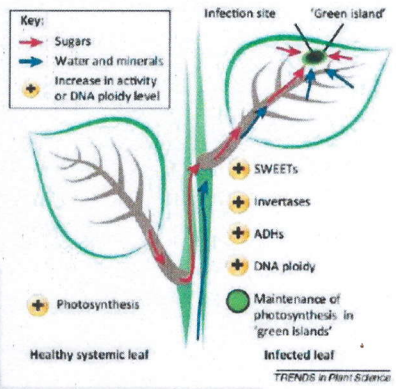
Plant Resistance to Pathogens



Molecular and Genetic Bases of Plant-Pathogen Interactions.
Course material

Dr. Abdelmoumen Taoutaou

Botany Dept.

Ecole Nationale Supérieure Agronomique, Algiers

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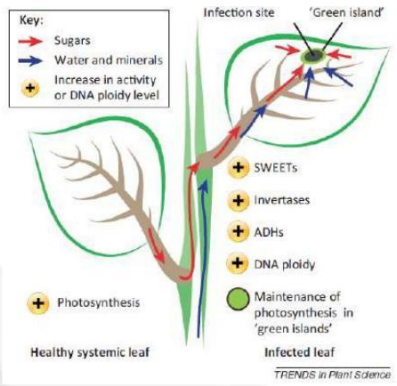

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Objectives

At the end of this course, students should be able to :

→ Explain plant defense mechanisms against pathogens

→Distinguish between biotrophic and necrotrophic defense mechanisms

→Distinguish between different types of host plants, as well as between different types of resistance

→Explain the inducers of defense reactions

→Explain the mechanisms of action of resistance and susceptibility genes

→Explain the transmission of information signals in plants for the induction of the immune response

→Distinguish between the different types of defense mechanisms and the different molecules involved

→defining the advantages and limitations of different resistance enhancement tools

Introduction

As primary producers (of nutrients, energy), plants are the target of many organisms (except carnivores). They are therefore under constant pressure to defend themselves. They have developed a sophisticated defense system. This has made them (plants), in general and in most cases, resistant to most pathogens. Disease, in fact, is the exception in a plant's life cycle. Plant-pathogen interaction is a series of events that result in a dialogue between the two living beings.

This document is the sequel to the first one: Mechanisms of Pathogenicity of Plant Pathogenic Fungi. The latter detailed the mechanisms used by different pathogens to successfully infect plants. In this work, we will outline the different mechanisms plants use to defend themselves against pathogen aggression.

I General Concepts about Resistance

1. Resistance not Host

k *Definition*

This is the resistance of all the genotypes of a plant species against all the genotypes of a pathogen.

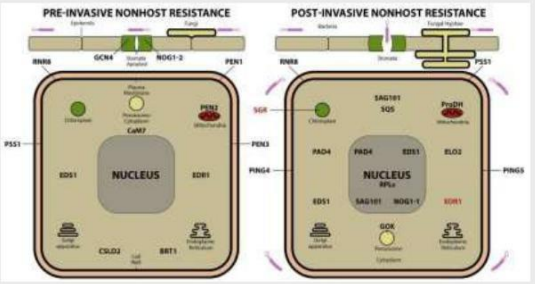
A *Example*

All genotypes (and individuals) of potato (*Solanum tuberosum*) are resistant to bean rust caused by *Uromyces fabae*.

All individuals of the *Triticum aestivum* species (soft wheat) are resistant to *Phytophthora infestans.* (agent of potato and tomato late blight).

Non-host resistance can be pre-formed or induced.

Fig. 1.1. non-host resistance before and after invasion by the pathogen (Fonseca & Mysore, 2019).



Fundamental

This is a pre-existing fundamental mismatch between the host and the potential pathogen, preventing germination or penetration of the pathogen or infectious molecule into the plant (presence of pre-formed substances in the host).

Example

Morphological or biochemical maladjustment of its surfaces or natural openings (see the chapter on infectious recognition and structure in the pathogenicity mechanisms course), absence of temporal or spatial coexistence of the protagonists, absence of certain molecules essential to the parasite's early stages of development, etc.).

A few cases of resistance linked to morphological structures or biochemical features of the host surface are sometimes cited:

- Cuticle thickness and topography,
- Stomata location and shape,
- Presence of surface inhibitors acting on parasite germination (substances produced/stored in trichomes)

Figure 1.2. Lotus leaf topography. Leaves have hydrophobic properties due to their high wax content (Garbone & Mangialardi, 2005*).

