Introduction

Natural defense mechanism of plants

Plants are primary producers and they are able to convert the energy of sunlight into chemical energy such as carbohydrates, proteins, and lipids. These characteristics are extremely important because, directly or indirectly, they provide most of the energy necessary for other organisms to live. Archaeological evidence indicates that around 10,000 years ago humans started to practice agriculture, and since then, and probably before, plant diseases started to be a human concern. From the germination of their seed, plants grow in one place and are therefore unable to escape unfavorable environmental conditions. Any circumstance that affects normal growth and development can be described as disease. Diseased plants may be toxic for humans and animals, and they reduce food quality and production. The causes of disease are many and can be divided into biotic and abiotic stresses. The latter originates from soil (e.g. nutrient supply), climate (e.g. temperature) and pollution (e.g. ozone), while biotic stresses are inflicted by parasitic organisms that live off the plant. Through evolution viruses, microbes and animals have developed mechanisms to access plant resources. If this becomes detrimental to the plant, such organisms are known as pathogens.

Until now, the control of plant diseases has been dependent on pesticide application to protect economically important food crops against disease and herbivore destruction. The application of these toxic compounds is harmful for the environment. The negative impact of these chemicals on human health and the natural environment has led to a public outcry against the use of chemical fungicide and pesticide resulting in a demand for more environmentally friendly and even organically grown food crops. Legislation, design around consumer concerns have forced farmers to use lower concentration of pesticide. These together with the emergence of fungicide resistant plant pathogen (Hayashi et al., 2002), leaves the farmer with a limited and sometimes ineffective means of protecting our valuable food resource. Over the last two decades scientists have been searching for replacement to chemical fungicide that would lessen the impact on human health and the environment. These strategies included the identification of biological control agents (Elad, 1996), breeding for new resistant cultivars and the use of genetic engineering to design more resistant crop species with similar or enhanced nutritional
value (Sawada et al., 2004). Some of these strategies have proven successful in combating certain insect pests. Genetic engineering of various crops with BT toxin from *Bacillus thuringiensis* has led to enhanced protection against some worm and insects pests (Akhurst et al., 2003). This toxin is very specific due to its narrow spectrum of activity. When it comes to designing new strategies for protecting crop against microbial pathogens scientist have however been less successful. Therefore, other environmentally less harmful approaches for controlling plant diseases and improving food quality and production are needed. At this moment, conventional breeding and genetic engineering for disease resistance seem to be good strategies for approaching these problems. As a part of developing better strategies for controlling plant diseases, a good understanding of the plant-pathogen interactions at the molecular level is needed. The new strategies for the protection of crops against microbes are centered around the utilization of natural defense system against these pathogen that are already present in plant and other living organisms.

Plants have been exposed to adverse environmental conditions, including biotic stresses, since their origins. Biotic stresses include fungi, bacteria, and viruses, which use different types of molecular mechanisms as virulence strategies to infect plants and eventually cause diseases. Because of these stresses, plants induce defense responses to protect themselves from the infecting microbes. Therefore, plant defense and microbe virulence strategies have a long history of constant co-evolution. Plants, in turn, have evolved strategies to counter pathogen attack and these are expressed both as preformed barriers and induced defences. Such a multilayered defence system confers effective resistance on the majority of plants to most pathogens and their interaction is known as ‘incompatible’. Once a pathogen has evolved the capability to overcome plant defences, the interaction is ‘compatible’. Incompatibility (resistance) and compatibility (disease) are not absolutes but more of a continuum that arises from pathogen and plant diversification in an evolutionary ‘arms race’ of attack and counter-attack. Plant inducible defences can be expressed by individual cells and, as such, are comparable to the innate immune response of animals. Indeed, some of the underlying principles of animal and plant innate immunity are the same and in both systems the rate and amplitude of transcriptional activation of defense genes appear to be decisive in controlling the extent of local and systemic immune responses. Plants are constantly challenged by pathogens, but the onset of plant disease is the exception
rather than rule, the reason for this being that the constant arms raised between pest and plant host has lead to the development of an efficient plant defense system. The interaction of the pathogen with a plant can lead to one of two responses. Plant defense to pathogens results from a complex combination of structural plant characteristics and induced biochemical reactions. The structural characteristics, including the cuticle and the cell wall, constitute a preformed plant defense that acts as a physical barrier to prevent the entrance and spreading of pathogens throughout the plant. In addition to this constitutive defense, plants may perceive directly or indirectly the presence of a pathogen and subsequently induce plant defense responses. These inducible plant defense responses include the synthesis of signals such as salicylic acid, ethylene and jasmonates that regulate gene expression, and the production of defense molecules such as reactive oxygen species, phenylpropanoids, phytoalexins and pathogenesis-related proteins. All these inducible biochemical reactions tend to create protective physiological conditions to limit pathogen growth and invasion in the host tissues. The final result of the host-pathogen interaction, plant disease (compatibility), or plant resistance (incompatibility), depends on the combination of a number of different variables. These variables include the genetic characteristics and physiological state of both the plant and the pathogen, and several environmental conditions including light, temperature, and humidity, among others.

In compatible interaction the pathogen evades the plant defense system and establishes disease, while in an incompatible interaction the plant recognizes the pathogen and elucidates a defense response through various signal cascades. The general plant defense system can be broadly divided into a preformed defense system and an induce defense system. The preformed defense mechanism is a major component of the host defense system and may play an important role in determining the host range of certain plant pathogenic fungi (Morrissey and Osbourn, 1999). The preformed defense is the first line of defense against pathogen infection and consists of preformed biochemical and proteinaceous molecules with antimicrobial activity (Broekaert et al., 1995). These antimicrobial molecules form protective barriers which prevents the onset or spread of pathogen infection. These antimicrobial barriers are usually present in the outer cell layer of plant organs, which represent the first plant cells to elicit a defense response upon pathogen infection (Osbourn, 1996). The most noticeable preformed defense barrier are present in roots and seeds where they protect
the plant material from the pathogen rich soil environment (Osbourn, 1996; Terras et al., 1995). The induced defense system relies upon recognition of the plant pathogen and elucidation of defense mechanisms activated by a signaling cascade. Early events in the induced defense usually involved hyper sensitive cell death and activation of the structural defense (Kiraly et al., 2006; Greenberg and Yao, 2004). The structural defense involved alteration of the plant cell wall and its structural sub components upon pathogen recognition. Either to prevent establishment of disease or to prevent further spread of disease the structure defense usually involved the strengthening and thickening of the plant cell wall through the deposition of lignin. These natural plant defense systems have been refined over millennia in the constant battle for supremacy between the pathogen and host. In general the plant defense system can be divided in to the constitutive or performed and induce defense system these defense system, can be further divided in to structural chemical and biochemical defense mechanism (Da Cunha et al., 2007). The structural defense components such as the cuticle and plant cell wall form the first barrier of defense in what is perceived as the passive defense system (Ferreira et al., 2007). The preformed defense system contains both chemical and biochemical compounds that accumulate at basal level within peripheral plant tissue to form microbial barriers and are most prevalent in nutrient rich plant organs such as flowers and seeds (Osbourn, 1996). One of the most important mechanisms of plant defense is the production of proteinaceous compounds with antimicrobials activity in response to pathogen attack (Koga et al., 2006). These compounds can range from fungal cell wall degrading enzymes to small antimicrobial peptides. In plant fungal interaction, most of these proteinaceous compounds have traditionally been classified in to two groups, namely pathogenesis related proteins (PR-proteins) and antifungal defense related proteins (Van loon et al., 2006).

**Perception of the pathogen**

To infect a plant, pathogens must be able to evade or overcome the constitutive plant defense formed by physical barriers such as for example the cuticle and the cell wall. Different pathogens use different strategies to face this constitutive plant defense and gain entrance into plant tissues. Some of the pathogens are specific for one plant variety while others affect a wide range of plants. Irrespective of the type of pathogen, plants might be able to perceive its presence and trigger a defense response. Plants achieve the perception of a pathogen through molecules of a diverse
nature called elicitors, which originate from the pathogen or from the plant, and are able to trigger plant defense responses. In some cases, the recognition of the pathogen by the plant is cultivar specific, as in the gene-for-gene type of interactions, while in other cases the plant non specifically recognizes the presence of the pathogen by general elicitors.

**Gene-for-gene interactions**

The gene-for-gene type of plant-pathogen interactions involve the specific plant recognition of a protein encoded by a dominant avirulence gene \((avr)\) from the pathogen, via a complementary interacting protein encoded by a dominant plant resistance gene \((R)\) (Flor, 1971). When one of the \(avr\) or the complementary \(R\) genes is not expressed, plant recognition does not occur, the plant-pathogen interaction is compatible and disease may be the result. When both interacting members, Avr and the complementary R proteins, are present, recognition occurs resulting in an incompatible interaction where plant resistance is the result. The hypersensitive response (HR) is a typical plant defense response associated with resistance in these incompatible plant-pathogen interactions, and is characterized by the local death of plant cells around the site of infection, which is thought to restrain pathogen growth and spreading (Hammond-Kosack and Jones 1996). Several plant \(R\) genes and the corresponding \(avr\) genes from the pathogen have been identified from many plant-pathogen interactions. Based on the structural domains that different \(R\) protein sequences exhibit, they have been classified into different groups. Most of the \(R\) genes encode proteins containing leucine rich repeats (LRR) that are thought to mediate protein-protein interactions (Takken and Joosten, 2000). In addition to the LRR regions, some \(R\) proteins contain nucleotide-binding sites (NBS) and they are classified as NBS-LRR. The NBS-LRR proteins are further subdivided according to the characteristics of their amino-terminal sequences, featuring either a coiled coil (CC) or a Toll-Interleukin receptor (TIR) homology domain (Bent, 1996; Takken and Joosten, 2000). Furthermore, some \(R\) genes contain kinase domains and some of these, as for example Xa21, are trans membrane receptor kinases (Song et al., 1995). In contrast to the \(R\) proteins, most of the \(Avr\) proteins show little or no homology to each other (Nimchuck et al., 2001; Bonas and Lahaye, 2002). However, some regions such as myristoylation encoding sites are present in several \(Avr\) sequences and they may be required for the proper localization and function of the \(Avr\) protein (Nimchuk
et al., 2000; Shan et al., 2000). There is evidence that Avr proteins play a role in pathogen virulence on susceptible hosts (Kjemtrup et al., 2000; White et al., 2000). For example, AvrPto enhances the capacity of *P. syringae pv. tomato* to induce necrosis in tomato plants lacking the R gene Pto, such a necrosis is correlated with an increase in bacterial growth (Chang et al., 2000). The simplest model for gene-for-gene interaction is where R proteins are receptors and Avr proteins are ligands. However, in some cases there is evidence that R proteins are not the primary receptor for the corresponding Avr protein. For example, the Ry-mediated resistance response in potato requires the intact active site of the protease elicitor NIa from potato virus Y (Mestre et al., 2000). Therefore, in this case the R protein may mediate the recognition of the product released by the Avr protein. Based on recent biochemical data, different models for R-Avr interactions have been hypothesized (Bonas and Lahaye, 2002).

