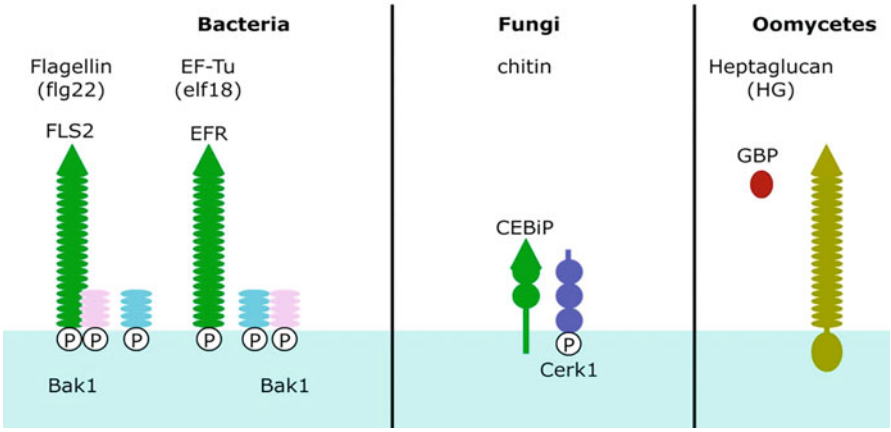


Fig. 32.10 Pathogen-associated molecular patterns (PAMPs) of the bacterial cells such as bacterial flagellin (flg22) and EF-Tu (elf18), fungal chitin and oomycetes heptaglucan. These microbial elicitors are recognized by pattern recognition receptors (PRRs) such as FLS2, EFR protein, and CERK1

membrane of the plant and possess an extracellular domain that contains LysM motifs which bind chitin fragments. The first chitin-binding PRR was identified in rice as the *lysine motif* (*LysM*)-*RLP* and was named *chitin* elicitor-*binding* protein (*CEBiP*). *CEBiP* is a glycoprotein localized on the plasma membrane. Intracellular protein kinase domain of CERK1 is involved in downstream signaling. CERK1 of *Arabidopsis* is also involved in the recognition of bacterial peptidoglycan, a polysaccharide that has a repeating N-acetylglucosamine unit.

Flagellin is a bacterial flagellar protein with highly conserved N-terminal domain, flg22, which is a 22-amino acid peptide derived from N-terminal fragment of flagellin. In *Arabidopsis*, the flagellin-sensing FLS2 protein present in plasma membrane detects flg22. Another receptor in *Arabidopsis* is EF-Tu receptor (EFR). The EFR protein perceives acetylated N-terminal 18 amino acids (elf18) of elongation factor-Tu.

PAMPs induce early responses, within minutes to hours, including rapid ion fluxes across the plasma membrane, oxidative burst, activation of mitogen-activated protein kinases (MAPKs), and calcium-dependent protein kinases (CDPKs), inducing defense-related genes involving pathogen cell wall/cell membrane-lysing enzymes, e.g., chitinases, glucanases, and defensin. Other responses may include formation of antimicrobial phytoalexins, plant cell wall modifications, e.g., formation of papillae rich in callose, lignin biosynthesis or change in cell wall proteins, and pectic polysaccharide structure. Once recognition of elicitors by PRRs takes place, it triggers an influx of calcium ions into the cell and production of ROS such as superoxide (O_2^-) and hydrogen peroxide. The plasma membrane-spanning NADPH oxidase transfers electrons from NADPH within the cell across the plasma membrane and uses them to convert extracellular oxygen to the superoxide anions

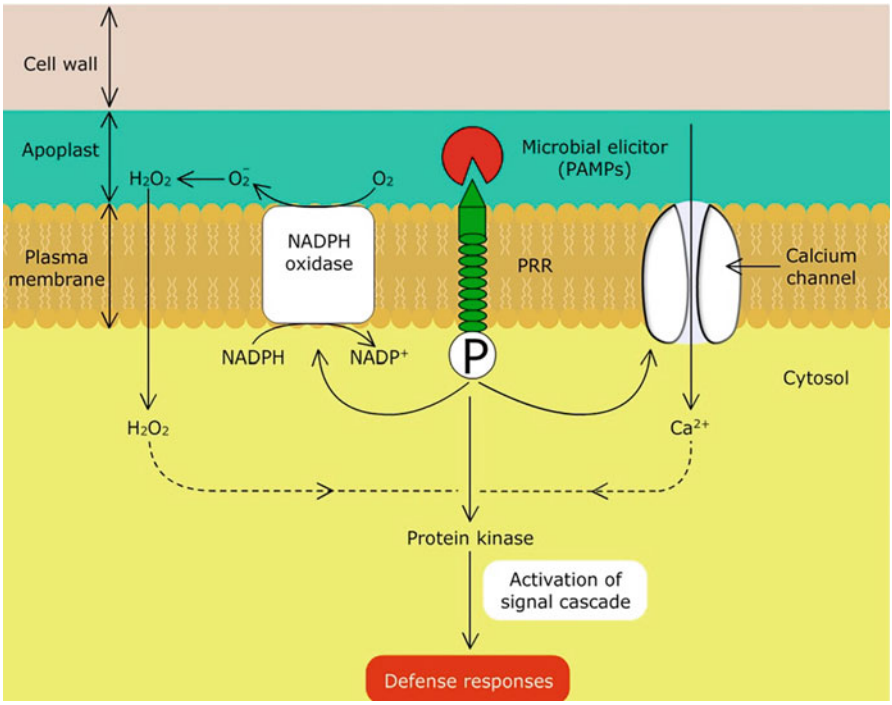


Fig. 32.11 Events in recognition of elicitor or PAMP by a plant cell. The elicitor or PAMP is recognized by pattern recognition receptor (PRR)

(O_2^-), which is rapidly converted into hydrogen peroxide (H_2O_2), either spontaneously or through the action of **superoxide dismutase**. Activation of NADPH oxidases involves the combined action of CDPKs and other protein kinases. The ROS generated by the extracellular peroxidases serve as antimicrobial agents and in cross-linking specific polymers to cell wall to improve rigidity. Thus, PAMPs trigger inducible defenses which include production of toxic chemicals and pathogen-degrading enzymes, and deliberate cell suicide is used conservatively by the plants (Fig. 32.11). Tomato plant produces tomatine, and only certain pathogens which produce a glucosidase that can toxify tomatine can infect tomatoes. For developing crops with higher resistance or inducible resistance, identification of several potential microbial molecules that act as PAMPs would increase chances of identifying more potential host plant PRRs.

32.3.2 Effector-Triggered Responses

Plant pathogens produce a wide range of molecules that enhance their successful colonization in the host plant. Any molecule made by the pathogen that enhances its

Table 32.3 Plant-pathogen effector molecules produced by pathogens and their role

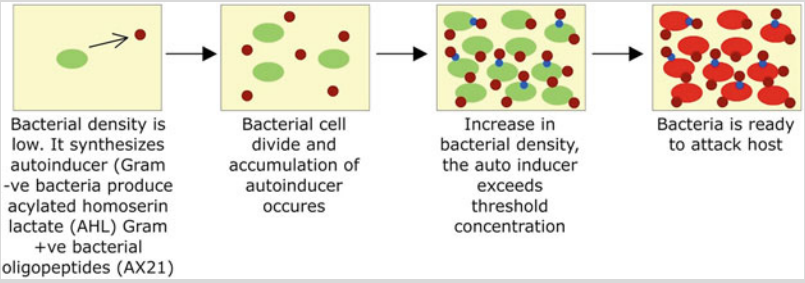
Organism	Effector molecule	Role of effector molecules in the regulation at cellular/ molecular level
Tobacco mosaic virus	P50	Viral replicase
Potato virus X	CP	Coat proteins
Phytophthora infestans	Avr3	Prevention of cell death
Pseudomonas syringae	Avrpto3	Protein kinase function to suppress defense response and enhance the bacterial growth and plant necrosis

ability to overcome basal defense of the plant and make it to be able to colonize, grow, and reproduce in host plant is called an **effector molecule**. These molecules manipulate host's cell structure and function, facilitating growth and reproduction of pathogens besides stopping the basal defense response in the plant. These can be either toxic or act as elicitors. In case interaction between plants and pathogens results in disease, these are referred as "compatibility factors." On the contrary, if the interactions do not lead to disease, these are described as incompatible. Many effector molecules either alone or in combination with other molecules suppress host pathogen-triggered immunity (PTI) or effector-triggered immunity (ETI). These molecules are produced by pathogen usually only in the presence of host. Plant-pathogen interaction involves effector molecules (Table 32.3) which target distinct sites in plants, either apoplastic or cytoplasmic, and are known accordingly. Apoplastic effectors are released in extracellular space and target the membrane surface receptors, while cytoplasmic receptors are released in the cytoplasm of the host cell through specialized structures. These belong to diverse classes, such as enzymes, toxins, and growth regulators.

There are 10–30 types of effector molecules required by the plant to detect and respond to PAMPs, which facilitate infection by interfering signaling pathways of the host plants. Several pathogenic bacteria produce enzymes that degrade components of plant cell walls. These include hydrolytic enzymes such as cutinases, cellulases, xylanases, pectinases, proteases, etc. Some may produce enzymes that regulate protein activity in the cell through phosphorylation and dephosphorylation. One common activity of the pathogens is their ability to act and protect against hydrolytic enzymes produced by the plants. One of the examples is an effector produced by *Cladosporium fulvum*, an extracellular pathogen of tomato which does not produce any haustoria or haustoria-like structures. *C. fulvum* enters either through stomata or subsists on leaking nutrients. The fungus is protected by the effector molecules which are small cysteine-rich proteins thought to function exclusively in the apoplast. The effector inhibits cysteine proteinases that are important components of plant defense in tomato. Some of the effectors act by binding to chitin fragments, so that their detection is avoided by the PTI of the plant. Some pathogens produce cell wall-degrading enzymes only when bacterial populations on the host

Box 32.4: Quorum Sensing

Bacteria use a survival strategy by sending signals to other bacteria to act in group for their successful colonization in the host. This survival strategy is known as "**quorum sensing**." The capacity to respond collectively as a group has obvious advantages such as to be able to migrate to a more suitable environment for better nutrient supply and antibiotic production and to adopt new modes of growth, such as sporulation or biofilm formation. Quorum sensing bacteria release chemical signal molecules called **autoinducers** that increase in concentration as a function of cell density. Gram-negative bacteria (e.g., *Pseudomonas syringae* and *Erwinia carotovora*) use acylated homoserine lactones (AHL), and gram-positive bacteria (e.g., *Xanthomonas oryzae*) use processed oligopeptides (Ax21) as autoinducers to communicate. The pathogenicity of *E. carotovora* depends on the production of various plant tissue-degrading enzymes, including pectate lyases, polygalacturonase, cellulase, and proteases necessary for colonization of bacteria in the host. Therefore, *E. carotovora* uses quorum sensing to ensure that exoenzyme production does not occur until sufficient bacteria have accumulated for successful tissue destruction and evasion of plant defenses. Quorum sensing is a novel target for antimicrobial therapy, e.g., biofilm production by bacteria makes them resistant, if signal involved in quorum sensing of biofilm production is blocked.



The process of quorum sensing

reach a certain density. The characteristic feature of bacteria to monitor its own as well as density of other bacteria is known as **quorum sensing** (Box 32.4). Cell wall-degrading enzymes may act both as elicitors and as enzyme. Xylanase released by fungi causes necrosis, ethylene synthesis, and electrolyte leakage in tobacco. Fungal **endopolygalacturonase** cause both necrosis and induction of phytoalexins biosynthesis. It degrades plant cell wall and induce production of oligogalacturonides as well which are responsible for inducing plant defense. *Erwinia*, a necrotrophic bacterium, uses pectic enzymes (pectate and pectin lyase) and causes cell death

and tissue disintegration by the hydrolysis of polygalacturonases. Pathogens produce phytotoxins that may be toxic to all plants or only to a particular plant species. Most pathogens produce at least one compound that is toxic to plants, while there are pathogens that produce many toxins. Majority of phytotoxins produced by phytopathogenic bacteria and fungi are non-specific. The site of action of non-specific toxins is known. Phytotoxins are mainly secondary metabolites though some are oligopeptides or proteins. Many elicitors of diverse nature are often phytotoxic. Some toxins are required for pathogenicity, whereas others elicit disease symptoms. For example, many of the bacteria damage plant tissues by secreting extracellular polysaccharides (EPSs) or cell wall-degrading enzymes. The extracellular polysaccharides (EPSs) secreted surround the growing bacterial colony aid bacterial virulence but are not required for pathogenesis. It may help in saturating intercellular spaces with water or blocking xylem to produce wilt symptoms.

Some pathogens such as ascomycetes fungal genera *Cochliobolus* (synonym *Helminthosporium*) and *Alternaria* produce host-specific toxins. *Cochliobolus carbonum* cause Northern leaf blight disease of maize. It makes a host-selective toxin called **HC toxin** (Fig. 32.12), which inhibits histone acetylase affecting expression of the genes involved in defense against the fungus. Mutant bacteria with impaired gene for the biosynthesis of HC toxin are not pathogenic. The TOX2 locus of *Cochliobolus carbonum* race 1 which controls production of HC toxin

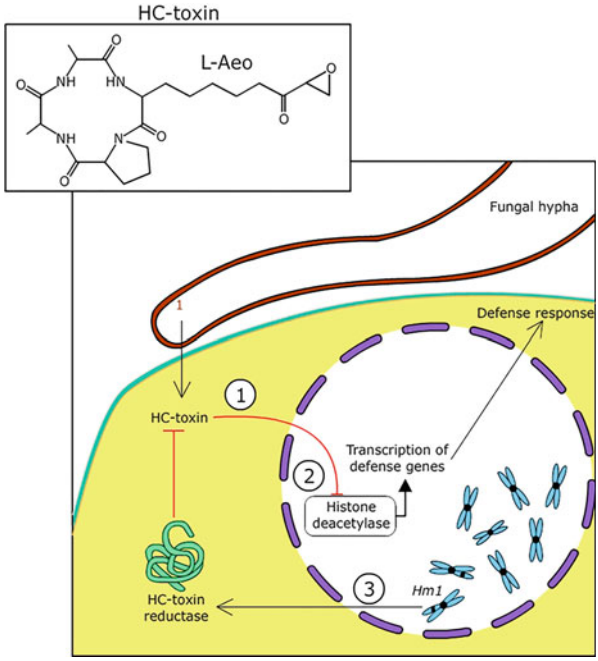


Fig. 32.12 Production of host-specific toxins (such as HC toxin) by nectrotrophic fungi. Toxin produced by *Cochliobolus carbonum* (a fungal pathogen on maize). **1.** HC toxin enters the responding plant cell and **2.** inhibits histone deacetylase activity which, in turn, inhibits transcription of defense genes. **3**. *Hm1*-resistant maize plants produce an HC toxin reductase which detoxifies HC toxin by reducing carbonyl group of the side chain of L-Aeo (2-amino-9,10-epoxy-8-oxo-decanoic acid)

includes a gene encoding HC toxin synthetase. The complementary resistance gene in maize, called Hm, encodes an enzyme called HC toxin reductase that provides resistance to maize by reducing production of HC toxin. Mutant strains of *C. carbonum*, which do not produce toxin, are nonpathogenic. Maize genotype with Hm1 gene, which encodes an enzyme that can detoxify HC toxin, is resistant *to C. carbonum.* On the contrary, there are non-specific toxins, such as **fusicoccin**, which are produced by *Fusicoccum amygdale*. Fusicoccin inactivates membrane-bound H^+-ATPase proton pump. As a result, rate of proton pumping across plasma membrane of guard cells cannot be modulated. Because of this, stomata will not respond to environment stimuli, and irreversible opening of stomata leads to wilting of plants. Another compound tabtoxin is produced by pathovar strain of *Pseudomonas syringae* var. *tabaci*, which is a dipeptide and consists of two amino acids, i.e., threonine and a non-protein amino acid tabtoximine**.** A peptidase from the plant hydrolyzes the tabtoxin resulting in the release of tabtoximine from the dipeptide which is a potent inhibitor of glutamine synthetase, an important enzyme required for nitrogen metabolism. This enzyme is important for re-assimilation of ammonia released during photorespiration in green leaves. In some instances, toxins released by the pathogen create pores in the membrane resulting in the leakage of nutrients. Toxins produced by biotrophs are under different selection pressures. These have to avoid detection by the host plant while maintaining the defense suppressive function. Although effector molecules are produced by pathogen genes, they function in cellular environment. These have been selected to mimic plant molecules. One of the examples is coronatin, secreted by several pathogens of *Pseudomonas syringae.* Structurally and functionally it mimics plant hormone jasmonyl isoleucine, thereby enhancing bacterial colonization. This also results in blocking induction of salicylic acid-mediated defense response and increase opening of stomata. Third class of effector molecules includes phytohormones, which may result in abnormal growth in infected plants. For example, infection by the fungus *Gibberella fujikuroi* causes rice shoots to grow much faster than their uninfected plants. Agrobacterium causes crown gall and hairy root disease in many eudicots by inserting genes encoding two of the enzymes involved in the synthesis of auxin and cytokinins. Pathogens like *Pseudomonas savastanoi* and *Agrobacterium tumefaciens* produce auxin and cytokinins, which causes characteristic growth abnormalities in plants. Fungus *Fusicoccum amygdale* produces fusicoccin which mimics many of the physiological effects of auxin.