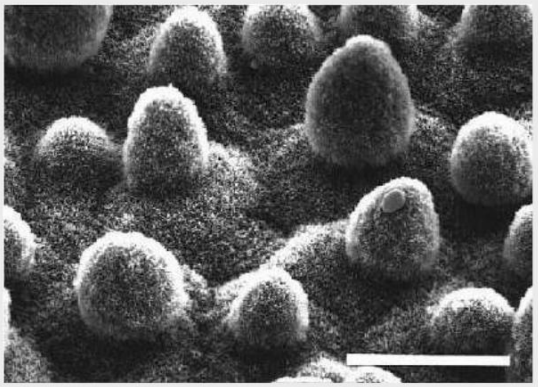
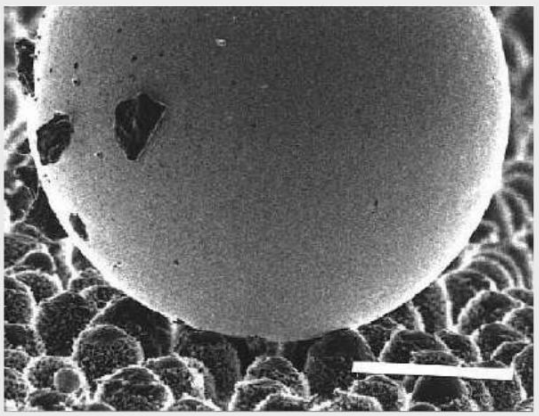


Figure 1.3. The texture and hydrophobicity of lotus leaves give them self-cleaning properties (Garbone & Mangialardi, 2005*).



2. The Host Resistance

k *Definition: Host plant*

A host plant is a plant that can be infected by a given pathogen.

k *Definition: Host resistance*

Is the resistance that certain individuals (one or more) of this host plant show to a given pathogen.

Figure 1.4. Beans resistant (left) and susceptible (right) to rust. caused by *Uromyces appendiculatus* (Schumann & D'Arcy, 2013*).

Figure 1.5. Potato susceptible (foreground) and resistant (background) to late blight caused by *Phytophthora infestans* (Schumann & D'Arcy, 2013).

Figure 1.6. Potato leaves with different levels of resistance. The most resistant genotypes (left) contain an extremely low number of lesions and are small in size, while the most susceptible (right) have a higher number of lesions and are large in size (Schumann & D'Arcy, 2013).

Qualitative Resistance

The resistance of a certain number of genotypes of a plant species against a certain number of genotypes (one or more) of a pathogenic species.

Note

Here, the plant is classified as either resistant (no disease) or susceptible (disease is present).

Attention

Qualitative resistance is controlled by a single gene: monogenic resistance

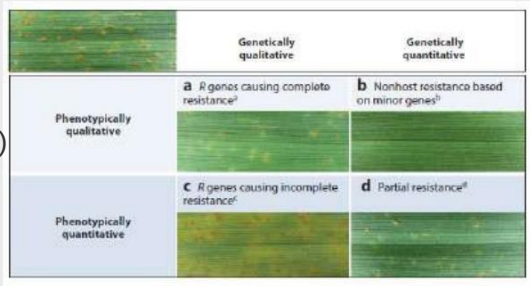
This gene confers total resistance (100%) against one race (genotype) of the pathogen.

Other genotypes of this species lacking this gene are susceptible to this race of the pathogen.

Quantitative Resistance

It is the resistance of all individuals of a species against all individuals of the pathogen up to a certain level. This resistance is not total. It is partial. It is controlled by several genes. Each gene contributes a part to this resistance. It's a resistance that can be quantified: plant resistance at 40%, for example.

Figure 1.7 Qualitative and quantitative resistance (Niks et al., 2015**)



Qualitative and quantitative resistance

3. Host Marginal

k *Definition*

These are species in which almost all genotypes are resistant to a pathogen, but only a few show levels of susceptibility, generally lower than in the pathogen's main host.

4. Resistance Preformed

k *Definition*

This is the set of characteristics that the plant develops naturally in the absence of any contact with the pathogen, and which contribute to the plant's resistance to the pathogen.