**General elicitors**

In addition to the specific pathogen-derived Avr elicitors, other types of general elicitors are generated during plant-pathogen interactions. These elicitors are called general because they occur in a number of different plant-pathogen interactions. These general elicitors are molecules of a diverse nature including oligosaccharides, lipopolysacharides, glycopeptides and peptides, derived either from the plant or the pathogen. All these general elicitors are somehow perceived by the plant cells and trigger several plant defense responses (Dow et al., 2000). Oligosaccharide elicitors were among the earliest elicitors characterized in detail (Darvill and Albersheim, 1984). These general elicitors are able to trigger several plant defense responses including oxidative burst (Apostol et al., 1989), induction of defense signal molecules such as jasmonic acid (JA) (Doares et al., 1995a) and ethylene, accumulation of phytoalexins and induction of pathogenesis-related genes (Darvill, 1992). Oligosaccharides, may originate from pathogen or plant structures. For example, hepta-β-glucoside, oligochitin and oligochitosan derived from pathogens while oligogalacturonides derived from plants (Ebel, 1998). Plant oligogalacturonides (OGAs) may be released by the action of cell wall degrading enzymes derived from the invading pathogen, which may generate OGAs with different degrees of polymerization. The size range of OGAs that activates plant defense responses is narrow. For example, OGAs with a degree of polymerization between 10 and 15 are
generally required to elicit most of the plant defense responses mentioned above (Darvill, 1992). However, OGAs with a degree of polymerization as short as 2 are also able to trigger induction of plant defense-related genes such as proteinase inhibitor and allene oxide synthase (Bishop et al., 1984; Norman et al., 1999). The perception of OGAs and other general elicitors most probably involve direct or indirect participation of membrane receptors (Hahn, 1996).

Receptors

Plant cell-surface receptors are key components that perceive extracellular stimuli from the environment. They are vital for an appropriate physiological role of the cells as functional units of the tissues and the whole organism. During the last decade, several types of receptors have been identified in plants. According to their structural characteristics, they have been classified into different categories including receptor-like protein kinases (RLKs), histidine kinase receptors, and receptors with different numbers of transmembrane domains (Walker, 1994; Grignon, 1999). RLKs are characterized by an extracellular domain probably involved in signal perception, a transmembrane domain, and a cytoplasmic kinase domain, which may initiate a signal transduction cascade into the cell. All plant RLKs identified are serine-threonine kinases and based on the structural characteristics of the extracellular domain they have been divided into different categories (Satterlee and Sussman, 1998, Walker, 1994). Several members of the RLK family contain a different number of leucine-rich repeats (LRR) in their extracellular domain. The LRR regions often participate in protein-protein interactions (Kobe and Deisenhofer, 1994) and hence, they may be involved in the binding of the protein ligands. For example, FLS2 an LRR-RLK from Arabidopsis (Gomez-Gomez and Boller, 2000), interacts with flagellin, a peptide component of the flagella of Gram-negative bacteria, and recently the MAP kinase signaling pathway and transcription factors activated by this receptor have been identified (Asai et al., 2002). CLAVATA1 represents another example of this class of RLK with a putative polypeptide ligand CLAVATA3 (Trotochaud et al., 2000). However, other regions of the extracellular domain of LRRRLKs, which are not LRR, might mediate binding to the ligand. The LRR-RLK, BRI1, perceives the signal of the steroid hormone brassinolide and a 70 amino acid island region, interrupting LRR regions of the extracellular domain, is required for brassinolide binding to the receptor (Wang et al., 2001). Another class of RLKs contains lectin-like motifs within their
extracellular domain. By their similarity to lectin proteins, these lectin binding motifs of RLKs are thought to bind sugars. For example, the *Ath.LecRK1* receptor (Hervé et al., 1996) from *Arabidopsis* and the SRK receptor from *Brassica oleracea* (Stein et al., 1991) contain a lectin-like motif (Shiu and Bleecker, 2001). A putative polypeptide ligand for SRK, *SCR*, has been identified (Schopfer et al., 1999); however, data about the extracellular domains interacting with the ligand remain elusive. Other members of the RLK family exhibit similarity to different types of plant and animal proteins. For example, the extracellular domain of the WAK (wall associated kinase) receptors from *Arabidopsis* contains epidermal growth factor-like (EGF) repeats (He et al., 1999) and CRINKLY4 from maize exhibit similarity to the ligand binding domain of the mammalian tumor necrosis factor receptors (TNFR) (Becraft et al., 1996). Interestingly, other RLKs contain extracellular domains with similarity to pathogenesis-related (PR) proteins from plants. Examples of this class are PR5K from *Arabidopsis* with similarity to PR5 (thauamatin) (Wang et al., 1996) and CHRK1 from tobacco with similarity to chitinase (Kim et al., 2000). On the other hand, a number of RLKs do not exhibit homology to a particular established sequence motif. Another classification for the different RLKs is through the location of cysteines in the extracellular domain. Cysteines may be involved in formation of disulfide bonds, which may determine the folding and final structure of polypeptides. In animals, the structural relationship of several families of growth factors has become evident through the analysis of their crystallized molecules and a common cystine knot structure has been described (Sun and Davies, 1995). In plants, different RLKs contain cysteine patterns in their extracellular domains (Kohorn et al., 1992; Walker, 1994), and recently a superfamily including a number of RLKs and other proteins with C-rich repeats has been described (Chen, 2001). For example, several RLKs classified into an S domain class (with the extracellular domain similar to the *Brassicaceae* S-locus glycoproteins) contain an array of cysteine residues in combination with other conserved motifs (Walker, 1994). The extensive diversity of plant RLKs and the large number of them present in the *Arabidopsis* genome suggest that RLKs may be involved in the perception of a wide range of stimuli including those occurring during plant-pathogen interactions. Indeed, some RLKs have been identified as R genes. For example, Xa21 (LRR-RLK) from *Oryza sativa* (rice) that confers resistance to *Xanthomonas oryzae* pv. *oryzae* (Song et al., 1995) and LRK10
from *Triticum aestivum* (wheat) that confers resistance to wheat rust fungi *Puccinia recondite* (Feuillet et al., 1997). In addition, several other RLKs have been associated with plant defense responses to pathogens. In these cases, the association is usually based on the expression pattern that the RLKs exhibit in plants treated with pathogens, elicitors, or signal molecules related to plant defense responses. For example, RLK3 from *Arabidopsis* is induced by oxidative stress, salicylic acid and pathogen attack (Czernic et al., 1999) and SFR1 from *Brassica oleracea* is induced by wounding and bacterial infection (Pastuglia et al., 1997). In addition to the RLKs, several high affinity binding sites for general elicitors including oligosaccharides, glycopeptides and peptides have been identified through biochemical approaches (Nürnberger et al., 1994). However, little functional data about these putative receptors are available. For example, similar 75 kDa proteins associated with the plasma membrane of *Glycine max* (soybean) and *Phaseolus vulgaris* (French bean) cells, bind β-glucan elicitors from *Phytophthora* species with high affinity (Umemoto et al., 1997; Mithöfer et al., 2000). However, none of these proteins exhibit any known functional domain that might be involved in signal transduction, suggesting that these β-glucan binding proteins may interact with other components to transduce the elicitor signal (Mithöfer et al., 2000).

**Early events of plant defense**

Following pathogen or elicitor recognition, a series of cytological changes and biochemical responses have been identified in plant cells. The cytological changes include papilla formation, increased cytoplasmic streaming and nuclear migration, which are associated with depolymerization of microtubules and microfilaments (Kombrink and Schmelzer, 2001). The biochemical responses include changes in the H+, K+, Cl-, and Ca2+ fluxes across the plasma membrane, and the formation of reactive oxygen species (ROS) that occur within 2-5 minutes after elicitor treatment (Low and Merida, 1996). Some of these biochemical reactions have been associated with the transduction of signals that lead to defense responses. For example, Ca2+ fluxes have been associated with the induction of phytoalexin accumulation (Dixon et al., 1994). Protein phosphorylation/dephosphorylation is another early event that follows pathogen recognition and is involved in signal transduction cascades that trigger plant defense responses (Dixon et al., 1994). The identification of several MAP kinases and kinase receptors associated with defense responses highlight the
relevance of the phosphorylation/dephosphorylation processes during plant defense signaling. Recently, all the components of the kinase-signaling cascade associated with the recognition of flagellin, a peptide of bacterial flagella, have been identified (Asai et al., 2002).

**Local and systemic defense responses**

Local defense responses to pathogens or elicitors involve the regulation of several genes, which contribute to generate protective physiological conditions against the invading pathogens. At the site of infection these responses include the generation of ROS (Wojstaszek, 1997), the synthesis of proteins involved in the production of signals such as salicylic acid (SA), jasmonates, and ethylene (Creelman and Mullet, 1995) as well as enzymes related to the phenylpropanoid metabolism (Hahlbrock and Scheel, 1989; Dixon and Paiva, 1995) and the biosynthesis of phytoalexins (Smith, 1996; Hammerschmidt, 1999), and pathogenesis-related (PR) proteins (Linthorst, 1991). Eventually, cells at the site of infection could undergo programmed cell death that often becomes visible as a hypersensitive response (HR) (Greenberg et al., 1994; Dangl et al., 1996). A local infection often leads to the induction of similar defense responses in uninfecte d systemic plant tissues that result in broad-spectrum disease resistance to subsequent infections (Kuc, 1982). Additionally, systemic resistance can also be induced by wounding (Schewizer et al., 1998) and non-pathogenic rhizobacteria (van Loon et al., 1998). Furthermore, the application of an extensive amount of natural and synthetic compounds including SA, jasmonates, ethylene, 2,6-dichloroisonicotinic acid (INA) induce similar defense responses leading to a subsequent plant resistance (Oostendorp et al., 2001). The different systemic defense responses associated with pathogen infections include the induction of several PR genes, accumulation of phytoalexins, induction of ROS and micro-HR (Kuc, 2001). Interestingly, the systemic resistance to virulent pathogens generated by previous treatment of a plant with biotrophic bacteria (*Pseudomonas fluorescens*) is not associated with induction of PR genes in systemic tissues (Pieterse et al., 1996). Different signal molecules originated at the local sites of infection are responsible for the systemic responses. The systemic responses induced depend on the pathogen and signal pathways triggered by this pathogen. For example, the systemic acquired resistance (SAR) has been associated with the signal molecule SA, which in turn is required for the induction of SAR genes associated with resistance (Mauch-
Mani and Métraux, 1998). However, other systemic responses resulting in resistance are SA-independent (Penninckx et al., 1996). In addition, an extensive amount of emerging evidence suggest that the induction of plant disease resistance results from a complex signaling network involving cross-talk between different pathways (Feys and Parker, 2000).

**The wound response**

Wound stress and pathogen attack are closely related. Pathogens may gain entrance into plant tissues through wounds produced by mechanical stress and pest injury, e.g. wind, rain, feeding insects, etc. Some of the plant defense responses induced by pathogens are similar to those produced by wounding. For example, defense responses to wound damage include generation of ROS, and induction of signals such as ethylene, and JA and derivatives such as methyl jasmonate (MeJA) (Bowles, 1998). These similarities may reflect the fact that common elicitors such as plant oligogalacturonides are generated during tissue damage produced either by wound or pathogens. Recently, an overlap in pathogen-specific resistance and wound response gene expression profiles has recently been shown (Durrant et al., 2000). The best characterized event during the wound response is the accumulation of proteinase inhibitor (PIN) in both local and systemic leaves. The induction of wound responses has been associated with several molecules including, oligogalacturonides (OGAs), systemin, abscisic acid, jasmonates, and electrical pulses (León et al., 2001). Oligogalacturonides have been shown to induce local and systemic expression of PIN and they are thought to be formed during leaf wounding. However, although oligogalacturonides may be responsible for the induction of PIN at the local level, it is unlikely that they could be the signal traveling from local to systemic leaves (Bowles, 1991). In contrast, there is evidence that systemin, a wound inducible 18-amino-acid oligopeptide inducing PIN locally and systemically, moves from local wounded leaves to systemic leaves (Ryan and Pearce, 1998). Electric pulses have also been suggested to be a mobile signal responsible for the systemic induction of wound responses (Wildom et al., 1992). However, it may be possible that both local and systemic wound responses result from a complex regulatory network that involves several components (León et al., 2001).
Reactive oxygen species