32.3.2.1 Effector-Triggered Susceptibility (ETS)

Diverse sets of effector molecules are the product of **avr genes** that determines pathogenicity of the pathogens. Genetic studies of diseased plants have revealed that product of **R (resistance) genes** present in them can recognize one specific effector molecule produced by the pathogen. When a plant has a dominant allele of a particular resistance gene (R) and the pathogen has dominant avirulence *avr* gene, i.e., no disease will occur. Avr products may be recognized intracellularly or extracellularly, depending on the pathogens. On the other hand, if there is no

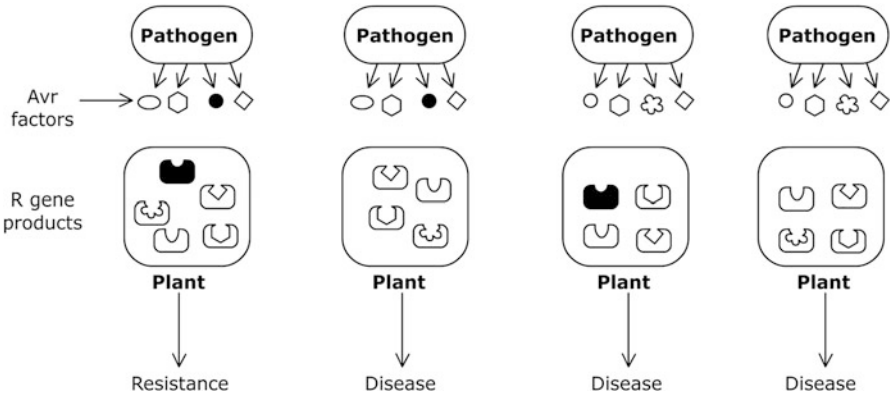


Fig. 32.13 Hypothetical representation of gene-for-gene model. Strains of pathogens produce diverse types of effector molecules. The first strain produces Avr effector (product of *avr* gene shown by solid circle) compatible with R gene product of the plant. As a result, plant shows resistance. Though the second strain of bacteria may produce effector molecule similar to first strain but compatible reaction with the host does not occur in the absence of compatible R gene product of the host. The remaining two figures also indicate incompatible reactions of the bacteria and the host either because of the absence of compatible effector produced by the bacteria or the compatible R gene produced by the plant. As a result, plant shows susceptibility to the pathogen as depicted in the last three figures

recognition of product of two genes (i.e., Avr of bacteria and R gene product of the host), then the plant is susceptible to pathogen. Thus, the plants that lack R gene will not be able to recognize the effector molecule produced by bacteria and hence will not initiate defense responses and thus become diseased. Presence of *avr* gene, complementary to R gene product of host plant, converts a pathogenic bacterium into an avirulent. H. H. Flor described this as **gene-for-gene model**, which explains the genetic basis for vertical plant resistance to pathogen (Fig. 32.13). He stated that genes in the pathogen (*avr*) that determine the pathogenicity have a one-to-one corresponding match to specific gene in the host that determines resistance (R gene). He further found out that host resistance is usually dominant to virulence.

The bacterial Avr effectors delivered in plants have been identified to be enzymes like phosphatases, proteases, phosphoserine lyases, or E3 ubiquitin ligases which induce signaling of defense in the host. Another gene or gene clusters that are required for pathogenicity on compatible host are referred to as hrp (hypersensitive reaction and pathogenicity). Bacteria which need to develop a secretion system (Type III) so as to inject the effector molecule inside the host cell are known as T3SS (Type III secretions system). **Hypersensitive response and pathogenicity cluster** (hrp) genes produce proteins in T3SS bacteria which form Hrp secretion apparatus. The Hrp secretion apparatus encircles the outer and inner bacterial membranes, and a pilus is produced which penetrates into the plant cell. Once Avr effector is injected into the plant cell, Hrp proteins are not needed. The transcription of both *avr* and *hrp* genes is nutritionally regulated. The hrpN gene of *Erwinia amylovora* encodes a cell surface-associated protein called harpin that causes

necrosis and other hypersensitive responses. The effectors produced by fungi may be secreted in the intercellular spaces and then taken up by the host cells. Some of the fungal pathogens have been found to induce synthesis of specific membrane transporters which have been found to aid in the secretion of non-protein toxins into the intercellular spaces of the plants.

32.3.2.2 Effector-Triggered Immunity (ETI)

Effector-triggered immunity (formerly called *R* gene-mediated or vertical resistance) refers to triggering of defense signaling in the plant as a result of incompatible interaction of Avr products of the pathogen and the R gene products in the host plant. It is based on highly specific, direct, or indirect interaction of pathogen and plant gene products (Box 32.5). The majority of R proteins (product of R gene) that mediate race-specific resistance and trigger ETI have diagnostic structural motifs**.** Different R proteins have two basic roles to play. First, they directly or indirectly recognize the effector molecule, and secondly, they activate downstream signaling, leading to induction of various defense responses. Most R genes are constitutively expressed at low level during normal growth. Once the pathogen attacks expression of R gene is upregulated. R proteins are involved in the detection of diverse pathogens, including bacteria, viruses, fungi, nematodes, insects, and oomycetes. ETI mostly relies on the endogenous NB-LRR protein products encoded by the R genes (resistance genes). The interaction is complicated as it involves two genomes, both of the plant and of pathogen. NB-LRR is named due to the presence of nucleotide-binding (NB) and leucine-rich repeat (LRR) domains. LRR regions are present in over 14,000 proteins in viruses, bacteria, archaea, and eukaryotes. Most

Box 32.5: Host Strategies to Detect Pathogen Effector

During infection, activity of the bacterial effectors on their target proteins goes unchecked in a susceptible host that lacks resistance to a particular pathogen. This renders the host susceptible to infection by that specific pathogen. On the contrary, in a resistant host, pathogen effectors can be sensed directly or indirectly by the products of **resistance (R) proteins** produced by the plant, which results in a protective immune response. However, many effector molecules are recognized indirectly by R proteins. According to **guard hypothesis**, the R proteins complex with the host target proteins. R proteins act as "guard" and the target molecules as guardee. The effector molecule released by the pathogen liberates the R protein (guard) from the complex, allowing the R protein to engage signaling pathways and induce a defense response. This hypothesis predicts that multiple pathogen effector proteins may interact with a common host target that can be guarded by more than one R protein. In *Arabidopsis*, RIN4 protein is targeted by three effector molecules delivered into cell by Type III secretion system. RIN4 is a 211-amino acid, plasma membrane-associated protein. It is guarded by at least two different R proteins.

LRR proteins are involved in protein-ligand and in protein-protein interactions. These LRR proteins include plant and mammalian immune response. Several classes contain LRR-containing receptor-like kinases (LRR-RLKs), LRR-containing receptor-like proteins (LRR-RLPs), nucleotide-binding site LRR (NBS-LRR) proteins, and PGIPs**.** They provide an early warning system for the presence of potential pathogens and activate protective immune signaling. In addition, they act as a signal amplifier in case of tissue damage. Most of R genes in plants encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins, the largest proteins known in plants, ranging from about 860 to about 1900 amino acids. These proteins have at least four distinct domains joined by linker regions. These are N-terminal effector domain, central nucleotide-binding site (NBS), and C-terminal leucine-rich repeat (LRR) domains as well as variable amino- and carboxy-terminal domains, which largely vary in plants. Two major subgroups that have distinct N-terminal domains are generally recognized: (1) one group with a toll-interleukin 1 receptor (*TIR*) domain are called TNLs, and (2) those with a coiled-coil (CC) domain are called CNLs. The crystal structures of more than 20 LRR proteins have revealed that LRR domains characteristically contain a series of β-sheets that form the concave face, shaped like a horseshoe or banana. The LRR motif of cytoplasmic and extracytoplasmic plant R proteins contains several leucine or other hydrophobic amino acids at regular intervals over 23 or 24 amino acid lengths, respectively. Within the part of these repeats, leucine residues occur at every second or third position, i.e., X-Leu-X-X-Leu-X-Leu-X, where X is another amino acid. This part of the repeats forms a structure within protein called a parallel β-sheet, in which leucine is in the interior of the protein, while X residues are exposed to the exterior and interact with other proteins. Single amino acid variation on the outer surface can affect the ability of the LRR domain to bind target proteins. Plant NBS-LRR proteins act through a network of signaling pathways and induce a series of plant defense responses, such as activation of an oxidative burst, calcium ion fluxes, mitogen-associated protein kinase cascade, induction of pathogenesis-related genes, and the hypersensitive response.

32.3.3 Signal Transduction

Once a signal is perceived either through recognition of elicitors or effectors, it is transduced in the form of protein activation. Protein phosphorylation plays an important role during pathogen attack. At least two classes of protein kinases are activated: the **mitogen-activated protein kinase (MAPKs)** and **calcium-dependent protein kinases (CDPKs)**. The CDPK class of protein kinases is present in plants only, whereas the MAP kinases are signaling molecule in all eukaryotes. Signaling cascades of MAPKs and CDPKs activate the expression of first set of genes that encode regulatory and signaling proteins. These proteins, in turn, activate expression of second set of genes which encode defense proteins that prevent pathogen attack. The *Arabidopsis* genome contains 110 genes for MAPK pathway components. *Arabidopsis* contains over 70 WRKY proteins which are responsive to

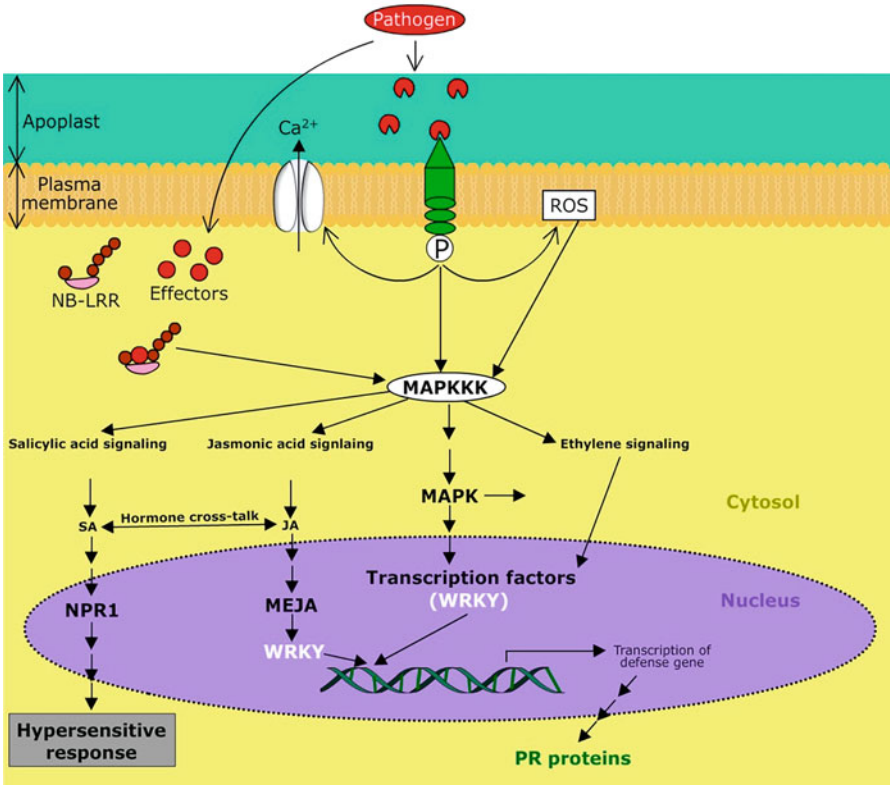


Fig. 32.14 Molecular changes during pathogen infection

pathogen infection or salicylic acid treatment. **WRKY proteins** are a large class of WRKY domain-containing sequence-specific, DNA-binding transcription factors found in plants. WRKY proteins regulate growth, hormone signaling, secondary metabolism, response to various stresses, seed germination, leaf senescence, and the synthesis of phytoalexins and other defense mechanisms against pathogens in *Arabidopsis*. All WRKY proteins recognize and bind to the core W box sequence TTGACC/T in the promoter of the responsive genes (Fig. 32.14).

32.3.4 Systemic Acquired Resistance (SAR)

Sessile nature of plants requires an efficient signaling system capable of detecting, transporting, and interpreting signals produced at the plant-pathogen interface, and **systemic acquired resistance (SAR)** and **induced systemic resistance (ISR)** provide a practical means to confer a fitness advantage to plants in conditions of high disease pressure, since plants which are primed more quickly and effectively activate their defenses ahead of pathogen attack. In SAR, defense proteins

accumulate not only at the site of infection but also systemically in uninfected tissues and/or uninfected plants. SAR provides long-term defense against a broad spectrum of pathogens. The SAR is analogous to the development of acquired immunity in animal systems. Another form of induced resistance which is similar to SAR in many aspects is ISR. SAR and ISR are two types of resistance which prepare plants against subsequent pathogen infection. Selected strains of plant growth-promoting rhizobacteria suppress infection by disease-causing pathogens by providing systemic resistance by antagonism, which is known as ISR. SAR and ISR resemble in the fact that in both the uninfected parts of the plant becomes resistant toward a broad spectrum of pathogens. SAR makes plant respond more quickly if it is attacked again. However, unlike human or mammalian immune response (antibodies persist in the body), SAR is neither very specific nor long lasting. In addition to SA production accompanying SAR, the accumulation of specific set of pathogenesis-related proteins (PRP) occurs both at the site of initial infection and in the uninfected tissue locally and at a distance from the site of infection. Induction of ISR may be due to induction of SA-triggered signaling pathway by the rhizobacteria. In ISR neither PR proteins nor SA seem to be involved. It is dependent on JA and ET (ethylene) signaling. MYC2 may be playing a potential role in signaling JA gene expression during rhizobacteria-mediated ISR against *Pseudomonas syringae* var. tomato DC3000. NPR1 gene is also needed besides MYC2 transcription factor. Combination of ISR and SAR can be commercially exploited to provide resistance against disease-causing agents.

32.3.5 Phytohormones in Plant Defense Response to Pathogens

Plant defense against pathogen attack involves many signal transduction pathways that are mediated by a network of phytohormones. Three most significant phytohormones for plant defense response against pathogens include salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Salicylic acid, a benzoic acid derivative, is an important phytohormone in regulation of the plant defense. It is derived from phenylpropanoid pathway and is similar in structure to aspirin (acetylsalicylate). It plays a role both in defense responses around the site of infection and in the establishment of SAR. In transgenic plants that constitutively express a bacterial *nah*G gene, which encodes salicylate hydroxylase responsible for converting SA into catechol and water, reduced level of SA is observed. Reduced level of SA results in weak R gene-mediated resistance. In *Arabidopsis*, activation of the SA pathway has been shown to be important in both basal and R gene-mediated biotrophic and hemibiotrophic pathogen defense. SA contributes HR-associated resistance. In tobacco, SA increases resistance in plants infected with TMV. Following treatment of *Nicotiana* plant with JA or SA, systemic resistance to TMV is enhanced. ETI can trigger SAR through both local and systemic synthesis of SA which, in turn, regulates transcription of genes encoding PR proteins. A change in amino acid homeostasis is responsible for SAR mediated by ETI. Amino acids are precursors of a large number of plant secondary metabolites involved in defense, including

signaling molecules, cell wall components, and anthocyanins. Plants use amino acids and their derivatives for SA synthesis in order to survive after pathogen invasion. SA is also stored in vacuoles as nontoxic glucose esters, i.e., salicylate glucose ester (SGE) which can be hydrolyzed to release free SA during pathogen attack. Methylation of SA generates methyl SA (MeSA) which is the mobile form that can travel from the infected leaf to the systemic tissues of the plant where it activates resistance after being converted back to SA. MeSA is believed to be directly emitted into the atmosphere, and only a small amount is retained in the leaves. MeSA is converted to SA by the enzyme methyltransferase. Under stress conditions accumulation of SA alters plant metabolism so as to reduce HR response to the biotic stressors and induce SAR. Metabolic plasticity refers to the capacity of the plants to alter their metabolism when subjected to any kind of stress and is characteristic for their survival. Mitochondrial respiration is adversely affected under stress conditions, and accumulated SA triggers alternative oxidase (AOX) which results in lowering of ATP production and reduction in ROS production, thereby lowering HR response. Continuous flow of electrons through SA-triggered AOX will result in continued operation of pentose phosphate pathway, resulting in generation of erythrose 4-phosphate and NADPH, which are required for the synthesis of phytoalexins. Additional requirement of NADPH is met through the action of NADP-specific malate dehydrogenase.