Pre-formed resistance is also known as constitutive or passive resistance. It is constituted by structures and molecules already existing in the plant before infection:

Trichomes

Wax on the leaves (ensuring a degree of hydrophobicity) to eliminate water stagnation on the leaves,... Cell wall: cellulose, pectins, etc.

Anticipins,... polyphenols, flavonoids, etc.

4.1. Roles

The role of pre-formed resistance (passive, constitutive) is to make the plant resistant to the majority of potential pathogens. It ensures incompatibility between the plant and the majority of potential pathogens.

4.2. Resistance types Preformed

There are two types, according to their nature:

4.2.1. Chemical Resistance

This refers to any molecule synthesized by the plant before the presence of a pathogen is detected, and which ensures a certain degree of plant resistance against the various potential pathogens.

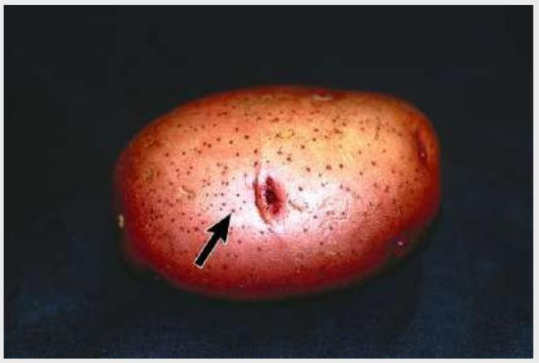
Figure 1.8. Onion anthracnose (caused by *Colletotrichum circinans*) mainly affects white onions. Red ones are generally more resistant because of the biochemical molecules that color their bulbs (Schumann & D'Arcy, 2013*).

4.2.2. Physical resistance

These are the plant's various physical structures, which also provide a defense against the majority of potential pathogens.

Figure 1.9. The wax-covered surface of this pepper makes the fruit hydrophobic, preventing water from stagnating on its surface and thus preventing pathogen spores from germinating (Schumann & D'Arcy, 2013).

Figure 1.10. Potato tuber epidermal cells contain suber, which reinforces their walls, providing a very good defense against many pathogens. Lenticels (arrows) are usually inconspicuous and invisible, expanding in moist soils, offering an entry point for pathogens (Schumann & D'Arcy, 2013).



5. Induced Resistance

k *Definition*

Resistance resulted from the plant's detection of the pathogen. It manifests itself as post-infectionnel (after contact with the pathogen).

Figure 1.11. Papilla formation is an induced physical defense mechanism. The papilla (arrow) is formed around the fungal hypha (penetration tip) at the site of penetration i n an attempt to prevent the pathogen from penetrating (Schumann & D'Arcy, 2013*).

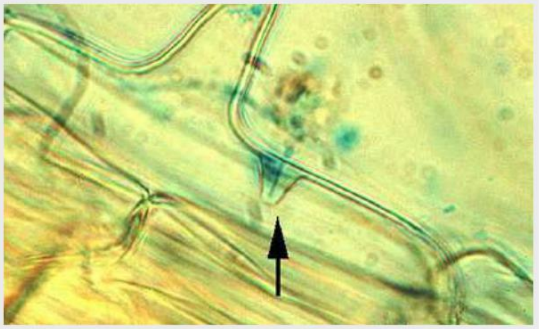
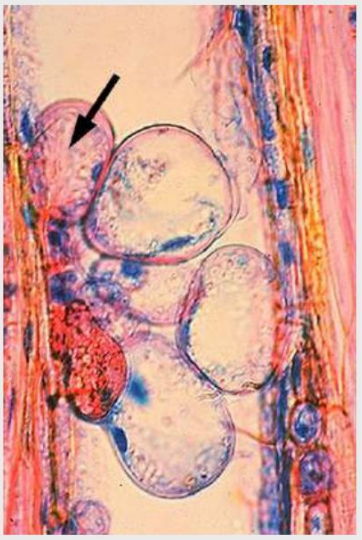


Figure 1.12. Tyllosis are also defense structures induced after pathogen detection (Schumann & D'Arcy, 2013*).