The production of reactive oxygen species (ROS) including hydrogen peroxide (H$_2$O$_2$), superoxide (O$_2^-$), hydroxyl radical (·OH) and singlet oxygen (O') normally occurs in the metabolism of plant cells. They are usually generated by the electron transport activities of chloroplasts, mitochondria and by enzymes in other cell compartments and the apoplast involved in reduction-oxidation processes of the plant cell (Mehdy, 1994). The hydroxyl radical is believed to be the most reactive species among the ROS mentioned, especially for its ability to initiate radical chain reactions responsible for the irreversible modifications of macromolecules and damage to cell organelles. At the other end, hydrogen peroxide is a relatively stable ROS and it is able to diffuse across cell membranes and reach cell locations remote from the site of its generation (Wojtaszek, 1997). ROS exhibit a dual function in the normal metabolism of plant cells. On one hand, they are toxic compounds that damage plant cells; on the other hand, they may be beneficial as for example, in the lignin formation in cell walls (Foyer et al., 1994). To control the action of ROS, plant cells contain several enzymatic and nonenzymatic scavenging systems in all subcellular compartments and the apoplastic space. The enzymatic antioxidative systems include enzymes such as catalases, superoxide dismutases, peroxidases, and the enzymes involved in the glutathione-ascorbate cycle. The nonenzymatic antioxidative metabolites include ascorbate, glutathione, flavonoids, carotenoids, and α-tocopherol (Polle, 1997). In plant cells under normal physiological conditions these systems provide sufficient protection against ROS. However, the increased generation of ROS induced by external stimuli, may overcome these systems and produce oxidative stress (Foyer, 1994). In host-pathogen interactions, accumulation of ROS has been associated with local defense responses (Levine, 1994). In some type of plant-pathogen interactions two phases of oxidative burst have been observed (Fig.1.1). The first phase occurs within the first hour after elicitor treatment of the plant cells and the second phase starts approximately after 4 hours of treatment and continues for several hours (Lamb and Dixon, 1997). In addition, accumulation of ROS has also been reported in systemic tissues of plants responding to pathogen infection (Alvarez et al., 1998). The most studied sources of ROS formation during plant pathogen-interactions are the NADPH oxidase complex in the plasma membrane and the generation of hydrogen peroxide by cell-wall peroxidases (Grant and Loake, 2000). The production
of ROS during plant defense responses to pathogens is associated with both local and systemic HR (micro-HR) (Kombrink and Schmelzer, 2001). Furthermore, the oxidative burst has been proposed as a prime candidate for triggering HR (Greenberg, 1997). Other plant defense responses associated with ROS include a direct antimicrobial activity, the crosslinking of plant cell-wall proteins, and the induction of defense related genes (Baker et al., 1997).

**Defense signals**

**Salicylic acid**

Salicylic acid (SA) is a phenolic compound that participates in several physiological processes of plant cells including defense responses to pathogens. The biosynthesis of SA is initially derived from the shikimate pathway. A number of studies carried out in several plants including tobacco, potato and *Arabidopsis* indicate that SA is synthesized from phenylalanine via cinnamic acid and benzoic acid, and that SA forms different conjugated products (Lee et al., 1995; Shulaev et al., 1997). However, recent studies in *Arabidopsis* have shown that SA is synthesized from chorismate via isochorismate and that SA made by this pathway is required for local and systemic defense responses against pathogens (Wildermuth et al., 2001). These proposed cascades together with the question marks that they still have and the complexity of the phenylpropanoid pathway, probably reflect the fact that SA may be generated from alternative pathways. Several pieces of data indicate that SA plays critical roles in plant defense responses. During plant defense responses to some pathogens an increase in SA levels has been observed in local and systemic tissues. For example, in tobacco plants treated with tobacco mosaic virus (TMV), SA increases 20 fold in locally treated leaves and 5-10 fold in systemic tissues (Malamy et al., 1990; Yalpani et al., 1991). The increase of SA during plant defense responses is correlated with the induction of PR proteins and resistance, and the exogenous application of SA or functional analogs of SA to plants induce the synthesis of PR proteins and resistance (White 1979; Ward et al., 1991). These lines of evidence suggest that SA plays a critical role during plant defense responses in local and systemic tissues. SA was originally proposed to be the mobile signal for systemic acquired resistance (SAR) (Malamy et al., 1990). Several studies supported this hypothesis. For example, transgenic plants expressing the *nahG* gene, encoding a salicylate hydroxylase that degrades SA to catechol, exhibit enhanced susceptibility to virulent pathogens and
were unable to develop SAR (Delaney et al., 1994). In addition, SA has been shown to be transported to uninfected leaves in tobacco and cucumber (Shulaev et al., 1995). However, grafting experiments using tobacco plants expressing napG or reduced levels of phenylalanine ammonia-lyase (PAL), and wild-type plants, suggested that other primary systemic signals are involved in the onset of SAR (Pallas et al., 1996). Scions of chimeric tobacco plants exhibited SAR after TMV treatment of napG rootstock leaves (Vernooij et al., 1994). Similarly, wild-type scions of chimeric tobacco developed SAR after TMV inoculation of PAL-suppressed rootstock leaves (Pallas et al., 1996). Because, PAL-suppressed scions did not exhibit SAR after TMV inoculation of wild type rootstock leaves, and SA only partially restored the SAR in PAL-suppressed plants, the authors suggested that, in addition to increased levels of SA the presence of other phenylpropanoid products may be important for SAR induction (Pallas et al., 1996). There is not a clear picture about the mechanisms of action by which SA induce PR genes and resistance. However, several studies suggest that SA action during plant defense responses may involve the inhibition of catalase and ascorbate peroxidase, which link SA action with the action of ROS. The identification of Arabidopsis mutants affected in SA-mediated responses is contributing in clarifying the mechanism of action of SA during plant defense responses (Cameron, 2000). For example, NPR1 encodes a protein with ankyrin repeats that function downstream SA in the SAR response and interacts with transcription factors that bind to promoter sequences required for SA-inducible PR gene expression (Zhang et al., 1999).

**Ethylene**

Ethylene is a gaseous signal molecule that regulates numerous processes in plants including normal growth and development, and defense responses to biotic and abiotic stresses (Bleecker and Kende, 2000). Ethylene is synthesized from methionine via S adenosyl-L-methionine (AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC). The conversion of AdoMet to ACC is catalyzed by ACC synthase, and ACC oxidase catalyzes the conversion of ACC to ethylene. Ethylene exhibits the capacity to stimulate its own synthesis via a positive feedback loop, and for many biological responses it is effective at nanomolar concentrations (Bleecker and Kende, 2000). ACC synthase is the key enzyme responsible for the regulation of ethylene production and it is encoded by a multigene family. The expression of ACC genes is
differentially regulated by diverse stimuli (Zarembinski and Theologis, 1994). Extensive data indicate that ethylene plays important roles in plant defense responses. The levels of ethylene have been shown to increase upon wounding, pathogen infection or treatment with elicitors of defense responses (Enyedi et al., 1992; O’Donnell et al., 1996). In addition, ethylene regulates the expression of several genes including those participating in plant defense responses to biotic stresses. For example, PR proteins such as glucanase and chitinase are induced by ethylene (Deikman, 1997). Ethylene perception and signal transduction pathway have been characterized in *Arabidopsis* through the studies of several mutants (Fig. 4). Ethylene is perceived by histidine-kinase receptors. ETR1, ETR2, ERS1, ERS2 and EIN4 constitute a family of ethylene receptors, and mutations of these genes produce plants insensitive to applied ethylene (Johnson and Ecker, 1998). CTR1 is protein kinase acting downstream of the ethylene receptors as a negative regulator of the ethylene response (Kieber et al., 1993). The *EIN2* gene encodes a 12-membrane-pass, metal-ion-transporter (Alonso et al., 1999) and is required for ethylene signaling. Although the role of this gene in the ethylene pathway remains elusive, genetic studies locate EIN2 between CTR1 and EIN3 (Bleecker and Kende, 2000). *EIN3* and the related *EIL1* and *EIL2* encode nuclear proteins (Chao et al., 1997) that regulate the expression of *ERF1* (Solano et al., 1998), a member of a large family of transcription factors referred to as ethylene-response-element-binding-proteins (EREBPs) (Ohme-Takagi and Shinshi, 1995).

**Fatty Acid–Derived Signals**

Fatty acids (FAs) consist of long hydrophobic, often unbranched chains of hydrocarbons, with hydrophilic carboxylic acid groups at one end. They are an important source of reserve energy and essential components of membrane lipids in all living organisms. FAs and their derivatives as signaling molecules, modulating normal and disease-related physiologies in microbes, insects, animals, and plants. For example, 18:1 and linoleic acid (18: 2) induces protein kinase C mediated activation of NADPH oxidase, resulting in the production of reactive oxygen species (ROS) (Cury-Boaventura and Curi, 2005). The T cell response to infection is modulated by eicosapentanoic acid, which induces anti-inflammatory effects (Denys et al., 2001). Free FAs also serve as alarm molecules to repel phylogenetically related or unrelated species in insects (Rollo et al., 1994). In plants, FA metabolic pathways play
significant roles in pathogen defense. Historically, FAs were only assigned passive roles in plant defense such as biosynthetic precursors for cuticular components or the phytohormone jasmonic acid. However, recent discoveries demonstrate more direct roles for FAs and their breakdown products in inducing various modes of plant defenses. Both 16- and 18-carbon FAs participate in defense to modulate basal, effector-triggered, and systemic immunity in plants. Studies of FA metabolic mutants also reveal an active signaling role for the cuticle in plant defense. Unsaturated FAs and their derivatives regulate sporulation, sexual structure development, and host seed colonization in mycotoxic Aspergillus spp. (Calvo et al., 1999; Wilson et al., 2004).

In plants, FAs modulate a variety of responses to biotic and abiotic stresses. For instance, polyunsaturated FA levels in chloroplastic membranes affect membrane lipid fluidity and determine the plant’s ability to acclimatize to temperature stress (Iba 2002; Routabou et al., 2000). Linolenic acid (18:3) is involved in protein modifications in heat-stressed plants (Yamauchi et al., 2008). FAs also regulate salt, drought, and heavy metal tolerance as well as wound-induced responses and defense against insect and herbivore feeding in plants (Tumlinson et al., 2008; Upchurch 2008). The FA-derived phytohormone, jasmonic acid (JA), is particularly well known for its role in wound responses and plant defense against insect pathogens (Creelman and Mulpuri 2002).

Jasmonates and C-6 volatiles

Jasmonic acid (JA) and other structural derivatives including methyl jasmonate (MeJA), function as plant signals involved in plant defense to wounding and pathogens and developmental processes such as tuberization and pollen growth (Creelman and Mullet 1995). Jasmonates are synthesized from linolenic acid that is oxidized by lipoxygenase (LOX) to form 13-hydroperoxylinolenic acid. Jasmonic acid is produced from 13-hydroperoxylinolenic acid via a series of enzymatic reactions including allene oxide synthase (AOS), allene oxide cyclase (AOC) and other enzymes (Creelman and Mullet, 1997). Increased levels of jasmonates have been found in several plant species after wound and pathogen as well as elicitor treatment (Creelman and Mullet, 1995). In addition, treatment of plants with JA or MeJA increases resistance to pathogens (Cohen et al., 1993). JA and MeJA regulate the expression of several genes including proteinase inhibitors, PR genes, and genes involved in phytoalexin biosynthesis (Farmer et al., 1992; Wasternack and Parthier,
Furthermore, octadecanoid precursors of JA and MeJA also activate the synthesis of proteinase inhibitors (Farmer and Ryan, 1992). On the other hand, photosynthesis related genes such as the small and large subunits of rubisco are downregulated by jasmonates (Creelman and Mullet, 1997). The JA signal transduction pathway is mainly unknown. However, several Arabidopsis mutants have been identified, which exhibit reduced or increased sensitivity to jasmonates (Vijayan et al., 1998). Another set of signal molecules, the C6-volatiles, is related to the jasmonate pathway. C6-volatiles are present in several plants and have been associated with defense responses upon tissue damage (Hatanaka et al., 1987; Turlings et al., 1995). C6-volatiles derive from 13-hydroperoxy linolenic or linoleic acids, which are formed by LOX from linolenic or linoleic acids respectively. The 13-hydroperoxides are cleaved by hydroperoxide lyase (HPL) to produce 12-oxododecenolic acid and either cis-3-hexenal or hexanal, depending on whether the precursor was 13-hydroperoxy linolenic or linoleic acid, respectively. These C6-volatile aldehydes are substrates for an alcohol dehydrogenase and an isomerization factor that produce other C6-volatiles such as trans-3-hexenal, trans-2-hexenal, cis-3-hexenol, trans-3-hexenol, trans-2-hexenol and hexanol (Hatanaka, 1993). These C6-volatiles are rapidly released from damaged tissue and are responsible for the ‘green odor’ released from damaged leaves (Bate et al., 1998).