Non-expressor of PR genes 1 (NPR1) is the key player of all the SA-mediated defense responses (Fig. 32.15). NPR1 protein is not the receptor of SA. Rather two proteins encoded by paralogues of the gene (i.e., related genes derived from gene duplication)—NPR3 and NPR4—are SA receptors which differ in their affinities for SA and function on the basis of SA concentrations. NPR1 promotes activation of

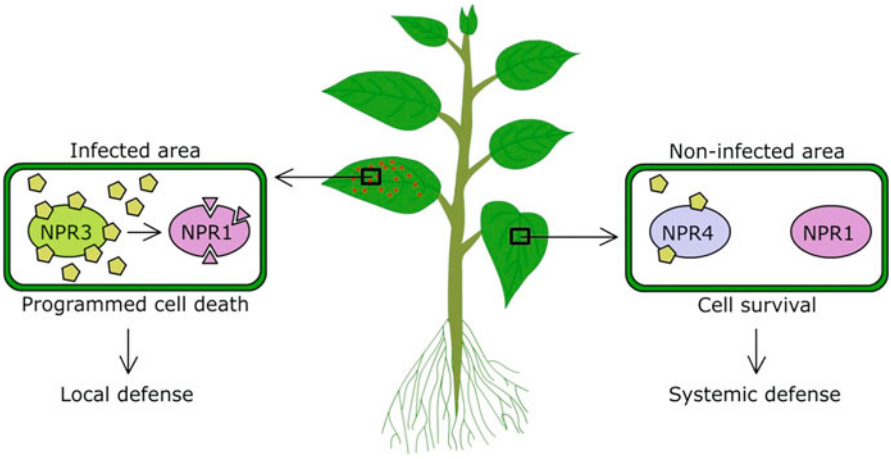


Fig. 32.15 Non-expressor of PR genes 1 (*NPR1*) is the key player of all salicylic acid-mediated defense responses. *NPR1* promotes activation of salicylic acid responsive genes involved in defense by promoting degradation of repressor proteins

salicylic acid responsive genes involved in defense by promoting degradation of repressor proteins. NPR1 exists in oligomeric or monomeric forms depending upon oxidizing and reducing conditions of cytoplasm, respectively. In the absence of infection, there will not be any SA accumulation and NPR1associated with NPR4 which is degraded via 26 proteasome pathway. As a result, no defense is triggered. In the infected tissue, SA alters the redox status of the cytoplasm. As a result, NPR1 is translocated to nucleus in monomeric form. In the infected cell, high concentration of SA in the nucleus promotes association of NPR1 with NPR3 promoting degradation of NPR1 through ubiquitin-proteasome pathway, triggering HR and ETI since at the distal site, SA concentration is low, it is unable to bind with NPR3, and cell death is blocked. Rather SA binds with high-affinity receptor NPR4, blocking degradation of NPR1. NPR1 upregulates expression of WRKY TFs which regulate many immunity-associated genes favoring cell survival and expression of genes associated with SAR (Fig. 32.16). SA also directly inhibits catalases resulting increased level of hydrogen peroxide induce peroxidase-catalyzed cross-linking of plant cell walls (Fig. 32.17). SA also interacts with other phytohormones, either synergistically or antagonistically. There is a connection between JA and SA signaling pathways and other defense pathways as part of the PR response. Plants with defective JA pathway fail to accumulate SA in the leaves or phloem and become highly susceptible to TMV. Conversely, SA pathway does not affect JA level but susceptibility is increased. SA-mediated signaling pathway is activated following infection by

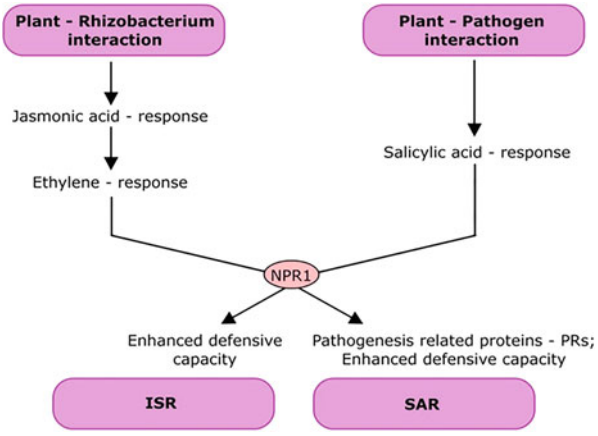


Fig. 32.16 Plant-pathogen interactions and molecular mechanisms involved in susceptible and resistance disease reactions by plant cell upon pathogen attack. If plant cells PRRs are unable to identify pathogen, then no signals are transduced, and hence defense-related genes are not activated, and plant becomes susceptible. On the other hand, if plant cell PRRs recognize pathogen, it shows hypersensitive responses (thickening of cell wall, tyloses, etc.), or defense-related responses are activated resulting in formation of PR proteins and ROS, which kill pathogen spores, and plant becomes resistant. *PRR* pattern recognition receptors, *PRs* pathogenesis related, *ROS* reactive oxygen species

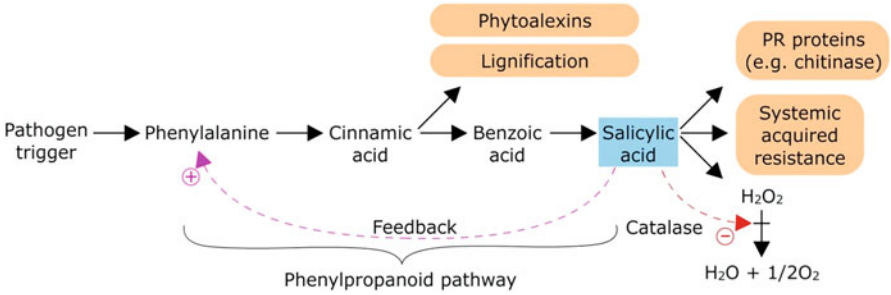


Fig. 32.17 Role of salicylic acid (SA) in induced plant defense responses. SA is a product of the phenylpropanoid pathway and activates genes for PR proteins and the development of systemic acquired resistance. It stimulates the phenylpropanoid pathway directly but inhibits catalase

biotrophic pathogens, while attack by necrotrophic pathogens induces distinct pathway regulated by JA and ethylene. SA- and JA-/ET-mediated defense pathways undergo crosstalk, and their interactions are generally antagonistic (Fig. 32.16). Biosynthesis of jasmonic acid (JA) and ethylene is initiated as PAMPs/MAMPs are detected by the plant. Ethylene is produced within 10 min in response to flg22, while JA level rises slowly. Ethylene signaling activates resistance against necrotrophic pathogens. In addition, abscisic acid, gibberellic acids, auxins, brassinosteroids, and cytokinins also have roles to play in defense mechanisms. ABA regulates numerous development and adaptive stress responses in plants. It can positively regulate plant defense at the early stage of infection by closing stomata or inducing callose deposition. If ABA pathway is activated at a later stage, it can suppress ROS induction and SA or JA signal transduction. Cytokinins are also involved in defense mechanism, including the induction of resistance against viruses, but suppress HR. Cytokinins act synergistically with SA signaling. These have been found to enhance production of two antimicrobial phytoalexins, scopoletin and capsidol, in tobacco plant. Brassinosteroids (BRs) are also considered important in plant defense against pathogens. In potato BRs are effective against viral infection. Application of BRs on tobacco plants decreases TMV viral infection and restricts infection by other biotrophs.

Nitric oxide (NO), like ROS, is an important signaling molecule that is rapidly generated after recognition of pathogens. It reacts synergistically with reactive oxygen species to increase cell death. It can also activate PAL (phenylalanine ammonia lyase), which amplifies the activity of phenylpropanoid pathway, leading to lignification of the cell wall, phytoalexin synthesis, and the production of salicylic acid which, in turn, amplify defense responses such as systemic acquired resistance and production of pathogenesis-related proteins, such as chitinase and glucanases. Plants produce NO from the amino acid arginine by the activity of a putative NO synthase enzyme which is stimulated by ion fluxes (Fig. 32.18).

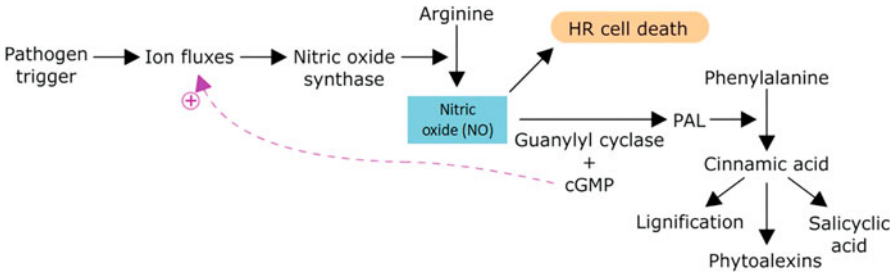


Fig. 32.18 The production of NO is stimulated by ion fluxes. NO is involved in inducing cell death (hypersensitive response) and activating PAL (phenylalanine ammonia lyase) which enhances the activity of the phenylpropanoid pathway, leading to wall thickening, phytoalexins synthesis, and production of salicylic acid. Production of NO is enhanced by guanylyl cyclase and cGMP

32.3.6 Pathogenesis-Related Proteins

Proteins which are induced in the host plant upon pathogen attack are generally described as pathogenesis-related (PR) proteins. These are low molecular weight proteins (6–43 kDa). SA induces a set of pathogenesis-related proteins by regulating the transcriptional activation of many PR genes. Seventeen families of PR proteins have been classified in most plants. These include hydrolytic enzymes, such as chitinase and glucanase, which degrade fungal cell wall structural polysaccharides inhibiting fungal growth and spore germination. Other classes of PR proteins include cell wall-modifying enzymes and antifungal compounds and components of signal pathway. PR protein, such as **lipoxygenase,** may contribute by generating secondary signal molecules such as jasmonic acid (JA) and lipid peroxidase besides triggering synthesis of a number of volatile or nonvolatile compounds having antimicrobial properties. The PR proteins have typical properties that enable them to resist acidic pH and proteolytic cleavage and thus survive in harsh conditions. These proteins occur in vacuoles, cell wall, or intercellular spaces.

32.4 Viruses as Plant Pathogens

Another class of pathogens includes viruses and viroids. Both of them require living plant cells for their replication. They rarely kill their host but weaken the plant by reducing growth and seed yield. Viruses also exhibit three levels of interactions with their host. First is their activity within an infected cell, second is spread to neighboring cells, and last pertains to transmission of the virus from one host plant to another. Viruses and viroids enter through wounds and are transmitted from one host to another by insects. Plant viruses are obligate biotrophs and have small genomes encoding only three to ten proteins. These proteins help in replication, cell-to-cell movement, and symptom development. All viruses (except Gemini viruses and nano viruses) encode replicases (RNA or DNA polymerases), which help in replication of virus in host cell. Plant viruses face challenges during initial replication, movement

to adjacent cells, and vascular system and in suppressing host defense systems. Most viruses encode one or more proteins called movement proteins, which modify the structure and function of plasmodesmata. During the movement of viruses, the size exclusion limit of plasmodesmata increases up to tenfolds to permit movement of large nucleoprotein complexes. In contrast, the dsDNA of cauliflower mosaic virus (CaMV) modifies the plasmodesmata into large tubular structures to facilitate the encapsulated virus particles through enlarged plasmodesmata. Another feature of cell-to-cell movement of virus is the involvement of the endoplasmic movement and membrane trafficking. In contrast to animal viruses, plant viruses never cross the plasma membrane of the infected cell. Many viruses also encode suppressors of the RNA-silencing mechanisms of the host. All these virus-encoded proteins can act as effector molecules similar to those of microbial pathogens. Once the virus enters the phloem cells, it can move at a speed of up to 1 cm/hour. Protein coat of virus and host-coated pectin methyl esterase are involved in the exit of the virus or viron from phloem into the distal leaves. Presence of certain protein motifs is a signal to plant cell about virus infection. NBS-LRR proteins are a major class of receptor proteins for recognition of virus infection. This signal reception leads to hypersensitive response. A 183 kDa protein from TMV interacts with a toll-like receptor in the host cell. This interaction of elicitor with the plant receptor results in the activation of a cascade of reactions leading to cell death. Plant mitochondria play an important role in detecting pathogen infection. Although viral patterns inducing PTI are well known from animal systems, there is no similar pattern reported for plants. Typical PTI cellular responses in plant-virus interactions include ion fluxes, ROS production, ethylene, salicylic acid (SA), MAPK signaling, and callose deposition. The virus-derived molecules (e.g., dsRNAs) act as PAMPs, which trigger PTI and RNA interference (RNAi). However, PTI is typically a form of innate immunity, whereas RNAi induces a form of adaptive immunity.

32.5 Plant Responses to Herbivory

Herbivores consume large quantities of plant leaves, seeds, fruits, etc. to acquire energy and nutrients. Some plant tissues serve as a better food source than others in terms of quantity and quality. For example, seeds and pollen have relatively high protein content. Similarly, meristematic tissues are made up of young, rapidly dividing cells that are energy and nitrogen-rich and are preferred by herbivores. Herbivore feeding causes mechanical damage to the plant tissue, and the degree of damage depends on how the food is taken up by the herbivore. Several classes of herbivores are known based on their feeding behaviors. Aphids and whiteflies, known as phloem feeders, insert their long narrow mouthparts into the conducting tissues of stem and leaves causing minimal damage to the epidermis and eliciting plant responses similar to pathogen attack. Phloem feeders also often act as vectors carrying viruses to other susceptible plants. In contrast, insects belonging to the orders Lepidoptera (moths and butterflies) and Orthoptera (e.g., caterpillars, grasshoppers and beetles) chew using their toothed mandibles to crush, cut and

macerate, and cause extensive physical damage to plants. Piercing and sucking insects, such as mites, thrips, and nematodes have short stylets and feed on epidermal and mesophyll cells. Vertebrates such as mammals use plants/plant parts in their diet and are classified according to their dietary specialization. Nectarivores feed on nectar and pollen; gumivores feed on exudates from trees; browsers feed on stems, twigs, buds, and leaves; and grazers feed on grasses.

Plants perceive herbivory also on the basis of specific pattern of damage caused by the feeding herbivore. Chemicals released from herbivores in their saliva as well as those released from the damaged plant tissues, **oviposition** fluids, etc., elicit plant defense responses against herbivores. In response to herbivore attack, plants secrete various chemicals that may act either as deterrents for herbivores or attractants for predators of herbivores. Resistance to insect pests can also be classified as constitutive or inducible. Preformed constitutive defense barriers include defensive proteins and metabolites that are expressed irrespective of the level of herbivore threat. In contrast, induced defenses are invoked in herbivore-challenged plants at the site of attack as well as systemically in undamaged tissues. It is important to note that induced defense traits evolve, as they require lower resource allocation costs than constitutive defense traits. Moreover, herbivores could evolve strategies to overcome constitute defense mechanisms of plant. Plants are also known to activate sequestration of sugars in underground parts of the plant, allowing them to tolerate herbivory better. Several defense mechanisms are induced in the plant in response to an insect attack that leads to different defense responses (Fig. 32.19).