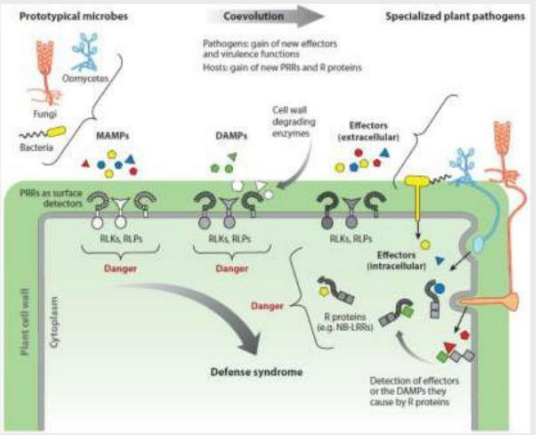
To trigger defense responses, plants need to detect and recognize pathogens. Over the course of evolution, they have developed different mechanisms for recognizing different microorganisms and classifying them as beneficial, neutral or pathogenic. Defensive responses are only triggered when pathogens attempt to penetrate (infect) the plant.

The plant recognizes pathogens by detecting their signatures, which are called elicitors. Because they induce plant defenses. There are several types of elicitors:

1. Microbe-Associates Molecular Patterns (MAMPs): These are molecules synthesized by different microorganisms. These molecular patterns are common to different groups of microorganisms.
2. Pathogen-Associated Molecular Patterns (PAMPs): These are molecules shared only by several groups of pathogens. Non-pathogenic microorganisms do not produce this type of molecule.

3. Damage-Associated Molecular Patherns (DAMPs)
   These are the molecules that result from the interaction between the pathogen and the plant. For example, cutin monomers are produced when the pathogen secretes cutinases to degrade the plant cell wall. These cutin monomers are recognized by the plant as DAMPs, indicating the presence of the pathogen.
4. Effectors

Figure 1.13. Elicitors such as MAMPs, even those resulting from the interaction between plant and pathogen (DAMPs) and effectors, are perceived by the plant as danger signals. Both MAMPs/PAMPs and DAMPS are detected by *Pattern-Recognition* Receptors (PRRs). In t h e course of evolution, pathogens acquire effectors as virulence factors, so plants evolve and develop resistance proteins whose role is to detect and recognize effectors (inter- or intracellular). After recognition of elicitors and effectors by PRRs and R proteins, the plant triggers defense mechanisms. RLK: *Receptor-Like Kinase*, RLP: *Receptor-Like Protein*, NB-LRR: *Nucleotide-Binding Site Leucine- Rich Repeat* (Boller & Felix, 2009*).



Fundamental: Elicitors and Effectors

In this document, the term elicitor is used as the English equivalent of MAMPs, PAMPs and DAMPs. The term elicitor does not include effectors.

There are two types of induced resistance, based on the type of molecule recognized by the plant and which betrays the presence of the pathogen:

5.1. Resistance induced by Elicitors

k *Definition: An Elicitor*

An elicitor is any molecule produced by the pathogen or resulting from the interaction between the pathogen and the plant, enabling the plant to detect the presence of the pathogen.

k *Definition*

This is resistance induced by the detection of the pathogen's presence through the plant's recognition of one or more elicitor molecules.

5.2. Resistance induced by Effectors

This is resistance induced by detection of the pathogen's presence through recognition of an effector by the plant.

Fundamental: Effectors and Avirulence Proteins

Now we consider Avr proteins to be a class of effectors.

Avr proteins are effectors that can be detected by the plant via R proteins, and consequently induce the plant's defense response to the pathogen. This type of resistance is known as *Effector Triggered Immunity* (ETI).

5.3. Exploiting Induced Resistance

After exposure to a stress (abiotic or biotic), plants develop responses that help them cope with the stress. Generally speaking, the latter will trigger resistance mechanisms that will be effective against a number of stresses.

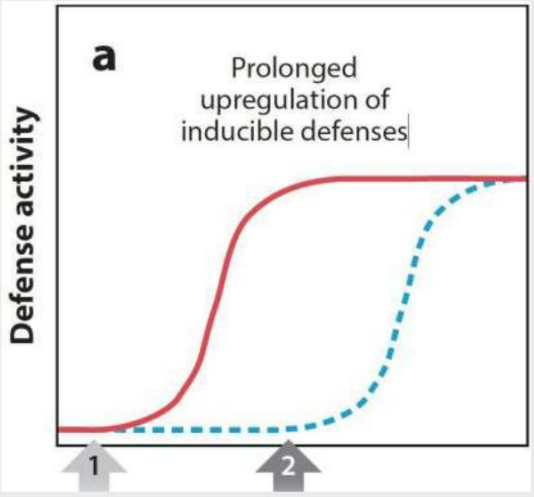


Figure 1.15. Resistance induction strategy (Wilkinson et al., 2019*).