The increased production of some of these volatiles including cis-3-hexenol and trans-2-hexenal has been shown during plant defense responses to pathogens (Croft et al., 1993). Some of the C6-volatiles have been shown to induce expression of genes (Bate and Rothstein, 1998) and the production of phytoalexins (Zeringue, 1992). Furthermore, C6-volatiles have been found to exhibit antimicrobial activity (Croft et al., 1993) and they reduce insect feeding rates (Hildebrand et al., 1993). Transgenic plants with reduced levels of HPL exhibited reduced levels of both hexanal and 3-hexenal and insects feeding from these plants exhibited a 2-fold increase in fecundity above those feeding on nontransformed plants (Vancanneyt et al., 2001).

**Cross-talk between signal pathways**

The signal transduction pathways triggered by signal molecules such as SA, JA and ethylene appear to be connected and form an intricate regulatory signaling network that modulates plant defense responses. Jasmonates and ethylene pathways
act synergistically for the induction of some PR genes. For example, in tobacco plants, ethylene and MeJA synergistically induce two PR genes, PR1b and osmotin (Xu et al., 1994), and in *Arabidopsis*, the expression of *PDF1.2*, a gene encoding an antifungal plant defensin, is concomitantly induced by ethylene and MeJA (Penninckx et al., 1998; Norman-Stetterblad et al., 2000). In contrast, SA and jasmonates have been shown to induce different set of PR genes (Thomma et al., 1998), and antagonism between SA and JA signal pathways has been shown during plant defense responses. For example, SA inhibits the synthesis of proteinase inhibitors induced by JA in tomato plants (Doares et al., 1995). This antagonism is also reflected by the existence of different types of systemic resistance responses, e.g. SAR responses are SA dependent whereas other induced systemic resistance (ISR) responses are JA/ethylene dependent (Pieterse and van Loon, 1999). However, in rice, the SA-analogue INA synergistically stimulates the expression of genes induced by JA (Schweizer et al., 1997), and vice versa, JA enhances the regulation of the SA-induced pathway (Genoud and Métraux, 1999). Recent studies in *Arabidopsis* with different mutant backgrounds indicate that SA dependent and JA/ethylene-dependent pathways may require components that are active in both pathways. For example, NPR1, a protein with ankyrin repeats that interact with transcription factors (Cao et al., 1997; Zhang et al., 1999), is required for SAR and for ISR responses (Cao et al., 1994; Pieterse et al., 1998). In another example, fumonisin B1, a fungal toxin from *Fusarium moniliforme*, triggers program cell death in wild-type *Arabidopsis* protoplasts, and this response is light-dependent and requires SA-, JA-, and ethylene dependent signaling pathways as well as other unidentified factors (Asai et al., 2000).

**Pathogen related proteins**

Plant–pathogen interactions have been studied extensively over the years from both the plant and pathogen viewpoints. An understanding of how plants and pathogens recognize each other and differentiate to establish either a successful or an unsuccessful relationship is crucial in this field of investigation. Looking at the defense mechanisms in plants, the recognition and signaling events that occur in plant cells in response to microorganism challenge need to be extremely rapid, reliable and specific, and are part of the strategy evolved by plants to survive attacks. The intracellular sensitive perception of pathogens and the recognition of pathogen-associated molecular patterns, such as lipo polysaccharides and flagellin, lead to the
activation of the plant basal defence (or resistance), which is the first defence response, and trigger a generic mechanism consisting of plant cell wall thickening, papilla deposition, apoplast acidification and signal transduction and transcription of defence genes (Alfano and Collmer, 2004). This generic basal defence mechanism has been observed in several incompatible plant–microorganism interactions, and is believed to corroborate the observation that most plants are resistant to invasion by the majority of pathogens. Therefore, successful pathogens must evolve mechanisms to interfere with or suppress basal defence to colonize the host and develop disease. Superimposed on the basal defence, some plant varieties express resistance proteins that guard against this interference and trigger a specific, genetically defined hypersensitive response and subsequent programmed cell death. The function of the hypersensitive response is to contain the pathogen, and it is typified by various biochemical perturbations, known as generic plant responses, including changes in ion fluxes, lipid hyper peroxidation, protein phosphorylation, nitric oxide generation and a burst of reactive oxygen species and antimicrobial compounds. This rapid incompatibility response effectively puts an end to pathogen invasion and prevents further disease development (Alfano and Collmer, 2004). With regard to plant pathogens, the capacity to overcome plant defence, by protecting themselves from the oxidative stress activated by the plant in response to pathogen perception, is of extreme importance. Therefore, pathogens induce several genes, such as catalases and superoxide dismutase (SOD), which are responsible for the inactivation of H$_2$O$_2$ and O$_2^-$. The importance of secretion pathways for pathogenicity has also been well established. Effector proteins expressed by the pathogen are predicted to collaborate in the suppression of basal resistance through the modification of specific host proteins. The secretion of extracellular enzymes, such as pectin esterases, polygalacturonases, xylanases, pectatolyases and cellulases, is another essential process for colonization and pathogenicity (Van Sluys et al., 2002). With the increase in genomic and postgenomic studies, a large amount of information is available, and advances have been achieved in the understanding of defence mechanisms in plants, as well as the pathogenicity strategies employed by microbial pathogens At present, the functional assignment of given proteins is considered to be the main challenge in postgenomic studies.
Inducible defense-related proteins were first discovered in tobacco reacting hypersensitively to *Tobacco mosaic virus* (TMV) and later shown to occur in plant species from at least 13 families upon infection by oomycetes, fungi, bacteria, viruses, and viroids, as well as nematode or insect attack (Van Loon 1999). The recognized PRs have been extensively reviewed (Broekaert et al., 2000; Datta et al., 1999; Kitajima et al., 1999; Kombrink et al., 1997; Yun et al., 1997) and currently comprise 17 families of induced proteins. A role of several families in limiting pathogen activity, growth, and spread fits with the identification of the PR-2 family as β-1,3-endoglucanases and the PR-3, -4, -8, and -11 as endochitinases, which could act against fungi. The chitinases, as well as the protease inhibitors (PR-6), could also target nematodes and herbivorous insects. Members of the PR-8 family also possess lysozyme activity and may be directed against bacteria, whereas defensins (PR-12) (Lay and Anderson 2005; Thomma et al., 2002) and thionins (PR-13) (Bohlmann 1994; Epple et al., 1997) both have broad antibacterial and antifungal activities. Some lipid transfer proteins (PR-14) have antifungal and antibacterial activities (García-Olmedo et al., 1995) and members of the PR-1 and the thaumatinlike PR-5 families have been associated with activity against oomycetes. Notably, the prominent PR-1 proteins are often used as markers of the enhanced defensive state conferred by pathogen-induced systemic acquired resistance (SAR), but their biological activity has remained elusive (Van Loon and Van Strien 1999). PR-7 is an endopeptidase that is the most conspicuous PR in tomato (Jorda et al., 2000). It might aid in microbial cell wall dissolution. PR-9 is a specific type of peroxidase that could act in cell wall reinforcement by catalyzing lignifications (Passardi et al., 2004) and enhance resistance against multiple pathogens. PR-10 shows homology to ribonucleases, and some members do have weak ribonuclease activity (Bufo et al., 1996). There are no other families of PRs that are directed specifically against viruses, and it has sometimes been assumed that the ribonuclease activity of PR-10-type proteins points to a role in defense against these pathogens (Park et al., 2004). However, recently an antifungal PR-4-type protein from wheat was shown to also possess ribonuclease activity (Caporale et al., 2004). The families PR-15, -16, and -17 have been added recently. PR-15 and -16 are typical of monocots and comprise families of germinlike oxalate oxidases and oxalate oxidase-like proteins with superoxide dismutase activity (Bernier and Berna 2001), respectively. These proteins generate hydrogen peroxide.
that can be toxic to different types of attackers or could directly or indirectly stimulate plant-defense responses (Donaldson et al., 2001, Hu et al., 2003). PR-17 proteins have been found as an additional family of PRs in infected tobacco, wheat, and barley and contain sequences resembling the active site of zinc metallo proteases (Christensen et al., 2002), but have remained uncharacterized so far. A putative novel family (PR-18) comprises fungus- and SA-inducible carbohydrate oxidases, as exemplified by proteins with hydrogen peroxide-generating and antimicrobial properties from sunflower (Custers et al., 2004). Not all families seem to be represented in all plant species and occurrence and properties of different members within a family may differ strongly (Table 1.1).

<table>
<thead>
<tr>
<th>Family</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-1</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>PR-2</td>
<td>β-1,3-glucanase</td>
</tr>
<tr>
<td>PR-3</td>
<td>Q Chitinase type I, II, IV, V, VI</td>
</tr>
<tr>
<td>PR-4</td>
<td>Chitinase type I, II</td>
</tr>
<tr>
<td>PR-5</td>
<td>Thaumatin-like</td>
</tr>
<tr>
<td>PR-6</td>
<td>Proteinase-inhibitor</td>
</tr>
<tr>
<td>PR-7</td>
<td>Endoproteinase</td>
</tr>
<tr>
<td>PR-8</td>
<td>Chitinase type III</td>
</tr>
<tr>
<td>PR-9</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>PR-10</td>
<td>Ribonuclease-</td>
</tr>
<tr>
<td>PR-11</td>
<td>Chitinase, type I</td>
</tr>
<tr>
<td>PR-12</td>
<td>Defensin</td>
</tr>
<tr>
<td>PR-13</td>
<td>Thionin</td>
</tr>
<tr>
<td>PR-14</td>
<td>Lipid-transfer protein</td>
</tr>
<tr>
<td>PR-15</td>
<td>Oxalate oxidase</td>
</tr>
<tr>
<td>PR-16</td>
<td>Oxalate-oxidase-like</td>
</tr>
<tr>
<td>PR-17</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Table 1.1 Recognized families of pathogenesis-related proteins**