Plants may adopt direct or indirect defense strategies. Direct defense strategies include both preformed (constitutive) as well as inducible response, while indirect response includes release of volatile organic compounds (VOC) by the plant in response to herbivory (Box 32.6). These VOCs attract the predators of the herbivores. This interaction among the plant, herbivores, and their natural enemies is known as **tritrophic interactions**. Defense response to herbivores in plants, such

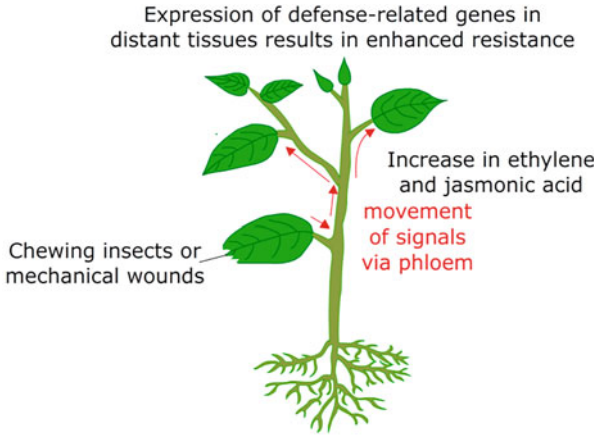


Fig. 32.19 Phloem-mobile long-distance signal induces the expression of defense genes in distal parts of the plant

Box 32.6: Herbivore-Induced Plant Volatiles: "The Watch Dogs"

Volatile organic compounds (VOCs) are natural products emitted into the atmosphere from marine sources and terrestrial environment. Many of the VOCs are of biogenic origin (BVOCs). Since plants are constantly exposed to various threat (including insects, pathogens, and parasitic plants), they arm themselves with molecular weapons and adopt different mechanisms to protect themselves. They also alert the neighboring plants against the potential damage. Primary function of plant produced VOCs is to defend plants against herbivory, pathogens, besides attracting pollinators and seed dispersers. VOCs also serve as signals in plant-plant communications. Plant VOCs consist of GLVs (green leaf volatiles), terpenoids, MeJA, MeSA, methanol, ethylene, and other substances. Herbivore-induced plant volatiles (HIPVs) are released by the plants on being attacked by the herbivores. These are released from the exposed parts of the plants, such as leaves and flowers, and provide airborne info-signals that either attract predators and parasitoids (indirect defenses) or deter oviposition (direct defenses). VOCs emitted by herbivore-challenged plants of *Brassica oleracea* are known to reduce oviposition preference of adult female moths of *Pteris rapae* and larval performance. These VOCs also attract larval parasitoid *Cotesia glomerata.* VOCs can eavesdrop to prime the neighboring plants so that they are able to protect themselves better against the attacking herbivore. Both intraspecific and interspecific signaling by VOCs are known. Green leaf volatiles (GLVs) are secondary metabolites produced by most of the green plants. These are low molecular weight hydrocarbons formed from the bio-catalyzed conversions of omega-6 fatty acid linoleic acid. GLVs are volatile C6 aldehydes, alcohol, and their esters that are derived from the octadecanoid pathway. The pathway starts with the formation of linolenic acid which is the hydrolytic product of membrane phospholipids catalyzed by lipases. Linolenic acid is oxygenated to form C13-hydroxyperoxy linolenic acid (13-HP), a precursor for both GLV, and jasmonic acid. 13-HP is cleaved to form basic volatile C6 aldehydes, which can be further processed into other volatiles. GLV formation is suppressed in the intact plant tissues but is activated when plant tissue is damaged. Smell of the freshly cut grass is due to the GLVs causing "green odor." There are several examples of GLVs being produced by the plants on being infected by pathogens. On being infected by pathogenic bacteria *Pseudomonas syringae* pv. *phaseolicola*, lima beans release *E*-2-hexanal and Z-3-hexanal. Since GLVs are immediately released from wounded leaves, release of GLVs provides reliable information about the location of herbivores. There are two kinds of receivers of the signals: the neighboring plants and the distal organs of

(continued)

Box 32.6 (continued)
the affected plant which trigger systemic defense. Within the same plant, the two types of signals (airborne signals as well as the one transmitted through vascular system) are responsible for triggering the **systemic defense**. Plasma membrane is the target of interactions with GLVs. On perceiving the signal, membrane is depolarized (V_m) due to activation of Ca^{+2} channels. Major product in the wounded leaves of *Arabidopsis* is (Z)-3-hexanal, while (Z)-3-hexanol and (z)-3-hexanyl acetate are formed in partially wounded leaves. GLVs also have antimicrobial properties. These restrict herbivory by attracting predators of the herbivores. GLVs make certain caterpillars attractive to the "big-eyed bugs" as a result of reaction with their saliva. GLVs may be the precursors for ozone formation. Thus, they may contribute to photochemical smog in urban area.

Plant volatiles	Chemical nature
Terpenes	3*E*-4,8-dimethyl-1,3,7-nonatriene (DMNT)
	3*E*,7*E*-4,8,12-trimethyl-1,3,7,11-trideca-tetraene (TTMT)
Alkaloid indole or phenylpropanes	Methyl salicylate, methyl anthranilate
Jasmonates	*cis*-jasmone and methyl jasmonate
Green leaf volatiles (GLV)	Cleavage products of fatty acids, e.g., (*E*)-2-hexenal, (Z)-3-hexenal, (Z)-3-hexenol, and (Z)-3-hexenyl acetate

as production of **proteinase inhibitors (PI)** and secondary metabolites by the plant, is induced either within a very brief period, known as short response, or the induction of response may occur after some time. Short-term responses occur within minutes to hours of herbivore attack and include reactions involving precursors already present in the leaf. Chewing of *Populus tremuloides* (quaking aspen) leaves causes enzymatic hydrolysis of two phenolic glycosides (salicocortin to salicin and tremulacin to tremuloidin) with the release of 6-HCH (6-hydroxycyclohex-2-ene-1-one) which is further converted to phenol or catechol (a potent toxin) in the gut of insects. As a result, insect cannot feed continuously on leaves; rather they must move constantly making them more vulnerable to predators. Variation in reallocation of resources in the plant also plays an important role. More carbon allocation is required by the plant for the production of the protective chemicals which possibly comes at the expense of investment of carbon in growth. Some plants cope up herbivory by attaining a high capacity to regrow. Plants that grow rapidly possibly invest less carbon in defense. Long-lived leaves of slow-growing plants should be well protected against herbivores to minimize tissue loss. According to optimum defense hypothesis, defense compounds should be concentrated more in those parts of plants

where they are needed more. The secondary metabolites having a role in defense are concentrated more in younger leaves than in the older ones. The older leaves undergo senescence, and these are not of much value to plants because of deterioration in their productive value. Younger leaves are more valuable to plant, and they tend to be more responsive to herbivory. Tropical trees that grow on the infertile soil have higher concentration of secondary metabolites than do trees that grow on more fertile soils. Fast-growing species utilize more carbon for their growth and invest less carbon in secondary metabolites rather than slow growing ones, making them more susceptible because of less allocation of carbon for defense chemicals. So, plants growing more rapidly invest less carbon in defense. Factors that inhibit growth more than inhibiting photosynthesis enhance secondary metabolite production. Physical features, such as leaf toughness and trichomes, form a physical barrier against herbivores. Defense strategies, both direct and indirect, provide dual protection to the plant against insect herbivores in natural ecosystems.

32.5.1 Defense Mechanisms in Plants Against Herbivory

Plants have evolved a number of traits to protect or tolerate herbivory. These include constitutive as well as induced defense mechanisms. Constitutive defense mechanisms include development of specialized morphological features that physically deter feeding by the herbivores as well as production of secondary metabolites, proteins, and enzymes in the plants that have toxics and/or anti-nutritional effects on the herbivores. First line of defense against herbivory **(constitutive defense)** is played by *plant structure* that forms a physical barrier preventing the pests through formation of a waxy cuticle and/or formation of spines, thorns, or trichomes. These morphological and anatomical traits offer an advantage to the plant by directly inhibiting the herbivores from feeding. These structural traits include cell wall reinforcement by deposition of cuticle resulting in toughened and hardened leaves (sclerophylly), waxes, etc.; presence of spines, thorns, prickles (spinescence), and trichomes or hairs (pubescence); and granular mineral inclusions in plant tissues. Nonglandular trichomes prevent small insects from accessing the leaf surface and, therefore, physically limit their movement. Wounding due to herbivores leads to *enhanced synthesis of chemicals* such as lignins, cellulose, suberin, callose, phenolics, and silica particles. These enhance toughness of leaves, thereby forming an important physical barrier to prevent the mouthparts of piercing-sucking insects from penetrating the plant tissues and causing mandibular wear and tear in biting-chewing herbivores. An increase in density of the trichomes is seen in response to insects feeding on plants. This may also be classified as an **inducible response**. Trichomes, in addition to serving as barriers, also act as sensors that send electrical and/or chemical signals to induce defense responses. Presence of mineral crystals such as silica crystals, called as **phytoliths**, present among the members of grass

family, adds toughness to the cell walls, making it difficult for insect herbivores to chew. Calcium oxalate crystals form needle-like structures called **raphides** (in specialized cells called as **idioblasts**) with sharp tips that can damage the soft tissue of the digestive tract of an herbivore. In addition to causing mechanical damage, raphides also facilitate the entry of toxic compounds produced by the plant through the damaged site. Latex-filled **laticifers** and **resin ducts** that store resins form a network of canals which constitute an anatomical defense mechanism in plants. Upon herbivore attack, the contents are exuded that entraps or poisons (such as toxic cardenolides) the herbivore. For example, the milkweeds (genus *Asclepias*) are known to exude latex that coagulates upon exposure to air and immobilizes the feeding insect larvae. Resin-based anatomical defense is seen in conifers. Resin is a mixture of terpenes that accumulate in resin duct network and are secreted out upon damage by herbivore attack. The highly volatile monoterpenes evaporate, trapping the insect in solidifying resins that also seals the wounded area. Sealing is important to prevent water loss and also to prevent bacterial and fungal infections at the site of infection.

A wide variety of **secondary metabolites**, including phenolics, terpenoids, alkaloids, cyanogenic glucosides, and glucosinolates, are secreted in response to herbivory that contributes toward plant defense against the pests. These metabolites inhibit herbivore activity by either reducing the nutritional value of plant food or by acting as deterrent to the feeding by herbivore or act as a toxin. A variety of **anti-nutritional proteins** are also induced in response to herbivory, which include serine proteinase inhibitors, several classes of proteases, oxidative enzymes, amino acid metabolizing enzymes, and lectins. Nitrogen-containing compounds produced by plants also act as defense molecules against pests. These include alkaloids, cyanogenic glycosides, glucosinolates, and some non-protein amino acids. Eating alkaloid-containing plants such as ragworts (*Senecio* spp. containing alkaloid senecionine) or lupin (*Lupinus* spp. containing alkaloid lupinine) can lead to poisoning among grazing animals.

Several alkaloids are known to be feeding deterrents against herbivores. Alkaloids derived from ornithine or arginine occur naturally in nontoxic forms in plants. These are reduced to toxic uncharged and hydrophobic tertiary alkaloids that can pass through membranes as they reach the alkaline digestive tracts of insect herbivores. Though stored in nontoxic forms, some compounds are converted to toxic forms when plant tissues are damaged. These include cyanogenic glycosides and glucosinolates. Cyanogenic glycosides and its hydrolytic enzymes are stored separately within the plant cells. Cyanogenic glycosides present in *Manihot esculenta* tubers enhance resistance against cassava burrower bug (*Cyrtomenus bergi*). *Sorghum* spp. which make dhurrin (a cyanogenic glycoside) is stored in the vacuoles of leaf epidermis, while the hydrolytic enzyme is stored in the mesophyll cells of the leaf. As the damage is caused to leaves, the two get mixed up resulting in release of hydrogen cyanide. Members of family Brassicaceae have got a characteristic smell which is due to glucosinolates. Similar to cyanogenic glycosides, these are also sulfur-containing nitrogenous compounds and are stored in vacuoles as conjugated with sugars, but sugar is attached to central carbon via sulfur atom. The

enzyme myrosinase is stored separately, and both are released during mechanical damage resulting in production of toxic isothiocyanates and nitriles. The glucosides of another nitrogen-containing compound, such as benzoxazinoid compounds (e.g., **DIOBA** and **DIMBOA**), are also stored in vacuoles which themselves are nontoxic, but a specific glucosidase is present in chloroplasts, which is released when mechanical damage is done to the plant and can cleave the molecule to release a toxic compound benzoxazinoid. Colored flavonoids are responsible for the coloration in plants; colorless flavonoids such as rutin and isoquercitin are toxic to a number of insect species and act as feeding deterrents to insect herbivores. Accumulation of high levels of certain non-protein amino acids provides protection against herbivores. Non-protein amino acids have defense functions through their misincorporation during protein synthesis. **5-Hydroxynorvaline** is one such non-protein amino acid identified from the leaves of maize (*Zea mays*). It accumulates in leaves during insect herbivory as well as in response to treatments such as with jasmonates, salicylic acid, etc. t-RNA of herbivores which consume plants containing non-protein amino acids become charged with these amino acids leading to disruption of protein synthesis or formation of unstable proteins. Canavanine, another non-protein amino acid that resembles arginine, is synthesized in *Canavalia* sp. (Jack beans). Canavanine cannot be distinguished by arginyl-tRNA synthetase since it closely resembles arginine when ingested by the herbivores. As a result, protein synthesis gets disrupted in the herbivores. However, arginyl-tRNA synthetase present in the seeds of *Canavalia* can distinguish between arginine and canavanine. They thus remain unaffected. In jack beans, t-RNA can discriminate canavanine from arginine. However, when ingested by the herbivore, canavanine proves to be lethal.

The intake of non-protein amino acids by the herbivores interferes with their metabolism in the following ways: 1. substitution in place of normal amino acid resulting in formation of defective proteins; 2. inhibition of the activity of aminoacyl-tRNA synthetases and other steps of protein biosynthesis, e.g., azetidine-2-carboxylaic acid and 3,4-dehydroproline; 3. inhibition of amino acid biosynthesis either by competitive inhibition or end-product feedback inhibition of key enzymes of a metabolic pathway, e.g., azaserine, albizzine, and S-amino ethyl cysteine; 4. targeting DNA and RNA-related processes, e.g., canavanine and mimosine. Mimosine inhibits collagen biosynthesis, and L-hypoglycine inhibits β-oxidation of lipids. Non-protein amino acids are also metabolized to form antimetabolites that prove toxic to herbivores. For example, in *Allium* sp. certain non-protein amino acids derived from cysteine are not incorporated during protein synthesis but are metabolized to form allicin *syn*-propanethial *S*-oxide which has antimicrobial effect. It is important to note that contrary to herbivores, plants are able to distinguish between protein amino acids and their analogs; therefore, they are able to tolerate antimetabolites. However, non-adapted herbivores are not able to discriminate and are therefore susceptible to toxic effect. Two early events in plants associated with insect herbivory are mechanical damage inflicted to plant tissues and the elicitors by which plants are able to identify "self" from "nonself." These include **damage-associated molecular patterns (DAMPs)** in host plants which

include structurally diverse groups of molecules. For example, oligosaccharide, such as oligogalacturonides, released from the cell wall acts as an elicitor. These appear in the apoplast and are involved in eliciting defense responses in plants. Another example of DAMP includes **systemin** which is a 20-amino acid peptide derived from prosystemin (200 amino acid long polypeptide). In the damaged leaf tissues of tomato, prosystemin is hydrolyzed to form systemin in phloem parenchyma. Systemin triggers a typical defense response in tomato plant. A 160 kDa plasma membrane-bound, systemin-binding LRR receptor kinase SR160/BRI1 has been identified on the surface of companion cells of phloem tissue in *Solanum peruvianum* which triggers the signal transduction pathway for jasmonate biosynthesis in CC-SE complexes. The second class of elicitors includes those which are generated in the oral secretions (OS) of insects. The resulting insect oral secretions (OS) recognized by plants are termed as herbivore-associated elicitors (**HAEs,** also called as **HAMPs**; herbivore-associated molecular patterns) that trigger and elicit defense responses in plants. One such elicitor is **violicitin**, a fatty acid-amino acid conjugate (FAC). Violicitin was initially discovered in oral secretions of *Spodoptera exigua* (beet armyworm), and it is composed of linoleic acid (hydroxylated at position 17) conjugated to glutamine. While the fatty acids are derived from the plant, an enzyme present in the gut of the insect facilitate conjugation of the fatty acids with insect-derived amino acids such a glutamine or glutamate after the insect ingests plant material containing these fatty acids.

32.5.2 Signal Transduction Triggering Plant Responses

As the insect feeds on the plant, a signal transduction cascade is initiated in the plant. It occurs both at the damaged site (local response) as well as a systemic response (at a site away from the damaged site). Signal transduction cascade is triggered after HAMPs/DAMPs are recognized. It involves intracellular changes such as $[Ca^{+2}]_{cyt}$ and changes in transmembrane potential leading to electrical signaling resulting in synthesis of hormones such as jasmonic acid (JA) and other defense responses of the plant.

Electrical Signaling First recognition of insect attack and initiation of signal transduction cascade occurs at the plasma membrane, which is in direct contact with the exterior. Evidences indicate involvement of electric signaling that is initiated in response to herbivore attack. Leaf injury caused by mechanical damage or herbivory induces the activity of two **glutamate-receptor-like (GLRs)** ion channels, such as calcium-ion channels, which results in generation and transmission of electrical signal, triggering defense responses at sites further away from injury. Double mutants from *Arabidopsis* for genes encoding two such channels (*glr3.3* and *glr3.6*) showed disruption of wound-induced electrical signaling, confirming the role of GLRs in wound-induced signal generation and transmission.