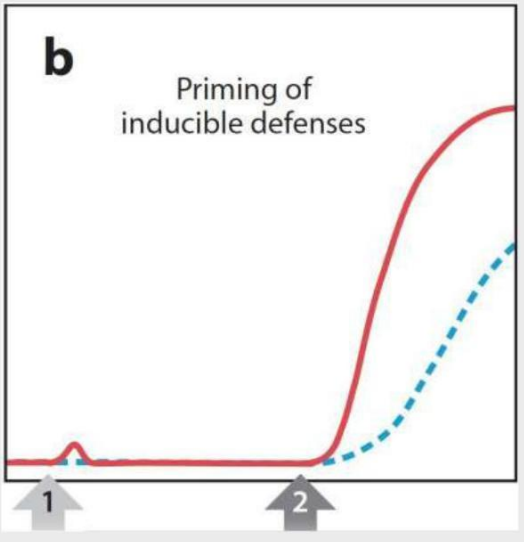


Figure 1.16. Resistance induction strategy (Wilkinson et al., 2019*).

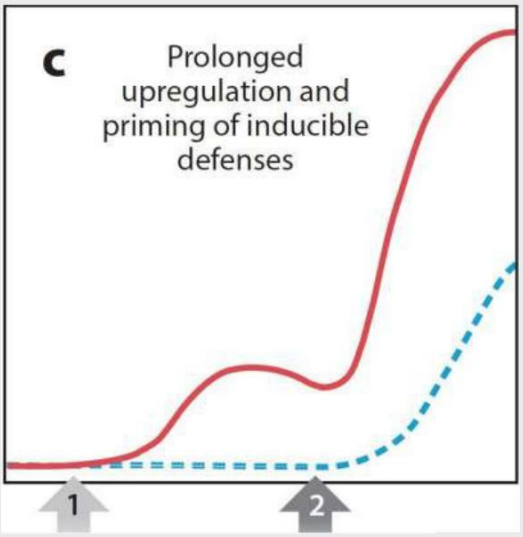


Figure 1.17. Resistance induction strategy (Wilkinson et al., 2019*).

6. The Durability of Resistance

Resistance can only be described as durable if

It is resistance that remains effective for a long period after large-scale use of the resistance and in an environment favorable to the pathogen.

7. Evolution of Host and non-Host Resistance

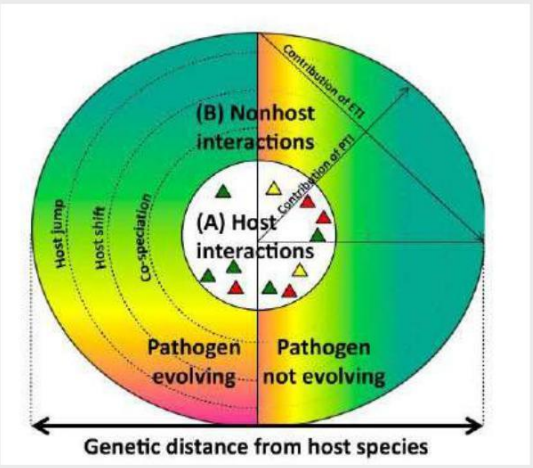


Figure1. 18. The evolution of host and non-host resistance (Gill et al., 2015).

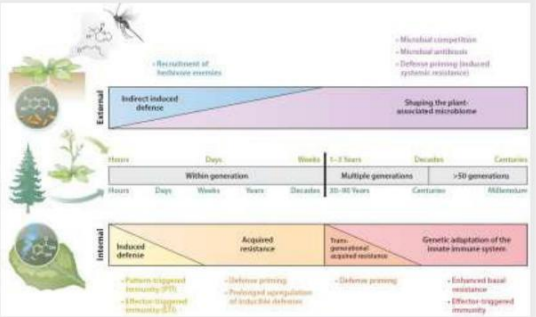


Figure 1.19. Long- and short-term strategies used by plants to adapt to different stresses
(Wilkinson et al., 2019*).