Upon infection with various types of pathogens, defense-related genes are coordinately activated and may be expressed in both infected and non-infected tissues concomitant with the development of SAR (Ryals et al., 1996). The association between accumulation of PRs, the products of the SAR genes, and SAR is often taken
to represent a causal relationship, with the proteins acting as the agents responsible for
the induced resistance against subsequent infection by a wide range of pathogens.
Moreover, pathogens such as *B. cinerea* on tobacco and *A. brassicicola* on *Arabidopsis* are virtually insensitive to SAR, but restricted by a different mechanism of induced resistance that is independent of the presence of inducible defense-related proteins in protected tissues (Thomma et al., 2001; Ton et al., 2002). This type of enhanced defensive capacity is elicited by specific strains of nonpathogenic, root colonizing bacteria and has been termed induced systemic resistance (ISR) (Van Loon and Pieterse, 2002). The expression of a PR-1 gene or protein in particular is usually taken as a molecular marker to indicate that SAR was induced. All PR-1 genes in plants appear to be inducible by SA, and endogenous production or exogenous application of SA has been shown to be both necessary and sufficient to elicit the induced state (Vernooij et al., 1994). Pathogen-induced synthesis of SA in tobacco is considered to occur from benzoate, whereas the evidence in *Arabidopsis* points to isochorismate as the immediate precursor (Durrant and Dong, 2004). Upon arrival of the mobile signal, the latter tissues must start producing SA, which induces the defense-related proteins locally (Verberne et al., 2003). The nature of the mobile signal has remained elusive so far. An *Arabidopsis* mutant, *dir1*, impaired specifically in the systemic character of SAR, implicates involvement of a lipid transfer protein (Maldonado et al., 2002), suggesting that the mobile signal may contain a lipid moiety. SA production has been suggested to be part of a feed-forward loop (Shah, 2003). Upon hypersensitive necrosis, not only does the level of SA increase, but also JA synthesis and ET production are strongly enhanced (Pieterse et al., 2000; Seo et al., 2001). As a result, in addition to SA-inducible defense-related genes, such as in *Arabidopsis* PR-1, -2, and-5, JA- and ET-inducible genes, i.e., PR-3-type basic chitinase, PR-4-type hevein-like protein, and PR-12 defensin PDF1.2, become activated (Thomma et al., 2001). PDF1.2 in particular is often used as a marker for the induction of the JA and ET-dependent defense-signaling pathway (Lay and Anderson, 2005). Induction of PDF1.2 can be limited, however, because accumulation of SA inhibits JA synthesis and action (Spoel et al., 2003). ET sensitizes the tissue to respond to SA, as evidenced by a lowering of the concentration of SA that is required for PR-1 expression when *Arabidopsis* is exposed to ET (Lawton et al., 1994). On the other hand, in tobacco induction of PR-1a by SA was reduced by simultaneous
application of JA (Niki et al., 1998). The nature and extent of the cross-talk between
the three defense-regulating hormones depend on the timing and magnitude of their
increases, which, in turn, can be modulated through the action of the attacking
pathogen (De Vos et al., 2005). Biotrophic pathogens are dependent on live tissues
and avoid triggering necrosis. The enhanced disease susceptibility to biotrophic
pathogens, such as the oomycete *H. parasitica*, of *Arabidopsis* mutants that are
impaired in SA synthesis or signaling indicates that SA-dependent defenses contribute
to basal resistance against these types of pathogens (Thomma et al., 2001). Exogenous
application of SA leads to induction of PR-1, -2, and -5 mRNAs. Thus, SA-regulated
defense-related proteins may be directed primarily against pathogens with a
biotrophic lifestyle rather than oomycetes as such (Oliver and Ipcho, 2004). Little
information on the effect of these proteins on biotrophs other than oomycetes is
available and further clarification is needed. *Arabidopsis* plants impaired in JA or ET
signaling are, in general, more susceptible to necrotrophic pathogens (Geraats et al.,
2003; Thomma et al., 2001; Vijayan et al., 1998). SA may induce resistance against
these pathogens also, but it is likely that SA-regulated defenses are important only at a
stage in which the pathogen (still) behaves as a hemibiotroph. *B. cinerea*, a pathogen
that is completely dependent on its necrotrophic lifestyle, kills the tissue in advance of
tissue colonization, is insensitive to SA-regulated defenses—at least in *Arabidopsis* and
tobacco are not affected by SAR. In contrast, necrosis in *Arabidopsis* as a result of
infection by *P. syringae* pv. *tomato* follows a phase of spreading chlorosis, in which
the bacterium multiplies abundantly in the infected leaves. Thus, *P. syringae* pv.
*Tomato* has a mixed biotrophic/necrotrophic lifestyle, and both SA- and JA/ET-
regulated defenses contribute to basal resistance against this pathogen (Van et al.,
2000). JA-/ET-dependent defenses in *Arabidopsis* are boosted upon challenge
inoculation of plants expressing rhizobacteria mediated ISR and are most effective
against pathogenic bacteria and fungi with mixed biotrophic and necrotrophic
lifestyles (Ton et al., 2002). Many PR-type proteins are JA- and/or ET-inducible and
their occurrence can be further modulated by abscisic acid (Audenaert 2002;
Rezzonico et al., 1998; Zhu et al., 1993). Whereas in *Arabidopsis* a distinction
between the SA-inducible PR-1, -2, and -5, and the JA/ET-inducible PR-3, -4, and -12
seems clear (Thomma et al., 2001), in tobacco it has been demonstrated that different
members within the same protein family are differentially regulated by SA and JA/ET
Thus, the acidic PR-1, -2, -3, and -5 proteins, which are inducible by TMV and accumulate in the apoplast, are regulated primarily by SA, with ET and/or JA acting sometimes in a synergistic manner. The basic isoforms that are developmentally regulated and are present in the vacuole appear to be regulated and are inducible further by JA and ET, acting alone or in concert. For instance, ET-insensitive tobacco does not express basic PR-1g, -2d, and -5c in response to TMV infection, whereas local expression of the acidic isoforms is not affected (Verberne et al., 2003). In other plant species, similar differential induction has been noted but not systematically investigated. In tomato, the basic orthologues of tobacco PR-1a, -1b, and -1c are present in the apoplast and inducible by SA, but in Arabidopsis and rice, the specific induction characteristics of the many PR-1-type proteins have not been investigated. Plants are able to recognize microbial invaders through specific surface determinants, collectively called pathogen-associated molecular patterns (PAMPs) and to react through defense signaling cascades (Asai et al., 2002; Nurnberger et al., 2004). Although a causal connection between recognition of nonpathogenic microorganisms and specific inducible defense-related proteins has not been established so far, broad-spectrum effectiveness of the induced resistance responses suggests that PR-type and similar proteins are part of an immune surveillance mechanism that protects the plant primarily against invasion by microorganisms that are generally perceived as nonpathogenic. Many saprophytic fungi appear more sensitive to the action of lytic enzymes, such as glucanases and chitinases, than are pathogens that are adapted to attack on living plants (Schlumbaum et al., 1986). Indeed, certain pathogens have been shown to be insensitive to the action of defense-related proteins from their host. For instance, Cladosporium fulvum is not sensitive to the chitinase and β-1,3-glucanase of its host, tomato (Joosten et al., 1995). Phytophthora sojae specifically inhibits the glucanase activity of its host, soybean, by producing inhibitor proteins (Rose et al., 2002). Fusarium solani f.sp. eumartii degrades PRs in the intercellular fluid from potato (Olivieri et al., 2002). Similarly, constitutive expression of various PRs in tobacco did not affect colonization by the beneficial mycorrhizal fungus Glomus mosseae (Vierheilig et al., 1995).

**Phytoalexins**

Phytoalexins constitute a large group of diverse compounds that are both synthesized and accumulated in plant cells undergoing defense responses against
biotic stresses. The extensive variety of their structures makes it difficult to give a chemical definition for the group and usually phytoalexins are defined as low molecular weight compounds with antimicrobial activity (Smith, 1996). More than 300 phytoalexins have been characterized including phytoalexins with structures like flavonoids and other phenylpropanoid derivatives, sesquiterpenes, polyketides, etc. (Hammeschmidt, 1999). The biosynthesis of phytoalexins may involve a single metabolic pathway or precursors from several different pathways (Kuć, 1995). Germinated peanuts have been shown to produce phytoalexins, such as resveratrol, arachidins and isopentadienyl-3,5,40-trihydroxystilbene, and up to 45 stilbenoid phytoalexin derivatives after inoculation with the food grade fungus Rhizopus oligosporus (Wu et al., 2011). Analysis of phytoalexins produced at different distances from the site of infection of peanut kernels with different Aspergillus fungal strains revealed temporal, spatial and strain-specific differences in phytoalexin profiles. Higher concentrations of phytoalexin accumulated with longer incubation, and the composition of phytoalexins varied significantly by layer (Sobolev, 2008). Challenge of peanut seeds with an Aspergillus caelatus strain produced known stilbenes as well as new stilbenoids (arahypin-1, arahypin-2, arahypin-3, arahypin-4, arahypin-5, arahypin-6 and arahypin-7) and pterocarpenes (aracarpene-1 and aracarpene-2), which have a defensive role against pathogenic organisms (Sobolev et al., 2006, 2009, 2010, 2011). Again in peanut, a comparison of fungi and chemicals on the induction of trans-resveratrol and trans-piceatannol found fungi to be the most effective (Yang et al., 2010). Ganoderma lucidum mycelium-treated peanut callus was proposed to be a good source of bioactive components. A new peanut hairy root line that produces resveratrol and arachidin-1 and arachidin-3 upon sodium acetate mediated elicitation was generated (Condori et al., 2010). Sodium acetate elicitation resulted in 60-fold induction and secretion of trans-resveratrol and, to a lesser extent, of other stilbenes, including trans-pterostilbene, into the medium of peanut hairy root cultures (Medina-Bolivar et al., 2007). These studies demonstrated the benefits of hairy root culture systems in studies of the biosynthesis of stilbenoids, and their use as an effective bioprocessing system for valued nutraceuticals, such as resveratrol and its derivatives (Condori et al., 2010; Medina-Bolivar et al., 2007).
Phenylpropanoids

The phenylpropanoid pathway includes an intricate set of reactions producing an extensive and diverse amount of products that participate in several cellular processes including those induced by biotic stresses. Phenylpropanoids are derived from cinnamic acid, which is formed from phenylalanine by the action of phenylalanine ammonia-lyase (PAL) (Dixon and Paiva, 1995; Hahlbrock and Scheel, 1989). A core set of reactions including the formation of cinnamic acid, its conversion into 4-coumaric acid, and the conversion of the latter into 4-coumaryl-CoA derivatives is common to the general phenylpropanoid metabolism (Hahlbrock and Scheel, 1989). Individual branches of the pathway may diverge at different points of these core reactions and lead to the formation of diverse phenylpropanoid derivatives including phytoalexins, anthocyanins, flavones, lignin, suberin, cell-wall bound phenolics and signal molecules such as SA (Keller et al., 1996).

**Differential defense response of plant to necrotrophic and biotrophic pathogen.**

Plant pathogens are often divided into biotrophs and necrotrophs, according to their lifestyles. In the case of biotrophs, it is easy to imagine that \( R \) gene–mediated resistance and SA signaling could result in resistance. The HR response would deprive such pathogens of a food source. However, in the case of necrotrophs, it seems that programmed cell death in the host would merely make life easier for the pathogen. Support for this idea was provided by experiments in which *Arabidopsis* mutants with defects in various defense-related signaling pathways were tested for defects in resistance to various pathogens. The mutation \( npr1 \) and the transgene NahG, which block SA signaling, result in loss of resistance to the biotrophic oomycete *Peronospora parasitica*, but have no effect on resistance to the necrotrophic fungus *Alternaria brassicicola*. Conversely, the \( coi1 \) mutation, which blocks JA signaling, severely compromises resistance to the necrotrophic fungus *A. brassicicola*, but has no effect on resistance to *P. parasitica* (Thomma et al., 1998). Such observations led to the suggestion that plant defense responses may be tailored to the attacking pathogen, with SA-dependent defenses acting against biotrophs, and JA- and ET-dependent responses acting against necrotrophs (McDowell and Dangl, 2000). As is often the case with simple, elegant explanations, further testing has revealed that while this idea is generally true, the actual situation is rather more complicated.
**Difference between Biotrophs and Necrotrophs**

Biotrophs are pathogens that derive nutrients from living host tissues, and necrotrophs are pathogens that derive nutrients from dead or dying cells (Agrios, 1997). Some pathogens can be clearly assigned as biotrophs or necrotrophs. However, many others behave as both biotrophs and necrotrophs, depending on the conditions in which they find themselves or the stages of their life cycles. Such pathogens are called hemi-biotrophs. Many fungi that are commonly considered as necrotrophs may really be hemi-biotrophs, as they have a biotrophic stage early in the infection process. The obligate oomycete pathogen *P. parasitica* is perhaps the clearest example of an *Arabidopsis* biotroph.Compatible infection begins with germination of conidia on the leaf surface. Appressoria form, resulting in hyphal penetration of epidermal cells and formation of haustoria within these cells. Haustoria are separated from the host cytoplasm by a host membrane that is continuous with the plasma membrane. Hyphae subsequently grow throughout the leaf, penetrating mesophyll cells and forming additional haustoria there. After about one week, conidiophores emerge from stomata, and mature conidia are formed. Sexual reproduction also occurs, resulting in formation of oospores. Throughout this process, infected plant cells remain alive. Heavily infected seedlings sometimes die, but only after sporulation is complete (Koch and Slusarenko, 1990). The ascomycete fungi *Erysiphe orontii* and *Erysiphe cichoracearum* are also good examples of obligate biotrophs. Compatible isolates of these fungi infect epidermal cells, develop haustoria, and sporulate profusely, without causing host cell death (Reuber et al., 1998; Vogel and Somerville, 2000). The bacterial pathogen *P. syringae* is often considered a biotroph, occasionally considered a necrotroph (Butt et al., 1998), and should probably be considered a hemi-biotroph (Thaler et al., 2004). The bacteria infect through wounds and stomata and multiply in the intercellular spaces. In the early stages of compatible infections, host cell death does not occur, but later stages of infection are associated with host tissue chlorosis and necrosis. Many strains, including *P. syringae pv. tomato* DC3000, which infects *Arabidopsis*, produce toxins that contribute to pathogenicity (Bender et al., 1999). Like many bacterial pathogens of animals, *P. syringae* actively transports dozens of proteins into host cells through a specialized system known as type III secretion. These proteins are called type III effectors and are thought to contribute to virulence. Several of them have been shown to contribute to virulence in *Arabidopsis* (Alfano...
and Collmer 2004; Espinosa and Alfano 2004). The fungal pathogens *Botrytis cinerea* and *Alternaria brassicicola* are considered necrotrophs. Both of these fungi kill host cells at very early stages in the infection and cause extensive tissue damage. They also produce a variety of phytotoxins that likely promote host cell death (Colmenares et al., 2002).