Role of Ca^{+2} as Secondary Messengers Several signaling pathways are known to involve fluctuations in Ca^{+2} concentrations following herbivore attack. Cytosolic concentration of Ca^{+2} is much lower than that in the apoplastic fluid and in the cellular organelles, creating a driving force for the $Ca^{+2}$to enter through the membrane channels. The calcium ions are later pumped back through Ca^{+2}-ATPase pumps. An increase in cytosolic Ca^{+2} concentrations has been observed in response to oral secretions of *Spodoptera littoralis* (Egyptian cotton worm) feeding on *Phaseolus lunatus* (lima bean) and *Gingko biloba.* An increase in cytosolic Ca^{2+} precedes membrane depolymerization through voltage-gated channels located in the plasma membrane and other intracellular membranes. Calcium signal activates calcium-sensing proteins, including calmodulin, calcineurin, and Ca^{+2}-binding protein kinases (CDPKs). This alters protein phosphorylation and gene expression patterns. CDPK signaling pathways further involve those that interact with mitogen-activated protein kinases (MAPKs) and form jasmonates (JAs).

Intracellular Wound Signal Mechanical damage of the leaf tissue induces the formation of systemin from prosystemin in the phloem parenchyma cell that functions as DAMP triggering response in the adjacent companion cell. Systemin enters into the apoplast and binds to a membrane-bound receptor of the companion cell and initiates an intracellular signaling cascade that activates a MAPK and, finally, leads to the biosynthesis of JA in the companion cells (Fig. 32.20). JA is then transported as methyl jasmonate to long distances via the phloem and activates target gene expression in distal undamaged leaves. Salicylic acid also plays a role in signaling in plants in response to aphids feeding through phloem. It acts as a negative modulator as it represses JA.

JA-Mediated Induction of Defense Gene Expression as a Systemic Response As mentioned earlier, defense responses are not only triggered at the damaged site but also in undamaged parts of the plant. While earlier systemin was proposed as the mobile signal, it is now known that systemin is involved only in initiating wound signaling and not in long-distance signaling. Long-distance signaling is mediated by JA (Fig. 32.21). Mutants deficient in JA are easily killed by insect pests and exogenous application of JA in such mutants has been shown to restore resistance. This indicates the vital role played by JA in herbivore-induced plant defense responses. JA is derived from its precursor α-linolenic acid via **octadecanoid pathway**. JA is the key regulator that modulates the expression of defense-related genes, such as those that code for proteins that interfere with the herbivore digestive mechanism. These include α-amylase inhibitors, lectins, proteases, and **proteinase inhibitors (PI)** (Fig. 32.22). Legumes produce α-amylase inhibitors. In some plants, defensive proteins such as lectins are produced that bind to oligoproteins. Upon ingestion by the herbivore, lectins bind to epithelial cells of the digestive tract and disrupt the absorption of nutrients. The gut epithelial membrane of the digestive tract is also directly affected by the ingestion of proteases that are produced by the plant in response to herbivory. Other important proteins that interfere with the digestion of the insect herbivore are the proteinase inhibitors,

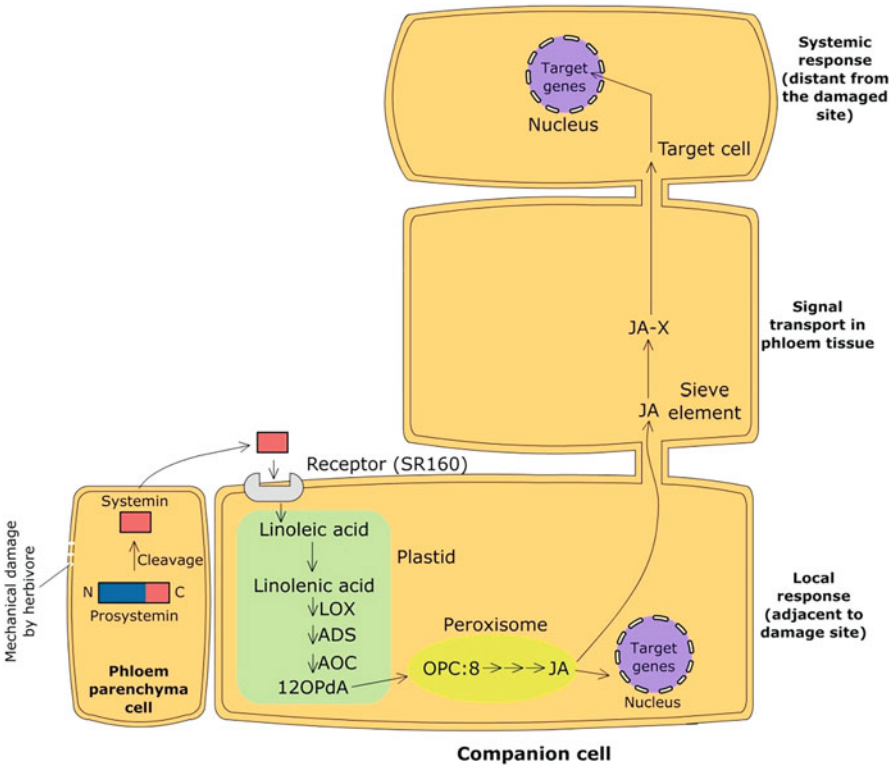


Fig. 32.20 Schematic model showing the role of JA in systemic wound signaling. Chloroplastic and peroxisomal JA biosynthetic enzymes are located in vascular bundles of the leaf. Binding of systemin to its receptor (SR160) activates JA accumulation. JA produced in the companion cell-sieve element complex is transported in the phloem via plasmodesmata connections between cells. JA, or a covalently modified form of JA (JA-X, e.g., JA-Ile), activates target gene expression in distal undamaged leaves

which bind to and inhibit protein hydrolyzing enzymes such as trypsin and chymotrypsin in insect gut. This results in deficiencies causing delayed development, reduced fecundity, and mortality. In addition to inducing the synthesis of PI, it also activates the genes responsible for the synthesis of compounds such as terpenes, alkaloids, phenylpropanes, and glucosinolates which act as deterrent for herbivores. JA not only induces defense-related genes but also suppresses growth. Growth inhibition allows reallocation of resources for metabolic pathways involved in defense.

Role of Ethylene Ethylene controls the production of constitutive defense compounds after herbivore damage and stimulates the production of JA and volatile compounds **[Herbivore-induced plant volatiles (HIPV)]**. For example, it has been shown in *Medicago truncatula* that ethylene modulates herbivory-induced early

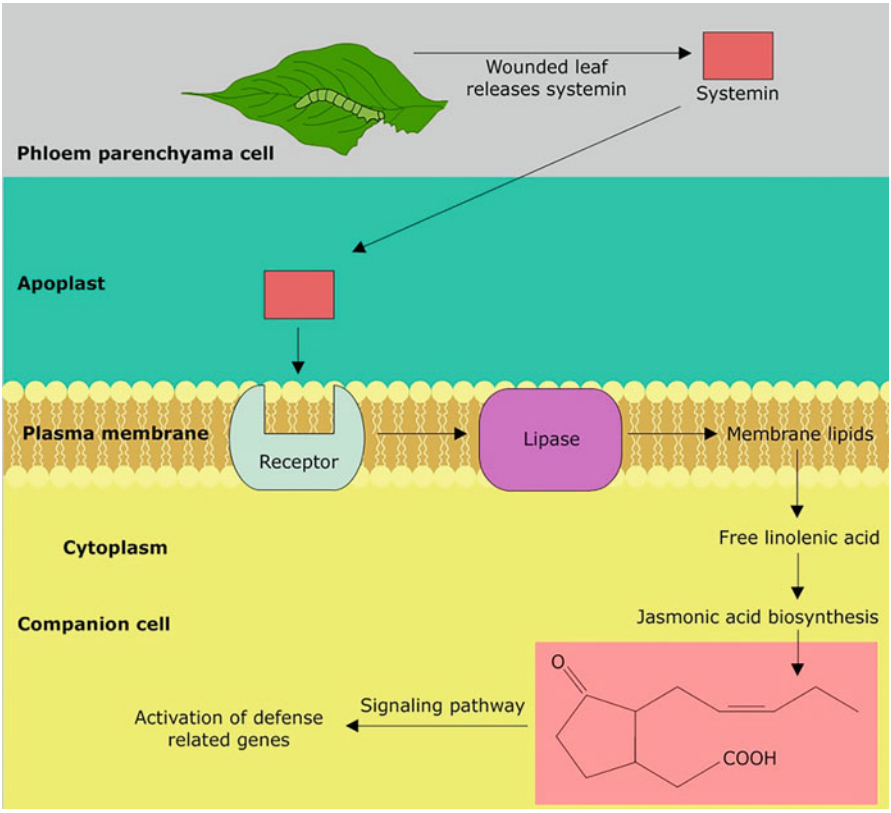


Fig. 32.21 Wounded leaf signals the proteolytic cleavage of prosystemin to release systemin. Systemin signaling pathway is initiated upon binding of the peptide to a 160-kDa plasma membrane-bound receptor (SR160). Systemin binding activates lipase activity that releases linolenic acid, a JA precursor, from lipids in the plasma membrane. Following JA synthesis, defense-related genes are activated

signaling events such as Ca^{+2} influx and downstream, JA-dependent biosynthesis of terpenoids. The crosstalk between JA and ethylene controls local cell expansion and growth following herbivore attack. This allows resource allocation toward induced defense responses against herbivores. Both JA and ethylene were found to suppress growth in attached leaves of tobacco. However, expression of trypsin proteinaceous inhibitors requires only JA signaling that is independent of ethylene. Ethylene modulates responses such as emission of specific VOCs, accumulation of phenolic compounds, and PI activity (Fig. 32.23). Ethylene also elicits several defensive proteins such as the enzymes polyphenol oxidase and peroxidase which form quinines. Quinines react with nucleophilic side chains of amino acids, thus causing formation of protein cross-linkages. Quinines interfere with digestion in insect gut.

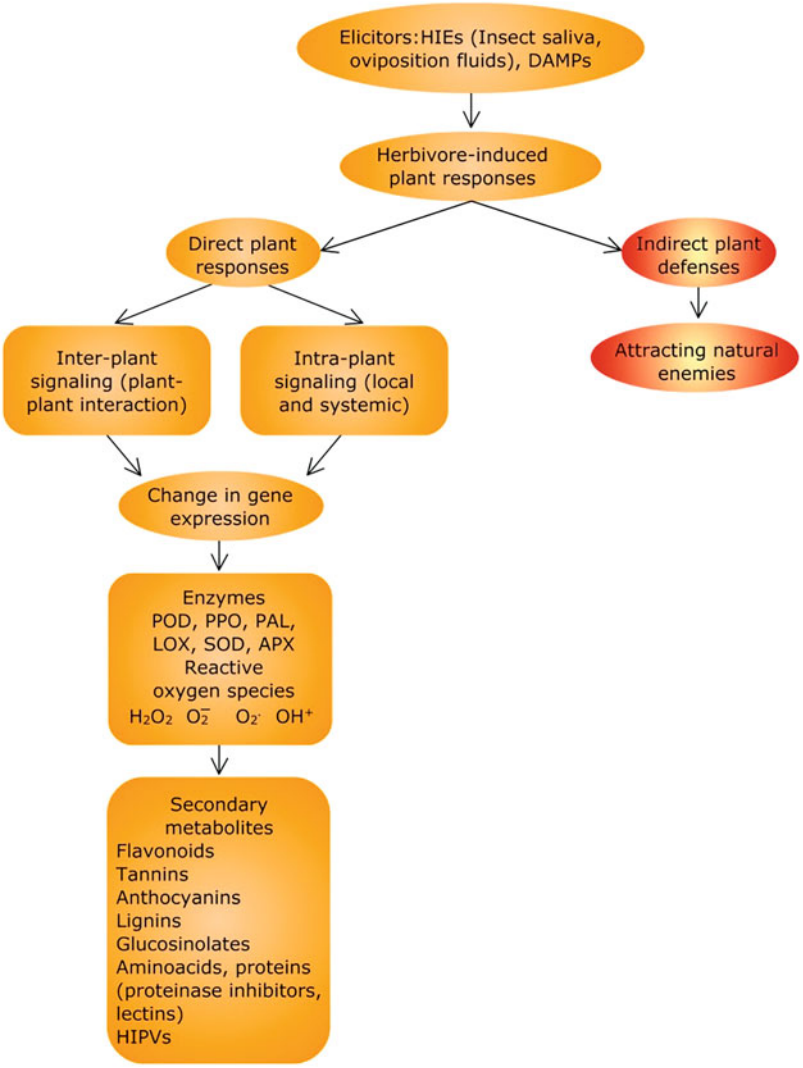


Fig. 32.22 Classification of herbivore-associated responses in host plants and the resistance factors

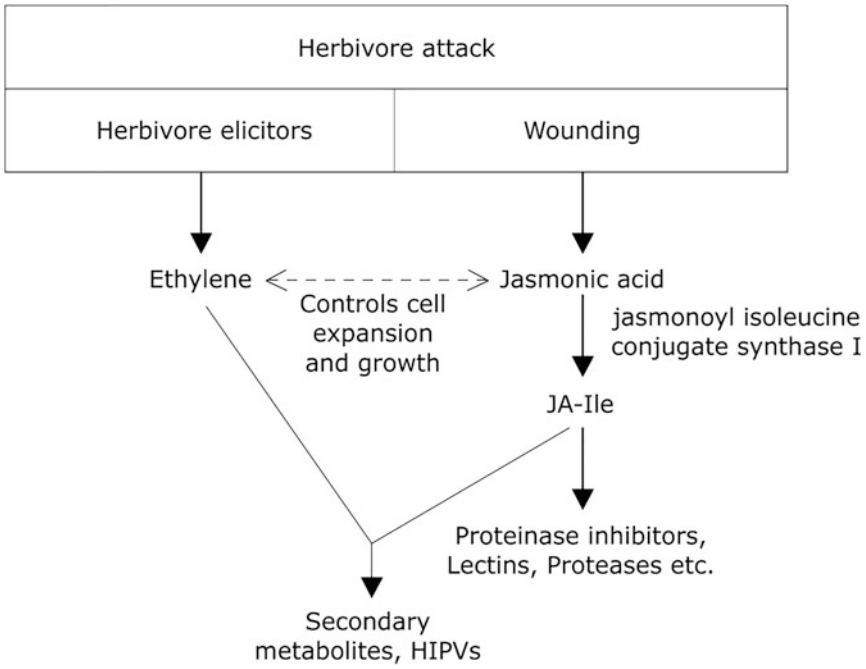


Fig. 32.23 JA and ET interaction in the leaves attacked by herbivores. Though both signals suppress growth inhibitors of protease, functions require only JA signaling pathway that is independent of ET signaling. However, both JA and ET may be needed for accumulation of secondary metabolites in response to herbivory in both local as well as for systemic response

32.5.3 Herbivore Response to Plant Defense

Herbivores have evolved counter strategies to resist or manipulate plant defense responses. For example, a bug, *Pameridea roridulae*, is adapted to walk on the sticky surface of *Roridula gorgonias*, a carnivorous plant. Several insect species are also known to remove leaf hairs and cut leaf veins or latex channels that prevent insect feeding. Specialist herbivores adapt mainly by two ways, by detoxification of the toxic chemicals produced by the plant or by prevention of defense activation. Suppression of host defenses often involves secretion of molecules called as effectors that modulate host defenses. The salivary secretions of insect *Helicoverpa zea* secrete the enzyme glucose oxidase (GOX) which acts as an effector molecule since it protects the herbivore against pathogen and also suppresses plant defense**.** Glucose oxidase oxidizes D-glucose from the host plant to D-gluconic acid and H_2O_2.

$$\mathrm{D-glucose} + \mathrm{O_2} \rightarrow \mathrm{H_2O_2} + \mathrm{D-gluconic\ acid}$$

GOX is a potent O_2 scavenger. GOX provides the initial oxidative burst of H_2O_2 which suppresses the induced defenses of the host plant. D-gluconic acid is known to scavenge free radicals and inhibit polyphenol oxidase. H_2O_2 is responsible for elicitation of salicylic acid induced response and associated negative crosstalk that attenuates JA and ET signaling. Herbivore-associated pathogens have also been associated with their roles as effectors. Since pathogens elicit SA-regulated defense, which often negatively crosstalk with jasmonate signaling, plants are unable to fully activate the jasmonate-mediated resistance against herbivores. Not only the chemicals of OS act as elicitors, eggs of some herbivores may cause local suppression of JA pathway via induction of SA pathway.