**Mechanism of defense against Biotrophs**

Gene-for-gene resistance is an important form of resistance against biotrophs. It is associated with activation of SA-dependent signaling and SAR. As SA and JA/ET signaling tend to be mutually inhibitory, JA/ET signaling is expected to have deleterious effects on resistance to these pathogens. Results from studies of *P. parasitica*, *Erysiphe* spp., and *P. syringae* support the idea that SA signaling is important for resistance. However, especially in the cases of *P. parasitica* and *Erisyphe* spp., JA signaling may also be effective if it is activated. More gene-for-gene resistance interactions are known for *P. parasitica*-Arabidopsis interactions than for any other *Arabidopsis*-pathogen system. The effectiveness of gene-for-gene resistance makes intuitive sense, as death of the first infected cell should stop growth of this biotrophic pathogen. Production of reactive oxygen species (ROS) associated with the HR does not appear to be required for resistance. AtrbohD/F double mutants do not produce peroxides, yet show more cell death than wild-type plants in a weakly resistant interaction (Torres et al., 2002). The mutant plants are also more resistant than wild-type, suggesting that the critical resistance factor is cell death, not ROS production (Torres et al., 2002). Mutations in *eds1* or *pad4*, which are associated with SA signaling, weaken resistance to some *P. parasitica* isolates (Feys et al., 2001). This effect may be due to a requirement for EDS1 and PAD4 for wild-type levels of cell death, rather than loss of some other SA-dependent defense response. In *eds1* plants, the HR fails completely, and the pathogen becomes fully compatible (Feys et al., 2001). In *pad4* plants, the HR does not occur as rapidly as in wild-type plants, and hyphae spread ahead of a trailing necrosis, culminating in sparse sporulation (Feys et al., 2001). SA-dependent defenses do play a role in limiting *P. parasitica* growth, even in compatible interactions. Mutations in *eds5* and *sid2* result in increased growth of the compatible isolate Noco2 (Nawrath and Metraux JP 1999). No enhanced susceptibility is detected in *npr1-1* (Col-0 background) (Bowling et al., 1997), but strong alleles of *npr1* in the Ws background result in a slight increase of susceptibility.
to the compatible isolate Emwa and to the incompatible isolate Wela (Delaney et al., 1995). In the case of Wela, the interaction is not fully compatible, and trailing necrosis is observed (Delaney et al., 1995). No enhanced susceptibility to compatible Noco2 is observed in the tga2/5/6 triple mutant (Zhang et al., 2003). Collectively, these results demonstrate the existence of SA-dependent, NPR1- and TGA2, 5, 6-independent resistance responses that limit growth of P. parasitica. Some of these responses may be controlled by AtWhy1, as mutations in this SA-dependent, NPR1-independent transcription factor compromise local resistance to the incompatible Emoy2 and SAR against the compatible Noco2 (Desveaux et al., 2004). Consistent with the idea that SA signaling is effective against P. parasitica, SAR is effective against this pathogen. Artificial induction of SAR by treatment with SA or SA analogs results in resistance to normally compatible isolates (Ryals et al., 1997; Uknes et al., 1992). Induction of SAR by infection with an avirulent pathogen is also effective (Nawrath and Metraux, 1999). Mutations in eds5, sid2 (Nawrath and Metraux, 1999), Col and Ws alleles of npr1 (Cao et al., 1997; Delaney et al., 1995; Ryals et al., 1997), and the tga2/5/6 triple mutant all show strong loss-of-induced-resistance phenotypes (Zhang et al., 2003). This illustrates that responses under control of this pathway are required for SAR against P. parasitica. Mutants with constitutively active defense signaling are resistant to P. parasitica. Not surprisingly, mutants with constitutively elevated levels of SA, including but not limited to mpk4 (Petersen et al., 2000), cpr1 (Bowling et al., 1994), cpr5 (Bowling et al., 1997), and cpr6 (Clarke et al., 1998), are resistant. Mutations in cpr5 and cpr6 cause constitutive expression of the SA-dependent gene PR-1 and the JA/ET-dependent gene PDF1.2. In cpr5 plants, expression of PR-1 is stronger than in cpr6, and expression of PDF1.2 is weaker, suggesting that cpr5 results in stronger activation of SA signaling and weaker activation of JA/ET signaling relative to cpr6 (Clarke et al., 2000), suggesting that either SA signaling or JA/ET signaling is sufficient to confer resistance to this pathogen. In the case of cpr6, cpr6 npr1 double mutants retain resistance, but cpr6 ein2 and cpr6 jar1 do not (Clarke et al., 2000), suggesting that in cpr6, resistance is due mainly to the activation of JA/ET signaling. These results should be interpreted with some caution, however, since the presence of ein2 increases SA levels in cpr5 and decreases them in cpr6, suggesting that complicated cross-talk is occurring between SA and ET signaling in these mutants (Clarke et al., 2000). Treatment with
exogenous JA can result in resistance to compatible *P. parasitica* isolates, although continuous exposure, as opposed to a single treatment, is required (Zimmerli et al., 2004). There is no evidence that JA/ET-dependent responses are normally active in limiting *P. parasitica*, although this has not been investigated extensively. Plants bearing *coil* do not lose resistance to the incompatible isolate Wela. *R* gene–mediated resistance and SA-dependent responses are effective against the biotroph *P. parasitica*. JA/ET-dependent responses do not seem to play a major role normally, but can be effective if they are induced prior to pathogen challenge. Camalexin does not seem to be involved in resistance, as *pad3* mutants do not show enhanced susceptibility to a compatible isolate (Thomma et al., 1998).

**Mechanism of defense against Necrotrophs**

According to the model, gene-for-gene resistance should not be observed in interactions with necrotrophs, as host cell death is not predicted to limit pathogen growth. Furthermore, SA-dependent responses and SAR are not predicted to play a role, whereas responses mediated by JA and ET are expected to play a role. All ecotypes of *Arabidopsis* that have been tested are highly resistant to the commonly studied *A. brassicicola* isolate, MUCL20297. Plants bearing mutations in *coil* are susceptible, indicating that JA signaling is required for resistance (Thomma et al., 1998). Production of camalexin is also required, as *pad3* mutant plants are susceptible (Zhou et al., 1999). The susceptibility of *coil* plants must be due to something other than a defect in camalexin production, as *coil* plants produce wild-type levels of camalexin in response to *A. brassicicola* infection (Van Wees et al., 2003). Treatment of *pad3* plants with exogenous JA reduces susceptibility, providing further support for the idea that JA signaling is required for resistance (Thomma et al., 1998). Several other mutations *bos1, bos3, bos4* and *esa1* compromise resistance to *A. brassicicola*. *BOS1* encodes an R2R3Myb transcription factor. Besides defects in disease resistance, *bos1* mutant exhibit reduced tolerance to drought, salinity, and oxidative stress. *BOS1* may act in JA signaling, as its pathogen induced expression is blocked in *coil* mutants (Mengiste et al., 2003). Expression profiling of *bos1* plants after infection is needed to test for a requirement for *BOS1* in induction of *COI1*-dependent pathogen-induced genes. Expression of *PDF1.2* is reduced in *bos3*, suggesting a defect in ET or JA signaling, whereas camalexin levels are reduced in *bos4* (Veronese et al., 2004). Plants bearing the *esa1* mutation have reduced *PDF1.2* expression,
suggesting a role for ESA1 in JA or ET signaling. Like bos1 plants, esal plants are sensitive to oxidative stress (Tierense al., 2002), suggesting a link between tolerance of oxidative stress on the one hand and JA signaling and resistance to necrotrophs on the other. There are no examples of gene-for-gene resistance against A. brassicicola in Arabidopsis. Several mutants with defects in SA signaling have been tested for enhanced susceptibility, including npr1 (Thomma et al., 1998), pad4 and sid2 (Van Wees et al., 2003), but no enhanced susceptibility was detected. Similarly, the mutation ein2, which blocks ethylene signaling, has no effect on resistance (Thomma et al., 1999; Van Wees et al., 2003). Thus, resistance to A. brassicicola depends on JA signaling and camalexin production, but R gene–mediated resistance, SA signaling, and ET signaling do not appear to play important roles. Like resistance to A. brassicicola, resistance to B. cinerea depends on JA signaling and camalexin production. Mutations that block JA signaling, including coi1 (Thomma et al., 1998) and jar1 (Ferrari et al., 2003) cause enhanced susceptibility. Expression of some JA-responsive genes is controlled by the MYC transcription factor JIN1 (Lorenzo et al., 2004). Interestingly, plants bearing jin1 mutations are more resistant to B. cinerea (Lorenzo et al., 2004). This result illustrates the need for caution in making broad interpretations about the roles of entire pathways in contributing to resistance against various pathogens. Apparently, JA signaling leads to expression of at least two classes of genes, those regulated by JIN1 that have a net negative effect on B. cinerea resistance, and those not regulated by JIN1, that have a net positive effect on resistance. Camalexin production also contributes to resistance to at least some strains, as pad3 mutant display enhanced susceptibility (Ferrari et al., 2003). Note, however, that another group, working with a different strain, did not detect enhanced susceptibility in pad3 plants (Thomma et al., 1999). Unlike resistance to A. brassicicola, resistance to B. cinerea requires ET signaling. The block in ET signaling caused by ein2 results in enhanced susceptibility (Ferrari et al., 2003; Thomma et al., 1999). Furthermore, overexpression of the transcription factor ERF1 enhances resistance (Berrocal-Lobo et al., 2002). It seems likely that genes playing an important role in B. cinerea resistance lie in the group co-regulated by JA and ET, and that ERF1 activates many of these genes. The data concerning the role of SA signaling in B. cinerea resistance are complex, but SA does appear to contribute to resistance. Plants bearing mutations in eds5, sid2, pad4, or npr1 do not exhibit
enhanced susceptibility (Ferrari et al., 2003). However, inhibitors that block phenylalanine ammonia lyase (PAL) activity do result in enhanced susceptibility (Ferrari et al., 2003; Govrin and Levine, 2002). Most of the SA produced in *Arabidopsis* is made through a pathway that requires isochorismate synthase, the product of *SID2*, and is thought not to require PAL (Wildermuth et al., 2001). However, it is possible that there is some synthesis through PAL, and that this is important for limiting the local spread of *B. cinerea* (Ferrari et al., 2003). Furthermore, sensitizing the system by using an *ein2* background revealed that an *ein2 npr1* double mutant is significantly more susceptible than the *ein2* single mutant (Ferrari et al., 2003). Treatment with exogenous SA or the SA analog BTH [benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] prior to infection also reduces lesion size (Ferrari et al., 2003; Zimmerli et al., 2001). Several other genes whose functions or positions in signaling pathways are not well understood are also required for resistance to *B. cinerea*. These include *BOS1, BOS2, BOS4*, and *ESA1*, as described above for *A. brassicicola* (Mengiste et al., 2003; Veronese et al., 2004). Mutation in *bos3*, which greatly reduces *PDF1.2* expression, also enhances susceptibility to *B. cinerea* (Veronese et al., 2004). Plants bearing *pad2* mutations are more susceptible, and this is probably not simply a result of reduced camalexin levels, as the phenotype of *pad2* is more severe than that of *pad3*, even though *pad2* produces more camalexin than *pad3* (Ferrari et al., 2003). *EDS4* contributes to resistance, possibly as a result of a defect in SA signaling (Ferrari et al., 2003; Gupta et al., 2000). No gene-for-gene resistance phenomena are known for *B. cinerea* interactions. On the contrary, Govrin & Levine have proposed that the cell death induced by *B. cinerea* is a form of the HR, and that this induction of cell death is an important component of virulence (Govrin and Levine 2000). Cell death induced by *B. cinerea* resembles the HR in that it is preceded by accumulation of ROI, and is associated with expression of the HR-associated gene *HSR203J* in tobacco (Govrin and Levine, 2000). Pre-infection with an avirulent *P. syringae* strain, but not a virulent one, leads to cell death and increased *B. cinerea* growth. The same treatment greatly reduces growth of a virulent *P. syringae* strain, as expected (Govrin and Levine, 2000). Further support for the idea that *B. cinerea* actively promotes a form of HR cell death has been provided by observations that inhibition of HR cell death by expression of animal anti apoptosis genes as transgenes in tobacco leads to enhanced
susceptibility to *B. cinerea* (Dickman et al., 2001). *B. cinerea* may promote production of ROIs as a virulence mechanism. High levels of ROI are present in infected tissue, and increasing hydrogen peroxide levels by application of glucose and glucose oxidase, or through catalase inhibition, results in enhanced growth of *B. cinerea* (Govrin and Levine 2000). The ROI sensitivity of bos1 and esal mutants also suggests that tolerance of ROI is important for resistance to necrotrophic fungi. Effective defenses against *B. cinerea* require JA and ET signaling. Induction of ROI and cell death in the host play major roles in virulence, and tolerance of ROI contributes to resistance. Production of camalexin may be an important defense mechanism. Although SA signaling does not appear to play a major role, it does contribute to some extent.