32.5.4 Nematodes

Nematodes are known to feed on plants using their stylets for obtaining food. Plant parasitic nematodes (PPN) can be classified as ectoparasites, migratory endoparasites, and sedentary parasites. All PPN possess a hollow protrusible stylet that punctures wall and is used for injecting secretions and ingesting nutrients from the plant cell. Ectoparasites remain outside the body of the host and use their stylets to feed on the content of the cell. This leads to wounding, necrosis and often gall formation in the host tissues. Migratory endoparasites have robust stylet that allows them to penetrate and continuously move through the root. Migration is made possible by the secretion of cell wall-degrading enzymes through the stylet. Infection by such nematodes results in extensive necrosis and formation of gall tissue. Sedentary endoparasites, such as the cyst nematodes and root-knot nematodes, induce the formation of permanent feeding cells inside the vascular tissue of the host plant after entering and migration. The young ones hatch close to the root tip and then migrate inter- and intracellularly toward the vascular cylinder. As they reach the vascular cylinder, they continuously feed on the cellular content while vigorously injecting the stylet secretions into the cell. This causes extensive changes in gene expression and morphology. The root tissue becomes hypertrophic and shows expansive proliferation of organelles (Fig. 32.24). Root nematodes result in the formation of six to seven giant multinucleated cells, while the cyst nematodes induce the formation of a **syncytium** after the initial feeding and fusion of adjacent cells. Unlike the migratory and ectoparasite nematodes, the endoparasitic nematodes maintain their feeding cells in a healthy and metabolically active state. Initially, as the PPN invade and remain inside the host for several weeks to months, they expose themselves to plant defense systems. However, to evade detection they adopt strategies such as repeated molting, creating a new challenge to the host immune system every time. The carbohydrate-rich outer surface coat is shed each time, and thereby the changes in composition create a variable target for plant immune system. In addition, PPN cover themselves with plant-derived carbohydrates to escape recognition as a nonself entity. In spite of these strategies, presence of PPN can be detected by the host plant which responds by the production of reactive oxygen species, cell wall modification enzymes, callose deposition, cell wall thickening, etc.

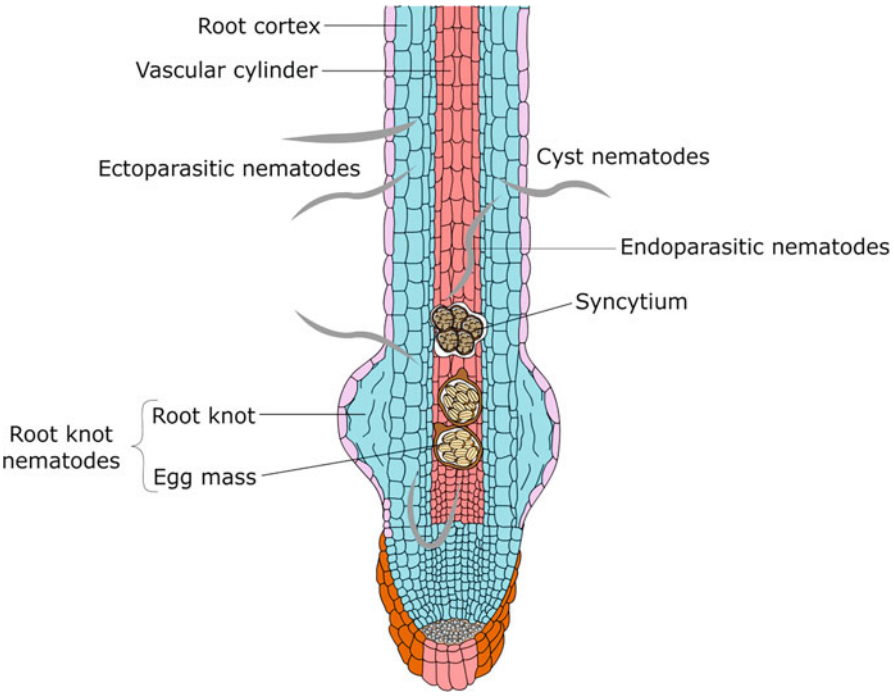


Fig. 32.24 Plant parasitic nematodes are classified as ectoparasites and endoparasites. Ectoparasitic nematodes do not enter the plants but use their stylets to feed on plant tissue. They cause least damage to the root tissue. In contrast, the endoparasitic nematodes completely enter the host and may either move through various tissues (migratory endoparasites) or reside as sedentary endoparasites in the host tissue. The latter includes the root-knot nematodes and cyst nematodes in which the infective stage (juveniles) moves into the vascular cylinder and induces the formation of feeding structures for drawing the nutrients. The cyst nematodes induce the formation of syncytium of hundreds of fused cells. The root-knot nematodes on the other hand induce the formation of several coenocytic giant cells

It is yet to be deduced whether these responses resemble JA or PAMP-induced responses. A number of effector proteins are secreted by the nematodes, which includes cell wall-degrading enzymes such as cellulases, pectate lyases, polygalacturonases, xylanases, and expansions (Fig. 32.25). In addition to these, enzymes such as glutathione peroxides and peroxiredoxin secreted by the nematodes protect them from ROS formed in response to infestation. Effectors suppress SA or JA production or interfere with plants ubiquitin-proteasome pathway to suppress plant immune system.

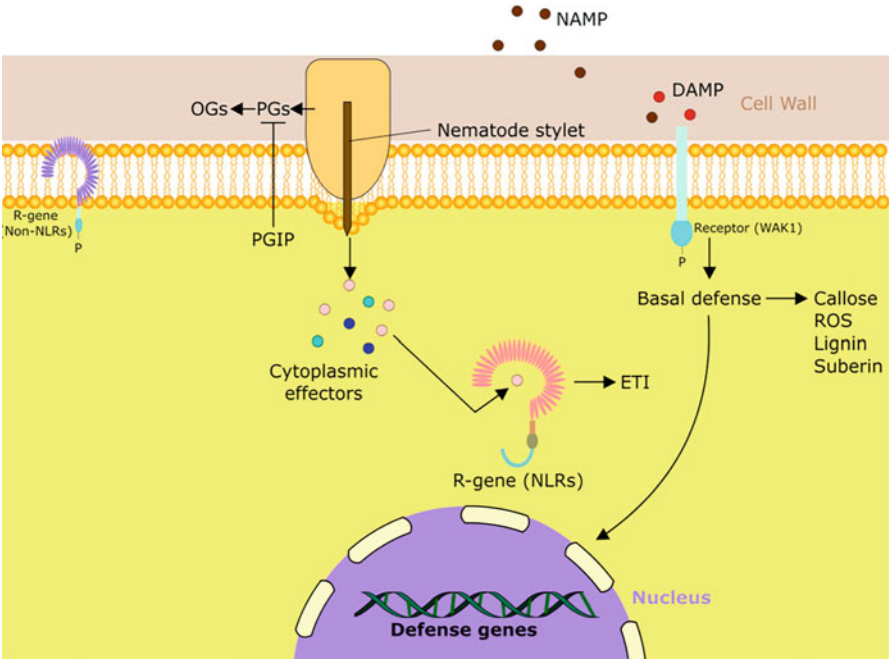


Fig. 32.25 Molecular events during plant-nematode interaction. Invasion of the nematode caused cell wall damage producing damage-associated molecular patterns (DAMPs) that initiate plant defense responses by binding to DAMP receptors, such as WAK1. Nematodes also secrete cell wall-degrading enzymes such as polygalacturonases (PG) that hydrolyze cell wall. In response plant produces PG inhibiting proteins (PGIPs). Nematode-associated molecular patterns (NAMPs) released from the nematodes are recognized by plasma membrane receptors and trigger pattern-triggered immunity (PTI) that includes callose, reactive oxygen species (ROS), lignin, and suberin. Nematodes, in response to plant defenses, secrete effectors to counter the NAMP-/DAMP-based immunity. Plants, in turn, have R genes that recognize effectors and initiate effector-triggered immunity (ETI)

32.6 Plant-Plant Interactions

32.6.1 Parasitic Plants

Approximately 4000 species of plants mostly from distantly related families of dicotyledons are parasitic on other plants. These are biotrophic pathogens requiring living plants for their survival since these are either partially or totally dependent on host plants for their requirements including water, mineral nutrients, or photosynthates. Some of them have completely lost their capacity to photosynthesize as well as to assimilate inorganic nitrogen and are dependent on host plants for carbon and nitrogen nutrients. These are obligate parasites also called **holoparasites**. These types of plants have lost their total capacities to complete their life cycle without the host. They do not contain appreciable amount of chlorophylls and are unable to photosynthesize efficiently. Their CO_2 compensation point may be as high as 200 Pa. They also do not

possess roots. One of the common examples is *Cuscuta* sp. which neither can photosynthesize nor has got true roots. It is dependent on host plants for photosynthates as well as for water and minerals. Other types of parasitic plants are known as **hemiparasites** which contain chlorophyll and have some photosynthetic activity. They, however, depend on the host for the supply of minerals and water. Distinction between holoparasites and hemiparasites is not very sharp, e.g., *Striga* sp. is a hemiparasite since it contains very little chlorophyll and possesses very little photosynthetic activity but is totally dependent on the host plant. Hemiparasites may either be **facultative or obligate parasites**. Some of them are attached to stem, while others are attached to roots of the host plant. Example for the latter type is *Rafflesia arnoldii.* Stem parasites include both holoparasites, e.g., *Cuscuta*, and hemiparasites, such as *Viscum* sp. (mistletoe). Root parasites also include both holoparasite, e.g., *Orobanche* sp., and the hemiparasite, e.g., *Striga* sp. (witchweed). *Striga* sp. parasitizes tropical plants, such as sorghum and maize, while *Orobanche* parasitize more temperate plants such as sunflower and tomato. Parasitic plants also include both woody species and small herbaceous species. Examples for these are *Exocarpos cupressiformis* and *Rhinanthus sclerotinus*, respectively. Some parasitic plants are host-specific, while others can parasitize a range of hosts. Facultative parasites have a broad host range, while obligate parasites tend to be more host-specific. *Striga* sp. is parasitic to either monocots or dicots while *Orobanche* sp. parasitize dicots only. Life cycles of *Striga* sp. and *Orobanche* sp. are quite similar. In both cases various mechanisms ensure coordination of the parasite and the host. This includes germination of the seeds of parasite plant, formation of haustoria to provide attachment, establishment of successful connection with vascular tissues of the host, and compatible interactions thereafter.

32.6.2 Establishment of Contact Between Parasitic and Host Plants

Seed Germination Seeds of *Striga* sp. and *Orobanche* sp. have very little reserves. They run the risk of dying if germinated without the availability of suitable host since their reserves would be exhausted before the plant starts getting nutrients. Interaction of parasite begins with release of a secondary metabolite by the roots of host plant. Seeds of *Striga* sp. germinate in response to a signal released from the roots of cotton (*Gossypium hirsutum*) plants even though it is not the host. The stimulant has been identified a sesquiterpene called strigol (Fig. 32.26). Strigol is active in concentrations as low as 10^{-12} M in soil solution. Many analogues of strigol have been synthesized and tested for their capacity to stimulate seed germination. Besides strigol, other stimulants have also been isolated and identified. Several stimulants for germination seeds of *Striga* sp. have been identified which collectively are called **strigolactones**. Strigol might be regulating synthesis or action of ethylene since there is absolute requirement of this for germination of seeds of *Striga* sp. Another member of the family, i.e., sorgolactone, has also been identified from sorghum. A gradient of germination stimulant may be responsible for the growth of radicle toward the host. Though the seeds of *Cuscuta* sp. which is an obligate stem parasite germinate even in absence of host, the seedlings are unable to survive for a longer period of time because of presence of limited reserves in the seeds. It is observed *Cuscuta* sp. perceives

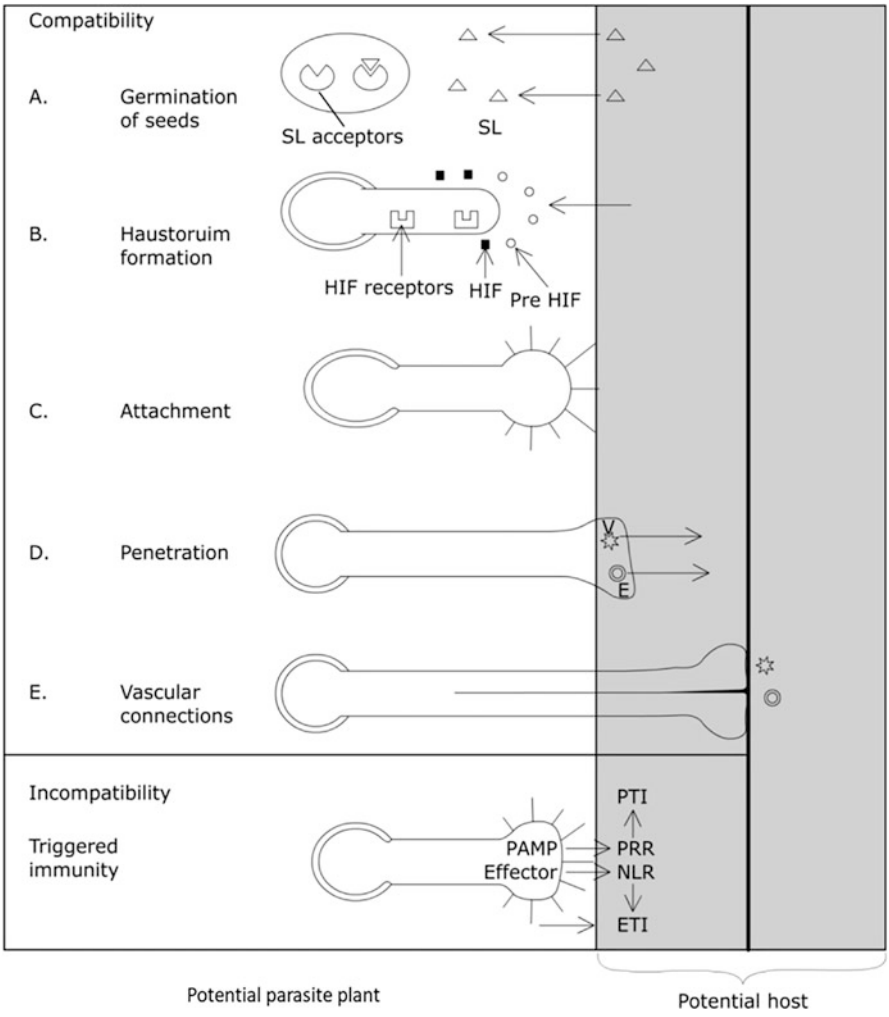


Fig. 32.26 (**a**) Interaction between a parasitic plant and the potential host: *SL* strigolactone, *HIF* haustorium inducing factors, *V* virulence factor, *E* effector, *PTI* pattern-triggered immunity, *ETI* effector-triggered immunity; upper box represents development stages of parasitic plant (**a**–**e**) during a compatible interaction. Lower box represents host-invading stage during which molecules (PAMP and effector) trigger immunity in host plants. (**a**) Seeds of parasitic plants start germinating on receiving stimulus (SL) from the potential host plant; (**b**) oxidoreduction processes enable production of haustorium inducing factors (HIF) from precursors of HIFs (pre-HIF). Perception of active HIFs by HIF receptors activates the formation of haustorium; (**c**) haustorial hairs and certain chemicals enable attachment of parasitic plant to the host; (**d**) further growth of haustorium and probable secretion of unknown virulence factors (V, small compounds) and/or effector proteins (E); (**e**) vascular connection is established with the host roots for nutrient uptake. Lower box: demonstrating incompatibility, parasitic plant-derived molecules such as PAMPs and effectors may activate PTI and ETI by nucleotide-binding domain leucine-rich repeat domains (NLR) and by pattern recognition receptors (PRRs), respectively

vicinity of the host plant by sensing the volatile terpenoids and chemicals such as pinene, β-myrcene, and β-phellandrene. These compounds act as chemo-attractant. Similar to insect herbivores some parasites may locate preferred host plant based on the defensive compounds produced by them. These compounds serve as attractants at high concentrations, while at low concentrations they serve as defensive compounds.

Haustorium Haustorium is a defining feature of all parasitic plants. The word is derived from Latin word, "haurire" which means to drink. Haustorium is a modified root which functions in attachment, penetration as well as transfer water and solutes. It is a morphological and physiological bridge between host and the parasitic plant. Three main regions of haustorium are recognizable. These include a swollen-rounded structure (which connect root or stem of the host plant to the main haustorial tissue, *hymenium*) located close to the host tissue and the parasitic tissue that penetrates into the host to form physiological connections with the vascular tissues of the host. This is known as endophyte. Haustorial development requires an adhesive stage, intrusive stage, and conductive stage which includes development and adhesion of haustorium with host plant, penetration of haustorium into the host, and establishment of physiological connection in between the vascular tissues of host and conducting tissue of the parasite, respectively. After finding suitable host, prehaustoria develop in the parasitic plants which serve as an adhesive disk. Prehaustorium secretes adhesive substances, such as pectins and other polysaccharides, which reinforce adhesion and develop into a specialized structure called **haustorium.** In *Cuscuta*, proximity to host results in an increase in cytosolic Ca^{2+} which lasts for about 48 h after the initial contact. *Cuscuta* induces host plant to produce sticky substance **arabinogalactan** proteins, to promote adhesion. These glycoproteins, produced by the host plant, are localized in the cell wall where these can force adhesion together with other sticky substances, such as pectins. Attachment phase is followed by penetration phase as prehaustoria develop into parasitic haustoria. Initiation of haustorium development occurs in response to signals produced by the host plant which are known as HIF (haustorium induction factors). Stimulus for haustorium formation may be initiated simply by the contact with the host surface, which causes stoppage of further elongation of parasitic root system. This is followed by isodiametric expansion of cortical cells of the parasitic roots. Inner cells divide first followed by cell division of peripheral cell resulting in formation of noticeable bump at or near the root tip meristem in parasites within 24 h. Epidermal cells elongate to form long, densely positioned haustorial hairs which are responsible for firm attachment of parasite with the host plant. After the parasite is firmly attached to host plant, a penetration peg invades host epidermis and cortex by a combination of physical and chemical processes until it reaches the vascular tissue of the host plant. In *Striga hermonthica*, haustorium formation is stimulated by phenolic acid, such as sinapic, vanillic, and syringic acids produced by the host plant. Enzymes (peroxidases and hydrolases) produced by the parasitic root system convert the inactive parahydroxyacids (by oxidative carboxylation) into active quinones and a functional haustorium is established. *Striga asiatica* is a hemiparasite which grows on the roots of plants such as *Vigna unguiculata* or *Sorghum bicolor*. In *Striga asiatica*, haustorium formation is induced in response to a signal, i.e., 2,6-dimethoxy-p-benzoquinone (DMBQ) which is produced by the

host roots in response to an enzyme produced by the parasite. It is not produced by *Sorghum* sp. roots rather is a product of lignin decomposition by the enzyme laccase produced by root tip of *Striga* sp. The compound is probably not released in the root environment rather is tightly bound with the cell wall. Benzoquinones are produced in plants in shikimate acid pathway by the oxidative carboxylation of phenolics or by enzymatic degradation of cell wall phenols by peroxidases and laccase. It was demonstrated that H_2O_2 generated at *Striga* sp. radicle tip activates host plant peroxidases which convert cell wall phenols into benzoquinone. Proximity of host is ensured before haustorium development in *Striga* by this mechanism. The haustorium penetrates through the host tissue effected by mechanical pressure and is supported by the biochemical degradation of the host cell caused by the secreted hydrolytic enzymes, such as methylesterase, or lytic enzymes, such as pectinase or cellulose.

Walls of parasitic cells are thickened with polysaccharides rather than with lignin. Enzymes from the parasitic cells soften the surface tissues of the host, and haustorium penetrates the epidermis, bark, and parenchyma to reach vascular tissue to absorb nutrients. Vascular cells of parasite contact vascular tissues of the host. As a result, contents of xylem are diverted into the parasite. Cell walls of some of the endophytic cells rupture, producing holes through which constituents of the host xylem can pass through forming a conducting vessel between the host and the parasite. Water and solutes are transported to haustorium through xylem pits. Most of the hemiparasites are xylem tappers. Contrary to this holoparasites are phloem tappers. For phloem connections, conducting tissues of haustorium terminate some distance from the interface. As a result of this, solutes have to travel some distance through parenchymatous cells. After contact with sieve cells, hyphae grow around the cell-like fingers resulting in increase in the parasitic surface around 20 times. A cytoplasmic **syncytium** is built up between *Cuscuta* sp. and the host plant. These cells, which are adjacent to haustorium, are similar to transfer cells. Since no symplasmic connections have been observed between the two partners, transfer of solutes via haustorium may occur via apoplast. Presence of mitochondria, dictyosome, ribosome, and well-developed endoplasmic reticulum in the parenchymatous cells suggests a role of active transport in solute transfer from the host plant to the haustorium of the parasitic plant. Mannitol is present in xylem sap of *Striga hermonthica*, while it is not present in host plant *Sorghum bicolor.* Asparagine is the predominant nitrogenous compound in *Sorghum*, while in *Striga* sp. major nitrogenous compound is citrullin. Malate and citrate are the organic acids present in xylem sap of *Sorghum*, while shikimic acid is a predominant organic acid in xylem sap of *Striga.* Even carbohydrate concentrations have been found to be five times higher in xylem sap of parasitic plant than that of host plant.

32.6.3 Physiological Interactions Between the Parasite and Host

Water and Mineral Relations In order to maintain a continuous influx of water into the parasite, an osmotic gradient is required. Potassium seems to be a preferred

osmoticum in some of the parasitic relationships. Movement of solutes and metabolites is mainly unidirectional. This is evident in case of phosphates which may be retained by the parasites despite of severe deficiency experienced by the host. Most of the xylem tapper parasitic plants do not have a mechanism to selectively import specific ions that arrive via xylem or to export the excess ions imported; these might be stored in parasitic plants. Parasitic plants have lower **water use efficiency (WUF)** than the host plants since these have higher transpiration rates in comparison to their counterparts. High rate of transpiration accounts for lowering of temperature around their leaves by as much as 7 °C. Water potential gradient in between the leaves and root is steep which accounts for their high transpiration rates. Sensitivity of parasitic species to ABA is much less in comparison to that of the host plant. Maize plants infected with the parasite *Striga hermonthica* have higher ABA content than the uninfected plants. High transpiration rates of parasitic plants allow rapid import of solutes through xylem. Reduced stomatal conductance in host plant after infection by the parasite may be attributed to change in their (host's) ABA content. Parasites generally maintain a water potential lower than their host plants by accumulating amino acids (e.g., proline, arginine), organic acids, carbohydrates, and mannitol besides accumulating immobile cations in the xylem.

Carbon Relations Carbon transfer from host to parasite is inversely proportional to photosynthetic efficiency of the parasite. Higher is the photosynthetic efficiency of parasite lower is the amount of carbon transferred from host to parasite. Obligate holoparasites obtain their entire carbon supply from the host since these do not have any photosynthetic efficiency. They predominantly import compounds from the sieve tubes of the host plants and have distinctly lower ratio of calcium to potassium in comparison to hemiparasites since calcium is present in lower concentrations in phloem sap. However, the value of carbon supply seems to be variable in case of facultative hemiparasites. In case of *Striga*-sorghum relationship, almost 30% of the parasitic carbon seems to be derived from host at low nitrogen supply, while in high nitrogen availability and at higher photosynthetic rates, the value declines to around 6%.

32.6.4 Competition for Resources

One of the ways parasites influence growth and reproduction in host is by reducing the amount of resources available for growth. Parasites rarely kill their hosts, but in most of the cases, host suffers reduced growth and reproductive performance. In many cases root parasites have the capacity to reduce crop production to zero. Parasite may provide a sink for photosynthates produced by the host plant, and in turn it increases the rate of photosynthesis in the host. The compensatory mechanisms in the host plant may include increase in leaf area, increased Rubisco content, and delayed leaf senescence or general reallocation of the carbon. Parasites prove to be a long drain on the nitrogen sources of the host plant and cause deficiency in the plant. A high demand for nitrogen by the parasites is explained

by the observation that their growth is profuse when they are attached to legume crops. Accumulation of NH_4^+, NO_3^-, and amino acids in the host may occur due to demand by parasites. Upon attachment to host, cytokinin concentrations may increase in the parasitic plants which may diffuse into the host after infection.

Since crop plants are damaged by the parasites, they trigger immune responses in the host plant. Some plants are resistant to parasitic infection. Resistance to parasitic plants may occur before penetration of the host plant, during haustorium development, or during or after the establishment of vascular connections. Various defense responses, observed in the host plants leading to resistance to parasitic plants, include induction of immunity-related genes, ROS production, deposition of callose and other phenolic compounds, vessel blockage, as well as hypersensitive response which leads to arrest of invasion followed by necrosis of the parasitic structures. There may be involvement of multilayered surveillance system responsible for plant immune system similar to that employed against microorganisms, i.e., cell surface pattern recognition receptors binding to PAMPs, while nuclear and/or cytoplasmic resistance (R) receptors monitor the effectors. Identification and cloning of resistance protein suggest that the host plant may be following similar strategies against parasitic plants as those used against pathogens. Parasitic plants may be secreting effector proteins during infection. SA signaling pathway may also be playing an important role in resistance against parasitic plants besides upregulation of genes encoding PR proteins. Many resistant plants act by surrounding the invading endophyte and blocking its access to the vascular system of the host.

32.7 Allelopathy

Allelopathy is described as both beneficial and harmful biochemical interaction among plants and/or plants and microorganisms through the production of chemical compounds. Such chemical compounds, known as **allelochemicals,** escape into the environment and subsequently influence growth and development of the neighboring plants. Allelochemicals can affect several physiological processes, such as respiration, photosynthesis, and ion uptake. Allelopathy has been observed for over 200 years, and a document published in 300 BC mentioned about many crop plants inhibiting the growth of other plants. In 1937, the word allelopathy was coined in by Austrian plant physiologist Hans Molisch. The term originates from the Greek roots, *allelon*, meaning "mutual" or "among each other" and *pathos*, meaning "suffering" or "feeling." Mostly, allelopathy is used to refer to the harmful effect of one plant upon another. It was reinforced that the effects of one plant to another plant may be either stimulatory or inhibitory depending on the concentration of the released compounds. When a plant produces allelochemicals that are detrimental to the establishment of new seedlings of its own, the phenomenon is called **autotoxicity**. Autotoxicity is a specialized intraspecific form of allelopathy. Although allelopathic symptoms among plants have been observed for a long time but very few specific allelochemicals have been identified (Table 32.4). Allelochemicals are a suitable substitute for synthetic herbicides because they

Table 32.4 Some allelopathic plants, their secondary metabolite secretions and effects.

Plant	Allelochemicals produced	Effects
Juglans nigra L.	Juglone	Inhibit mitochondrial functions and electron transfer in photosystem II
Sorghum bicolor	Sorgoleone	
Callistemon citrinus	Leptospermone	Herbicide (low efficacy)
Centaurea maculosa (weed)	Catechins	Phytotoxic
Actinidia chinensis	Emodin	Feeding deterrent to a large spectrum of organisms
Festuca rubra	m-tyrosine	Phytotoxic
Salvia leucophylla	Mixture of monoterpenes (BVOCs[a])	Toxic for seed germination and root growth
Chrysanthemoides monilifera	Mixture of sesquiterpenes (C15) and diterpenes (C20) [BVOCs]	

[a]*BVOCs* Biogenic volatile organic compounds

(allelochemicals) do not produce residual or toxic effects, although efficacy and specificity of many allelochemicals may be limited.

32.7.1 Molecular Mechanisms of Allelopathy

32.7.1.1 Allelopathic Action Through Root Exudates

Allelochemicals are released from the roots in the form of exudates into the rhizosphere and help in nutrient acquisition. They also serve as signals to a number of microorganisms. Some plants secrete about 5–20% of carbon fixed in photosynthesis into the rhizosphere indicating their importance for plant's survival. Allelopathic interactions are one of the important factors which contribute to the distribution of species and their abundance within plant communities. These interactions are also important in success of the invasive plants, such as *Eichhornia crassipes* (water hyacinth), *Centaurea maculosa* (spotted knapweed) and *Alliaria petiolata* (garlic mustard). *Centaurea maculosa* belongs to Asteraceae family and is native to Europe where it is neither dominant nor problematic. However, in North America it has become an invasive exotic weed and releases phytotoxic secondary metabolites into the soil. In Montana state of Northwestern United States, it has infested over 1.8 million ha (~4.4 million acres). It releases a racemic mixture of (±) catechin, a phytotoxic secondary metabolite. In susceptible species, it triggers accumulation of ROS in the root meristem which initiates a Ca^{2+} signaling cascade. This leads to changes in genome-wide gene expression, causing death of root meristematic cells. In *Arabidopsis*, expression of approximately 1000 genes has been observed to double after 1 h of (±) catechin treatment.

Black walnut (*Juglans nigra*) produces juglone, a toxic naphthaquinone present in all parts of this species, but is released by living roots in high concentrations. *Sorghum bicolor* produces sorgoleone, a benzoquinone, which is specifically

produced in and secreted by root hairs. Both juglone and sorgoleone are implicated in the inhibition of mitochondrial functions and electron transport in photosystem II. Thus, they affect respiratory and photosynthetic pathways. Consequently, secretion of juglone and sorgoleone as root exudates suppresses growth and establishment of other species in substantial area around these plants, thereby reducing competition.

In *Festuca rubra*, a grass species, a different mode of action of allelopathic compound secreted by roots has been reported. Roots of *Festuca rubra* exude *m*-tyrosine, an isomer of *p*-tyrosine and a non-protein amino acid, which is phytotoxic to root growth in different weed species. It has been proposed that *m*-tyrosine interferes with amino acid metabolism via its mis-incorporation instead of phenylalanine, leading to disruptions of protein regulation. Several compounds, such as flavonoids, quinines, cytokinin, and p-hydroxy acids secreted by host roots, have been reported to induce formation of haustorium. These molecules are also implicated in host-parasite recognition and mycorrhizal associations. In S*triga*, the secondary metabolites secreted by host roots act as signal to initiate the development of haustoria. The elongating *Striga* radical perceives haustorial initiation factors (HIFs) secreted by host roots and forms a functional attachment organ.

32.7.1.2 Allelopathic Action Via Biogenic Volatile Organic Compounds

Allelopathic actions are not only mediated through secretion of soluble root exudates. Several **biogenic volatile organic compounds (BVOCs)** have also been implicated in impeding growth of potential competing neighboring plants. *Salvia leucophylla* produces a mixture of volatile monoterpenes which suppress growth of neighboring species. Monoterpenes, such as camphor, camphene, 1,8-cineole, α-pinene, and β-pinene, suppress root growth in *Brassica campestris*. At high concentrations, monoterpenes inhibit seed germination in *Brassica campestris*. Production of α-pinene causes inhibition of root growth in many other plants as well. In *Cassia occidentalis*, exposure to α-pinene leads to enhanced lipid peroxidation and elevation of H_2O_2 levels, leading to severe membrane damage. It is quite possible that similar toxicity mechanisms are responsible for allelopathic mechanisms of other species as well to become invasive. For instance, *Chrysanthemoides monilifera*, invasive in Australia, emits a blend of sesquiterpenes (C15) and diterpenes (C20) which inhibit root growth and germination of the native sedge, *Isolepis nodosa*. BVOCs are particularly toxic to germination and root growth, whereas shoot growth seems more resistant. Roots are also known to emit a variety of BVOCs.

Role of Microorganisms in the Rhizosphere Various microorganisms in the rhizosphere of the plant may modify the action of allelochemicals either by enhancing or minimizing phototoxicity of allelopathic donor toward allelopathic receiver by degradation or transformation of toxic allelochemicals or by modulation of the defense mechanism operative in the allelochemical receivers. These may induce expression of stress responsive genes. Microbes may also act by degradation of nontoxic glycoside allelochemicals to toxic allelochemicals in the rhizosphere.

32.7.2 Plant Physiological and Biochemical Processes Affected by Allelochemicals

Allelochemicals adversely affect the shape and structure of plant cells. Treatment with hordenine and gramine (allelochemicals from barley roots) causes disorganization of organelles, increase in size and number of vacuoles, and cell autophagy. Citral, a volatile essential oil from *Cymbopogon citrates*, causes microtubule disruption in the roots of wheat and *Arabidopsis*. Monoterpenoids, such as camphor, 1,8-cineole, β-pinene, and α-pinene, affect cell proliferation and DNA synthesis in plant meristems. Sorgoleone results in polyploid nuclei. Rye allelochemicals, benzoxazolinone (BOA) and 2, 4-dibydroxy-1,4(2H)-benzoxazin-2-one (DIBOA), significantly inhibit the regeneration of cucumber root cap cells, thus inhibiting root growth. Allelochemicals significantly inhibit the activity of antioxidant enzymes and increase free radical levels causing greater membrane lipid peroxidation and membrane potential alteration. Citral has been reported to damage the membrane system of *Echinochloa crus-galli* L. (barnyard grass), causing lipid peroxidation and electrolyte leakage.

Imbalance in the Antioxidant System Balance of the redox state in the cell is instrumental in allelopathic effects. Upon exposure to allelochemicals, the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and ascorbic acid peroxidase (APX) is altered to resist oxidative stress in the contact area of the recipient plants. Caffeic acid induces changes in the activities of proteases, PODs, and polyphenol oxidases (PPOs) during root development.