The idea that gene-for-gene resistance and SA signaling are generally effective against biotrophs whereas JA/ET signaling is generally effective against Necrotrophs appears to be correct, at least in interactions between *Arabidopsis* and its pathogens. Nonetheless, some subtleties should be kept in mind to avoid being misled into an over-simplistic view of plant disease resistance. Some biotrophs, including *P. parasitica* and *Erisyphe* spp., avoid triggering activation of JA-/ET-dependent defenses. If these defenses are triggered, they can be effective against these pathogens. So, while it is true to say that JA-/ET-dependent defenses are not important in these interactions, the ability of the pathogen to avoid triggering such defenses is important. The role of JA/ET signaling in *P. syringae* interactions is presently somewhat contradictory. There is abundant evidence supporting the idea that the *P. syringae* virulence factor coronatine is an inducer of JA/ET signaling, yet constitutive activation of JA signaling in *cev1* plants leads to resistance. Additional work is needed to resolve this contradiction. The necrotrophs *A. brassicicola* and *B. cinerea* are restricted by JA-/ET-dependent defenses. Camalexin contributes to resistance against these pathogens and against another necrotroph, *Leptosphaeria maculans* (Bohman et al., 2004). Gene-for-gene resistance effective against these pathogens has not been observed, and in the case of *B. cinerea*, there is evidence that host cell death may promote pathogen growth. Although SA signaling does not appear to play a major role, it does have some effect on resistance to *B. cinerea*. Testing of sensitized lines, as in the use of ein2 npr1 double mutants to detect a role for *NPR1* in
B. cinerea resistance, may result in detection of a contribution from SA signaling to resistance against other necrotrophs.

**The plant hosts: Arachis hypogaeae L. (Groundnut)**

Groundnut, an important cash crop, is an annual legume. India is the second largest producer of groundnuts after China. Groundnut is the largest oilseed in India in terms of production. It accounted for 35.99 per cent of the oilseeds production of the country. Gujarat is the largest producer contributing 25 % of the total production followed by Tamil Nadu (22.48 %), Andhra Pradesh (18.81 %), Karnataka (12.64 per cent) and Maharashtra (10.09 %) during 2006-07 (fig.1.2). Groundnut contains on an average 40.1 % of fat and 25.3% of protein and is a rich source of calcium, iron and vitamin B complex like thiamine, riboflavin, niacin and vitamin A. It has multifarious usages: It is used not only as a major cooking medium for various food items but also for manufacture of soaps, cosmetics, shaving creams and lubricants. In fact, it plays a pivotal role in the oilseed economy of India. About two thirds of world production is crushed for oil and the remaining one third is consumed as food. Its cake is used as feed or for making other food products and haulms provide quality fodder.

![Fig. 1.1 Groundnut growing areas in India.](image)
Taxonomy

The genus *Arachis* is a member of family Fabaceae (synonym: Leguminosae) subfamily Papilionoidea, tribe Aeschynomeneae and subtribe Stylosanthinae (Polhill and Raven 1981). At the time Linnaeus first named the cultivated groundnut *Arachis hypogaea* L., it was the only member of the genus. One hundred years later Bentham (1841) produced the first taxonomic treatment of the genus. These treatments are now largely outdated because of the number of new taxa that have been collected and described in the past forty to fifty years. Unfortunately during this surge in germplasm collection, many new taxa were invalidly described, other unofficial names came into common use and different names were sometimes assigned to the same species and vice versa (Resslar, 1980). The basis for the confusion was a lack of recognized differentiating morphological descriptors, as well as fragmentary early collecting and the representation of species by seedling specimens. The genus was in a state of chaos until 1994 when Krapovichas and Gregory published a taxonomic revision of the genus. This work took them nearly thirty-five years, and involved re-visiting and collecting specimens at the type locations of each known species. This taxonomic revision recognises 69 species in 9 sections. Distinctions are made on the basis of morphological characters and life cycle attributes, although eco-geographic distribution, crossability evidence, cytological information, plant form, as well as chromatographic and antigenic reactions were all considered in the groupings. All species, except the cultivated species and *A. monticola*, in Section *Arachis*, and certain species in Section *Rhizomatosae*, are diploid (2n=2x=20). Section *Arachis* contains the cultivated groundnut *A. hypogaea*, which is itself divided into two subspecies, subsp. *fastigiata* Waldron and subsp. *hypogaea* Krap. et Rig. Previously each subspecies was divided into two varieties (Gregory et al., 1973); subspecies *hypogaea* contained var. *hypogaea* and var. *hirsuta*, and subsp. *fastigiata* contained var. *vulgaris* and var. *fastigiata*. However in 1994, Gregory and Krapovickas proposed two new varieties of subsp. *fastigiata* in addition to the existing ones, namely var. *peruviana* and var. *aequatoriana*.

Climatic condition

Climatic conditions such as temperature and rainfall significantly influence the groundnut production. Warm and moist conditions are very favorable than cool and wet climate, which results in slow germination and seedling emergence, increasing
the risk of seed rot and seedling diseases. Temperature is a major environmental factor that determines the rate of crop development. Temperatures above 35°C inhibit the growth of groundnut. Optimum mean daily temperature to grow is 30°C and growth ceases at 15°C. For rapid emergence, soil temperature above 21°C is needed. The optimum temperature for the most rapid germination and seedling development is about 30°C. A minimum 100 - day optimum temperature growing season is necessary for successful groundnut crop production. Adequate and well distributed rainfall during the growing season, especially during flowering, pegging and pod formation stages, is essential for maximum yield and quality of groundnut. Groundnut is grown in areas receiving 600 to 1500 mm of rainfall. However, the crop can be grown successfully with a rainfall of 1250 mm.

**The amount of rainfall required:**

- pre-sowing operations (preparatory cultivation) 100 mm
- sowing 150 mm
- Flowering and pod development 400-500 mm

**Varieties**

The genus *Arachis hypogaea* has been divided into 4 different varieties as 'Virginia', 'Peruvian', 'Valencia', and 'Spanish'. These different types are believed to have thought to have originated in different locations. Virginia variety may have been developed in Amazonia. The Peruvian variety is the common type found in archaeological sites in the oases of Peru is believed to have been developed in Peru. The Spanish variety was grown by the peoples of northeastern Brazil. The Valencia variety may have been developed by the Guarani peoples of the Paraguay-Praraná basin. The subspecies hypogaea and hirsuta share similar morphological features as they don't have floral axes on main axis (Weiss, 2000; Bunting et al., 1985). The Virginia type is less hairy and branches are short, whereas Peruvian is more hairy with long branches. Similarly, the subspecies fastigiata and vulgaris share similar morphological features as floral axes are found on the main axis. There are continuous runs of multifloral axes along lateral branches. Valencia type is little branched whereas Spanish type is more branched. The Virginia and Peruvian varieties are prostrate, have seed dormancy, and require 5 to 10 months growing season. The prostrate varieties are commonly called as runners as lateral branches remain close to the ground, giving spreading appearance. The Valencia and Spanish varieties are
erect, have non dormant seeds, and mature in 3 to 5 months. Erect types are also called as bunchy type as the upright growth of branches give mature plant a tightly bunched, bushy appearance. Erect types often have lower individual nut yields per plant than prostrate types. However, erect types tend to have slightly higher seed oil and seed protein contents. Spanish variety is particularly rich in oil. In India, about 80% of total groundnut area is covered by Virginia runner type.

**Ecology**

The main range of peanut cultivation is between 350° S and 400° N, but it extends to 450° N in Central Asia and North America. It adapts to wide range of environments. It is normally grown commercially below 1250 m, although many varieties could be found at much higher elevations (Weiss, 2000). It is a day neutral plant and thus little affected by day length. However, plant growth is adversely affected by low light intensity. Bunchy types are generally more severely affected by climatic variation than runner types. Temperature between 25 to 30°C is optimum for plant development (Weiss, 2000). Once established, peanut is drought resistant, and to some extent it also tolerates flooding. A rainfall of 500 to 1000 mm will allow commercial production, although crop can be produced on as little as 300 to 400 mm of rainfall. Once pods are mature, rainfall will adversely affect the crop as some cultivars have a very brief dormancy and germinate under suitable condition. There are two calyx lobes, an awn like one opposite the keel and a broad opposite the back of the standard. The flower has 10 stamens, two of which are usually not fully developed. The pistil consists of an ovary, style, and stigma. Anthesis and pollination usually occur at sunrise with pollination taking place within the closed keel of the flower. The mature pod is indehiscent legume containing one to five seeds, enclosed in papery testas. The seeds do not contain an endosperm but have two large cotyledons, an epicotyl with three meristems, hypocotyls, and a primary root epidermis. A unique characteristic of the peanut plant is the nyctinastic movements of the leaflets (Coffelt, 1989). The leaf blade consists of four oval to obvate leaflets attached to the midrib by small articulations which allow for movement. During dark periods and hot sunny days, the paired leaflets are close together in a vertical position, and on a normal day leaflets are separated from each other in a horizontal position.
Flowers

Flowers are born on inflorescence located in the axils of the leaves. Flowers are never at the same node as vegetative branches, although very short internodes on some plants may make it appear that they are (Coffelt, 1989). Environmental conditions may cause the transformation of reproductive axes into vegetative axes, but not the reverse. The first flowers appear from 4 to 6 weeks after planting. Each flower is subtended by two bracts; the lower, on an axis of the inflorescence and the upper in the axil of the lower bract. The flower contains five petals: a standard, two wings, and two petals fused to form a keel. The first written account of the crop is found with the Spanish entry into Hispanola in 1502, where the Arawak cultivated under the name of mani (Sauer, 1993). Records from Brazil around 1550 showed the crop was known there with the name mandubi. Early Spanish and Portuguese accounts record the presence of crop through of the West Indies and South America.