Growth Regulator Homeostasis Allelochemicals can cause alterations in the contents of plant growth regulators leading to their imbalances. This results in inhibition of growth and development in plants with respect to seed germination and seedling growth. Phenolic allelochemicals stimulate IAA oxidase activity and inhibit the reaction of POD with IAA and bound GA or IAA to influence endogenous hormonal levels. Salicylic acid has been reported to inhibit the synthesis of ethylene in cell suspension cultures of *Pyrus communis*.

Enzyme Activity Synthesis, functions, contents, and activities of various enzymes are affected by allelochemicals. Activity of enzyme **γ-phosphorylase,** involved in seed germination, might be inhibited by chlorogenic acid, caffeic acid, and catechol. Tannic acid suppresses the activity of POD (peroxidase), CAT (catalase), and cellulase. Phenolics can increase the activity of phenylalanine ammonia lyase (PAL) and β-glucosidase and reduce the activity of phenol-β-glucose transferase, thereby inhibiting root growth. The activity of protease, invertase, and succinic dehydrogenase (SDH) is also suppressed by allelochemicals.

Respiration Different stages of respiration, such as transport of electrons in mitochondria, oxidative phosphorylation, CO_2 generation, and ATP synthase activity, are affected by various allelochemicals. These can reduce oxygen intake which prevents NADH oxidation, thereby inhibiting the activity of ATP synthase, and reduces ATP formation, disturbs plant oxidative phosphorylation, and ultimately inhibits respiration. Sorgoleone blocks electron transport at the b-c_1 complex. Juglone has been implicated in the disruption of root oxygen uptake. α-pinene acts under at least two mechanisms: uncoupling of oxidative phosphorylation and inhibition of electron transfer. It results in strongly inhibiting mitochondrial ATP production, decreasing mitochondrial transmembrane potential and impairing mitochondrial energy metabolism.

Photosynthesis Impact of allelochemicals on plant photosynthesis mainly involves inhibition of or damage to the machinery involving acceleration of the decomposition of photosynthetic pigments. Consequently, photosynthetic pigment contents are decreased, which lowers energy and electron transfer, reduces activity of ATP synthase, and inhibits synthesis of ATP. Stomatal conductance is also affected inhibiting the overall photosynthetic process. Allelochemicals affect photosynthesis mainly by influencing the function of PS II. For example, sorgoleone inhibits the decay of variable fluorescence, blocks oxidation of the PSII and primary electron acceptor, Q_A^- by PSII secondary electron acceptor Q_B^- by displacing Q_B^- from the D_1 protein of PSII, thus inhibiting photochemical reaction. Essential oil from lemongrass is found to decrease ratio of chlorophyll a/b and carotenoid content adversely affecting photosynthetic metabolism.

Water and Nutrient Uptake Several allelochemicals affect absorption of nutrients and induce water stress through long-term inhibition of water utilization. Allelochemicals can inhibit the activities of Na^+/K^+-ATPase involved in absorption and transport of ions at the plasma membrane. Subsequently, it leads to suppression of cellular absorption of K^+, Na^+, and other ions. The effect of allelochemicals on ion uptake depends upon their concentration. Sorgoleone and juglone have been reported to inhibit H^+-ATPase activity and H^+-pump across root cell plasma membrane in *Pisum sativum* which affects the solute and water uptake.

Synthesis and Metabolism of Proteins and Nucleic Acids Many alkaloids exhibit allelopathic effects. They inhibit DNA polymerase I preventing transcription of DNA and translation process. Integrity of DNA and RNA is affected by all phenolics. Allelochemicals have been shown to inhibit transport and absorption of amino acid, thereby interfering protein biosynthesis adversely affecting the cell growth. Protein biosynthesis has been reported to be affected by ferulic acid and cinnamic acid as well as many phenols and alkaloids. The observed allelopathic phenomenon may be partly a result of interaction of the allelochemicals with target molecules, such as DNA, RNA, and amino acids.

Summary

- Plants need to adapt themselves to environmental stress including the biotic environment. Not all biotic interactions are harmful. These can be beneficial also, as in commensalism, mutualism, or symbiotic associations. Plants develop defense mechanisms to counteract the biotic stresses which include pathogens (disease-causing microorganisms), viruses, tissue-damaging herbivores, nematodes, and parasitic plants and against allele chemicals produced by other plants.
- Disease-causing microbes are known as pathogens. Once pathogen enters the plant, it employs three different strategies for survival, viz., biotrophic, necrotrophic, and hemibiotrophic. Pathogens which can invade the host but are unable to cause disease are known as avirulent, and those which drastically affect the host and cause disease are known as virulent. The process of infection, colonization, and reproduction of pathogen is known as pathogenesis. Pathogens penetrate the epidermal layer of the plant cell or enter through the wound or stomata. Penetration involves molecular interaction between cell surfaces of both host and pathogen. Pathogen secretes enzymes such as cellulases and hemicellulases that enable them to digest cellulose and other cell wall polysaccharides for their growth and multiplication. Most of the pathogens also secrete toxins in the host tissues. In most cases microorganisms induce hypersensitive response in the plant without causing any disease. Hypersensitive response is a localized response of plant cells to the microorganisms so as to prevent further invasion in adjacent cells. Different hypersensitive responses are ROS formation, changes in cell wall composition and membrane permeability, callose deposition, and production of phytoalexins or phenolics leading to programmed cell death.
- Plants have innate immunity which depends on the ability of plant to distinguish between self and nonself molecules. Plants possess a wide array of defense mechanism which is broadly classified into constitutive and induced. The constitutive defense mechanisms are always present whether the plant is attacked or not. These include the structural barriers (cell wall, epidermis layer, trichomes, thorns, etc.) and accumulation of chemical compounds (such as phenolics, nitrogen compounds, saponins, terpenoids, steroids, and glucosinolates) and proteins and enzymes with inherent antimicrobial properties. Inducible defense mechanisms are apparent only when infection is underway. These include basal defense mechanisms or/and R gene-mediated defense mechanisms which are also known as microbial-associated molecular-pattern-triggered immunity (MTI) or pattern-triggered immunity (PTI) and effector-induced resistance often referred as effector-triggered immunity (ETI). PTI is a first-level immunity response triggered by plant pattern recognition receptors (PRRs) that recognize the molecular pattern of the pathogen. PTI develops in response to the molecular patterns of the pathogen which are common among them, i.e., it is triggered in response to most of the pathogenic bacteria. The basal defense mechanism puts selective pressure on potential pathogens to produce effector molecules which interfere with basal

defense mechanisms, therefore further enabling the pathogen to attack the plant. This is referred as effector-triggered susceptibility (ETS). ETI acts at the second level and is the most successful means of controlling pathogens that are able to evade basal defense. ETI is the immune response of plant to specific pathogens and occurs in response to specific molecules secreted by the pathogenic bacteria which are known as effectors, product of avr gene of bacteria. In plants, complementary to avr genes is the R (resistance) gene which produces R proteins. If pathogen is able to produce effector molecules which are not recognized by R gene-mediated defense mechanism, it will be able to attack and exploit the plant for its use causing disease.

- Once a signal is perceived either through recognition of elicitors or effectors, it is transduced in the form of protein activation. Protein phosphorylation plays an important role during pathogen attack. At least two classes of protein kinases are activated: the mitogen-activated protein kinase (MAPKs) and calcium-dependent protein kinases (CDPKs). This leads to release of WRKY33 transcription factor, which binds to the W box (region in promoters of various target genes) and induces pathogenesis-related (PR) genes. SAR (systemic acquired resistance) and ISR (induced systemic resistance) are two types of resistance which prepare plants against subsequent pathogen infection. In SAR, defense proteins accumulate not only at the site of infection but also systemically in uninfected tissues and/or uninfected plants. SAR provides long-term defense against a broad spectrum of pathogens. Selected strains of plant growth-promoting rhizobacteria suppress the infection by disease-causing pathogens by providing systemic resistance by antagonism which is known as ISR.
- Plant defense against pathogen attack involves many signal transduction pathways that are mediated by a network of phytohormones. Three most important phytohormones in plant defense response against pathogens include salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Proteins, which are induced in the host plant after pathogens have attacked, are generally described as pathogenesis-related (PR) proteins. SA induces PR proteins by regulating the transcriptional activation of many PR genes. Many plant viruses encode proteins that act as suppressors of RNA silencing and hence allow the virus to replicate in the plant.
- The host plant perceives herbivore attack by recognizing DAMPs (mechanical damage) and HAEs (oral secretions). Oral secretions of insect herbivores contain fatty acid-amino acid conjugate (FACs) such as volicitin, inceptin, etc. that are capable of eliciting defense responses in plants. Plants employ direct as well as indirect strategies against herbivory. Direct defense responses affect the interaction of the herbivore and the host plant directly. Direct defense strategies include resistance factors such as morphological and structural features (thorns, spines and trichomes, tissue toughness, secretory structures such as laticifers and resins) as well as biochemical (e.g., toxic and anti-nutritive metabolites and proteins). Defense responses can be constitutive or inducible depending on their mode of expression, i.e., the defense traits may be preformed resistance factors or

deployed after attack by herbivores. Indirect defense responses of plants rely on attracting natural enemies of the herbivores. Plants produce odors that attract predators of herbivores guiding them to their prey. Biochemical defense responses include production of secondary metabolites that act in multiple ways, as toxins, as feeding deterrents, as digestibility reducers, and also as volatiles in indirect defense. Such secondary metabolites include phenolics, terpenoids, alkaloids, cyanogenic glucosides, and glucosinolates. Anti-nutritional proteins are also synthesized such as proteases, proteinase inhibitors, lectins, etc. Herbivore induced VOCs function as external signals within and between plants. The defense responses are not only restricted to the site of attack but also extend to distal sites. Signaling molecules such as cell wall-derived oligogalacturonides, systemin, jasmonic acid, as well as electrical signaling have been implicated in the systemic signaling process. Sytemin is an 18-amino acid long peptide derived from proteolytic cleavage of a larger precursor prosystemin and acts as a primary wound signal generated in response to wounding triggers the production of JA via octadecanoid pathway. The long-distance signaling is mediated by JA-Ile conjugate that serves as a phloem mobile signal and induces the expression of defense-related genes in distal parts of the plant. In response to plant defenses, herbivores counterattack by secretion of molecules called effectors that modulate host cell defenses.

- Parasitic plants are biotrophic pathogens which depend on host plants for the nutrients, water, and minerals. Some parasitic plants lose the capacity to photosynthesize and are dependent on the host plant completely (holoparasites), while others retain some chlorophyll and are able to photosynthesize but are dependent on the host plants for water and minerals (hemiparasites). Some of the parasites are obligate, while others are facultative. Some are stem parasites; others are root parasites. *Striga* is an example of hemiparasite, while *Cuscuta* is holoparasite. Stimulant for germination of seeds of *Striga* has been identified to be a strigolactone which is for released from the host plant which is indication for the nearby presence of the host. On reaching the host prehaustoria develops, followed by the adhesion phase because of secretion of sticky substances such as arabinogalactones by the host plant. This is followed by development of haustoria from prehaustoria in response to haustoria induction factor (HIF) which is formed due to conversion of a compound from the host by the enzyme released by the parasitic plant. A quinone, BMBQ, has been identified which is responsible for development of haustorium inside the host tissues until a connection is established with the vascular tissues of the host plant.
- Allelopathic interactions include both beneficial and harmful interaction among plants through release of allelochemicals into the environment which affect various physiological and biochemical processes in plants. Mode of allelochemical action takes place either through release of soluble secretions in the form of root exudates in to the rhizosphere or via biogenic organic compounds.

Multiple-Choice Questions

1. Interaction among plants, herbivores, and their natural enemies is known as:
   (a) Biotrophic interactions
   (b) Pathogenic interaction
   (c) Tritrophic interactions
   (d) Allelopathic interactions
2. A non-protein amino acid which has a role in defense is:
   (a) Homoserine
   (b) Canavanine
   (c) Citrulline
   (d) Ornithine
3. Systemin is an example of:
   (a) Pattern-associated molecular pattern
   (b) Damage-associated molecular pattern
   (c) An effector molecule
   (d) A molecule which is translocated away from the damaged site of the plant
4. Elicitor refers to the molecules:
   (a) Produced by the plant in response to pathogenic infection
   (b) Produced by the pathogen at the time of infection
   (c) Present on the surface of plant cells by which pathogens are recognized
   (d) By which plant is able to identify "self" from "nonself"
5. HAMPs are:
   (a) Components from the plant feed which are modified in the guts of the insects
   (b) Molecules produced in plants in response to the damage caused by herbivores
   (c) Molecular pattern on the plant surface recognized by the insects
   (d) Compounds in herbivores identified by the plant
6. An example of HAMP is:
   (a) Linoleic acid
   (b) Volicitin
   (c) Glutamine
   (d) Caeliferins
7. Holoparasites are the plants which:
   (a) Contain chlorophyll and have little photosynthetic capacity
   (b) Have lost their capacity to complete their life cycle without the host
   (c) Get complete nourishment from the host plant
   (d) Are dependent on the host plant for the supply of water and minerals
8. Strigol is a (tick mark which is not true):
   (a) Compound which inhibits germination of the seeds of *Striga* sp
   (b) Stimulant for the germination of cotton seeds
   (c) Secondary metabolite released from the roots of host plant
   (d) Sesquiterpene released from the roots of cotton plant

9. Sticky substance produced by the host plant when induced by *Cuscuta* include (tick mark which is not correct):
   (a) Arabinogalactan proteins
   (b) Polysaccharides
   (c) Parahydroxyacids
   (d) Pectins
10. Parasitic plants have:
   (a) Lower water use efficiency than the host plants
   (b) Higher water use efficiency than the host plants
   (c) Lower transpiration rates than the host plants
   (d) Maintain high temperature around their leaves
11. Catechin, a toxic secondary metabolite, is produced by:
   (a) *Eichhornia crassipes*
   (b) *Alliaria petiolata*
   (c) *Centaurea maculosa*
   (d) *Juglans nigra*
12. An interaction when both partners of the association are benefitted is known as:
   (a) Commensalism
   (b) Mutualism
   (c) Amensalism
   (d) Parasitism
13. Necrotrophy is the pathogenic infection in plants when the cells:
   (a) Are killed prior to infection
   (b) Remain alive throughout infection
   (c) Are immediately killed after the infection
   (d) Are killed much later on after the infection
14. An effector molecule is:
   (a) Produced by pathogens which triggers basal defense in plants
   (b) Present on the surface of pathogen by which it is identified
   (c) A molecule made by pathogen to overcome basal defense of the plant
   (d) Produced by the plant in response to pathogenic infection
15. Effector-triggered immunity refers to:
   (a) Defense strategies by host plant in response to the molecular pattern at the pathogen's surface
   (b) Suppression of host-triggered immunity by the effector molecules produced by pathogens
   (c) Triggering of defense response in plant as a result of incompatible interaction of factors produced by pathogens and R gene products
   (d) Immunity in host plants in response to elicitors
16. Fusicoccin is a non-specific toxin which:
   (a) Inhibits histone acetylase thus affecting gene expression involved in defense against pathogen
   (b) Mimics action of jasmonic acid isoleucine and helps in bacterial infection by activating JA signaling pathway
   (c) Acts by damaging plant cell walls
   (d) Inactivates membrane-bound H+-ATPase proton pump

17. Toxicity of tabtoxin (a dipeptide) is due to:
    (a) Release of its hydrolysis product an amino acid threonine
    (b) Release of tabtoximine as a result of its hydrolysis, a potent inhibitor of glutamine synthetase
    (c) Due to pores created in the membrane resulting in leakage of nutrients
    (d) Irreversible opening of stomata which leads to wilting of plants
18. Effector molecules are:
    (a) Proteins required to develop Type III system in bacteria
    (b) Products of R genes in host plants
    (c) Molecules produced by pathogens that trigger infection of the host plant
    (d) Products of avr genes of pathogens
19. Systemic acquired resistance is:
    (a) Due to accumulation of defense proteins not only at the site of infection but also systemically in uninfected tissues and/or uninfected plants
    (b) Due to suppression of infection by disease-causing pathogens by providing systemic resistance by antagonism
    (c) Resistance in the host plant at the infected tissues by pathogens
    (d) Very specific and long lasting

Answers

1. c	2. b	3. b	4. d	5. a	6. b	7. b
8. d	9. c	10. a	11. c	12. b	13. a	14. c
15.c	16. d	17. b	18. d	19. a		

Suggested Further Readings

Alba JM, Allmann S, Glas JJ, Schimmel CJ, Spyropoulou EA, Stoops MV, Kant MR (2012) Induction and suppression of herbivore-induced indirect defenses. In: Witzany G, Baluska F (eds) Biocommunication of plants. Springer, New York, pp 197–212

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