Vegetative growth

The peanut (Arachis hypogaea L.) is an annual legume, unusual in its genus being polyploid (4x=40). It can interbreed only with another species A. monticola, the probable wild progenitor of the crop (Sauer 1993). The cultivated peanut plant is an erect or prostrate, usually 15 to 60 cm tall. It is sparsely hairy and, and has a well developed tap root system with many lateral roots. Roots are usually devoid of hairs, and a distinct.

Origin

The archaeological records support its cultivation between 300 and 2500 BC in Peruvian desert oases (Weiss, 2000). Although no archaeological evidence of peanuts has been uncovered in the area due its tropical climate, the Gurarani region of Paraguay, eastern Bolivia, and central Bolivia showed the greatest diversity of wild varieties of Arachis species. The cultivated peanut was likely first domesticated in the valleys of the Paraguay and Prarana rivers in the Chaco region of South America. The plant is believed to have been originally domesticated by predecessors of the Arawak speaking peoples who now live in its homeland.

Morphology

Annual legume and produce angular hairy stems with spreading or erect branches having root system with a well developed lateral (Secondary) root system. Root nodules start developing on top and lateral roots when plants are about 15 days
Plants are prostrate herbs leaves. Leaves first appear in the embryo and no new ones are formed until much later. They are almost always tetrafoliate except in a group "erectates having trifoliate leaves. The leaves of the cultivated groundnut are paripinnate with two piece of opposite, sub-sessile, obovate leaflets with entire ciliate margin. They are born spirals in a 2/5th phyllotaxy. The leaves are arranged on the main axis and higher order branches in a distichous fashion. Stipules are prominent, linear and adnate, becomes free of petiole at the pulvinus. The leaflets are borne on a slender, grooved and jointed rachis. The leaves exhibit nyctiotropic movements. Groundnut cultivars differ in their leaf colour, shape and hairiness and size. The stomata present on both sides of leaf (Fig. 1.2).

**Fig. 1.2 The Groundnut plant**

**Stem**

The young groundnut stems are angular, often pubescent and solid with a large pith. With aging, stems with hallow cylindrical and shed hairs. In annual species, twigs do not turn woody. The main axis develops from the terminal epicotyls bud and is flanked by two opposite cotyledonary laterals (n+1). The stem thickness is also a variable character, "bunch" types generally have thicker stems than "spreading" varieties. The internodes are short, highly condensed at the base but are large at the higher nodes.
Root system

In moist soils at 27°C, the primary root will emerge in 24-36 hours growing about 0.4-0.5 cm in 4 days. Lateral roots appear after second day and can be as many as 100 in 5 days. During the first few hours of germination, the radical consists of about half hypocotyl and half primary root, depends on planting depth. With its annual herbaceous nature, groundnut has a fairly well developed root system and a taproot. The adventitious roots commonly form from the aerial branches that come in contact with soil. A well developed tap root may penetrate to a depth of 130 cm but rarely goes beyond 90 cm. The root system is normally concentrated between a depth of 5 and 35 cm. The spreading confined to a radius of 12 to 14 cm. The spreading types have a more vigorous root system than the bunch types.

Inflorescence

• Solitary or in raceme, very irregular (Papilionaceous)

Flower

Orange yellow in colour, papilioneous with standard wing and keel, bisexual, zygomorphic, complete and sessile. Self pollinated crop and pollination takes place early in the morning. After pollination, meristematic region grows at the base of the ovary and become a stalk like structure (gynophore) referred to as Peg which bend downwards and forces the ovary into the soil. The peg carrying the ovary pushes itself into the soil. Fruit is an indehiscent pod containing one to five seeds. Each seed consists of two cotyledons. Seed coat known as testa is papery and thin. Cotyledons contain oil and other food materials.

Peanut uses

History of use

The early records on peanut states that mani was a common food to the Indians of South America before the arrival of Columbus and other Spanish explorers. It was consumed as raw or roasted. It was also considered of having soporific, and anti inflammatory effect (Smith 2002). In Peru and Brazil it was used to prepare peanut milk and products similar to traditional almond confectionary. It was taken by Portuguese to Africa where it became an important part of diet. The peanut paste was used to thicken soups, stews and similar dishes, and the oil was used for culinary purposes. Before 1800, in the Caribbean and in the British colonies in North America slaves grew peanuts on small garden. Peanuts were used to feed slaves almost from
the earliest days of the European slave trade. White colonists do not appear to have consumed peanuts directly, but they used peanut to fatten swine and poultry (Smith, 2002). Early records of peanut use in the US shows that it was used as beverage as a good substitute for chocolate. Throughout the nineteenth century, peanuts were mainly sold roasted in their shells. All parts of the peanut plant can be easily utilized. The vines with leaves make an excellent high protein hay for horses and ruminant livestock. The shells or pods can be used as feed for livestock, burned for fuel, made into particle board, and many other uses. The peanut is grown mainly for human consumption of the seed. The seed can be used directly for food and crushing to produce oil and a high protein meal. Nearly two thirds of all groundnuts produced are crushed for oil (Bunting et al., 1985). Peanut oil can be used in cooking, lighting, fuel and as a food constituent. Peanut oil has a better keeping quality than soybean, corn, and safflower oils and is a good source of Vitamin E. Used directly as food, peanut is a major crop for subsistence (Hammons, 1973). The multiple uses of the peanut make it an excellent cash crop for domestic markets as well as foreign trade. In most parts of the world the peanut is utilized primarily as whole seeds. The most common method of preparation for human consumption of whole seeds is dry roasting the seed (Coffelt, 1989). The peanut is well-established snack food as fresh cooked and roasted peanuts. In the USA, the major use of peanut is for grinding into peanut butter. Peanut is also used as peanut flour, concentrates, and isolates. These serve as potential extenders in many meat formulations. Peanut flour has been used to replace part or all of wheat flour or corn meal in making various types of breads and other bakery products. Peanut protein isolates have for many years been used in the manufacture of imitation milk as an extender to cow or buffalo milk. Peanut protein isolate and peanut oil have been used to make cheese analogs for the production of cream cheese and cheese spread products.

**Nutritional Quality**

It is estimated that the shell represents about 25% of the dry weight of unshell peanut, and the kernel comprises 75%. Cotyledons are the main storage tissues and are a concentrated source of protein, lipids, and dietary energy. Amino acid profile of raw peanut is in many respects inferior to the profile of raw soybean. Comparatively, the protein content of raw peanut is only about 70% of that of raw soybean. Peanuts
and peanut protein products are low in Sulfur based amino acids. Peanuts are a reasonable source of dietary minerals especially potassium, phosphorus, and magnesium. However, they are poor source of fat soluble vitamins like A, D and K. Peanut oil is an excellent source of mono- and polyunsaturated fatty acids, exceeding the levels of these fatty acids in soybean and corn oil, but significantly lower than in sunflower and safflower oil. Peanut oil contains about 1% palmitic acid and 80% oleic and linoleic acid.

Disease

Groundnut is a crop which is mainly cultivated under rain-fed conditions, thus, pathogens have more of a chance to attack the crop. Grover (1981) listed more than 55 pathogens in groundnut crop. Only a few, such as early leaf spot (*Phaeoisariopsis arichidicola*) late leaf spot (*Phaeoisariopsis personata*), rust (*Puccinia arichidis*), collar rot (*Aspergillus niger* van Tieghem), stem rot (*Sclerotium rolfsii* Sacc.), root rot (*Macrophomina phaseolina*), and aflaroot (*Aspergillus flavus*). are economically important in India. Nematode diseases like root knot and viral diseases like peanut bud and stem necrosis, groundnut mottle and clump (Ghewande and Reddy, 1986) are major diseases that limit groundnut production and productivity. In addition, the pre- and post-harvest aflatoxin contamination in the kernels and meal also reduces groundnut quality as well as export value.

Collar rot

The *A. niger* causing collar rot disease on groundnut seedlings was first reported by Jochem (1926). However, Jain and Nema (1952) first reported the *Aspergillus* blight of groundnut caused by *A. niger* in India. This disease appears in two phases viz, pre-emergence and post-emergence phase. In the pre-emergence phase, the seed may rot in the soil or be covered with sooty black masses of spore on germination, the emerging hypocotyls are rapidly killed by these spores. In the post-emergence phase, circular light brown lesions appear initially on the cotyledons and as they advance the hypocotyl tissue or stem lesion becomes water-soaked and shows light brown discoloration. The seedlings then collapse and die due to the rotting of the succulent hypocotyls (Fig. 1.3). Collar rot is prevalent in almost all groundnut growing states of India viz, Punjab, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Gujarat, Maharashtra, Rajasthan, Karnataka and Orissa. Collar rot is causing more damage in sandy loam and medium black soil. The most of the groundnut
cultivars are susceptible to this disease. *A. niger* may cause an average 5 per cent loss in yield but in some areas it may cause as high as a 40 per cent loss. Collar rot is a more serious problem in sandy soil (Gibson 1953; Chohan, 1965). In Punjab (India), the mortality losses of plants due to *A. niger* may amount to 40 to 50 per cent (Aulakh and Sandhu 1970). Joshi (1969) surveyed groundnut growing areas in the state of Gujarat (India) and found as high as 50 per cent seedling blight in some fields. Similarly, Ghewande *et al.* (2002) reported that losses in terms of mortality of plants due to collar rot range from 28 to 50 per cent. Thus, among the diseases associated with groundnut, collar rot (*A. niger*) is one of the most important. Collar rot causes heavy losses in pod and fodder yield of groundnut. Most of the varieties of groundnut are susceptible to this disease. Many seed dressing fungicides are reported to be effective against collar rot of groundnut (Gangopadhyay *et al.*, 1996; Karthikeyan 1996). But limited work has been done on successful exploitation of plant defence mechanism for the management of collar rot disease.

![Fig. 1.3 Collar rot disease of groundnut](image-url)
Leaf Spot

Each year, peanut leaf spot is the most prevalent peanut disease in India. It causes defoliation, and thus, can cause yield reductions of over 50 percent when not controlled and less than five percent when a total control program is utilized. Currently, it is estimated that peanut leaf spot is causing an annual 10-20 percent loss in India. Not too many years ago state-wide losses attributable to peanut leaf spot exceeded 50 percent. Actually, two leaf spot diseases occur but together they are called peanut leaf spot (Fig. 1.4). Early peanut leaf spot, caused by the fungus *Cercospora arachidicola*, usually is the first to occur. It is characterized by a round, brown-red spot and may have a yellow halo. Late leaf spot is caused by a related fungus, *Cercosporidium personatum*, and is characterized by a somewhat round spot that is black on the underside of the leaflet and it may or may not have a yellow halo. Lesions (spots) of either leaf spot disease may be found in leaflets, petioles, stems and pegs. However, lesions are not found in petioles, stems or pegs until later in the season or after numerous lesions are found on leaflets. Occasionally, chemical burns from insecticides or cracking time herbicides cause dark spots that may be confused with leaf spot. Microscopic spores which are produced on the surface of the lesions are disseminated by wind, rain or irrigation. When the leaf, petiole or stem surface is wet, the spores germinate and penetrate the tissue. Within 10 to 14 days after these infections occur, new lesions with more spores are produced. Leaf spot causes premature leaf drop. Fallen leaves with lesions will provide primary inoculum (spores) for the next season if peanuts are planted in the same or adjoining fields.

![Fig. 1.4 Leaf Spot disease of groundnut](image)
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