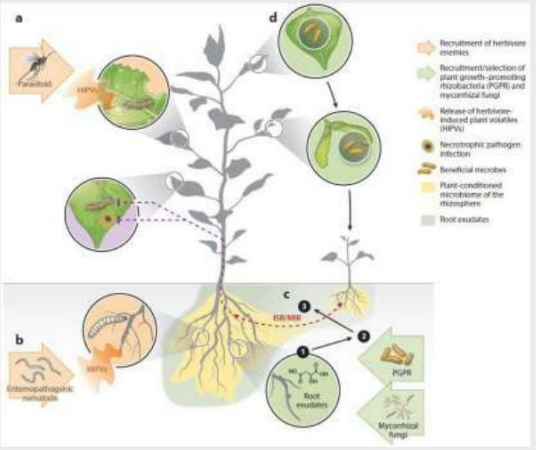


Figure 1.20. External strategies used by the plant to resist various biotic and abiotic stress factors

(Wilkinson et al., 2019*).

II Elicitors

1. Introduction

k *Definition*

An elicitor is any molecule produced by the pathogen or resulting from the interaction between the pathogen and the plant, enabling the plant to detect the presence of the pathogen.

To detect the presence of a pathogen, the plant needs one or more markers that are more or less specific to the pathogen. The degree of specificity of this molecule (marker) will enable the plant to identify the pathogen and respond in a specific and/or general manner.

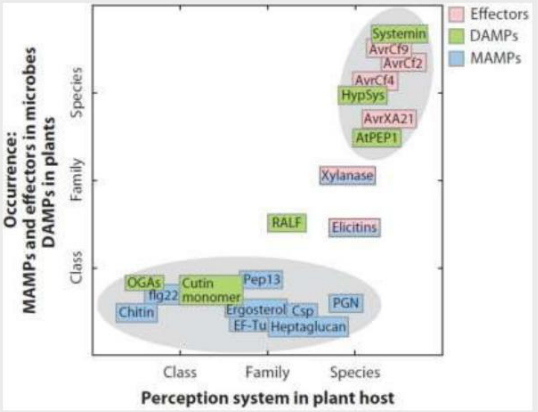
2. The different types of elicitors

elicitors can be classified according to their degree of specificity or their origin:

2.1. According to their Specificity

The specificity of elicitors varies according to the degree of conservation of these molecules in different taxonomic classes. It ranges from highly specific molecules, down to the race level, to highly ubiquitous molecules, common to all members of a kingdom.

Figure 2.1. The degree of specificity of different types of elicitors. MAMPs (and PAMPS) are present in several microorganisms, generally found in the taxonomic classes of pathogens, e.g. chitin in fungi,... DAMPS can be specific, as in the case of systemin, or common to several plant classes, as in the case of cutin monomers. Effectors are specific (Boller & Felix, 2009*).



2.1.1. General Editors

These are the molecules that indicate the presence of a pathogen.

These molecules are generally common to several pathogen species.

A *Example*

Chitin in fungi

Note

In English, we speak of PAMPS: Pathogen associeted molecular pathern.

These are elicitors common to several groups of pathogens. For example, molecules common to all pathogenic fungi, molecules common to phytopathogenic bacteria,...

Note

Common molecules between pathogenic and non-pathogenic microorganisms are known as "MAMPs".

MAMPS: Microbial Associated Molecular Pattern.

2.1.2. Specific elicitors

It is these molecules that enable the plant to recognize the pathogen, even at the level of the race.

A *Example*

Effectors are highly specific elicitors.

2.2. According to Their Origins

2.2.1. Pathogen-derived elicitors

These are the molecules that indicate the presence of the pathogen.

Their specificity varies according to the molecule (see previous point).

2.2.2. Elicitors from Plant-Pathogen Interaction

These are the molecules resulting from the interaction between the pathogen and the plant.

A *Example*

Glucose: This is the result of the degradation of cellulose (from the plant) by cellulases (from the pathogen).

a) Constituent Elicitors

These are molecules that exist in the plant even before the arrival of the pathogen and are released following the pathogen's action.

A *Example*

Glucose molecules, cellobiose, ...

b) Induced Elicitors

These are molecules sunthetized by the plant following infection by the pathogen, whose role is to induce (elicit) immune responses.

III The genes of Resistance

1. Introduction

Resistance genes play a vital role in plant resistance to disease. The majority of these genes are dominant. Only a small number are recessive. Resistance genes provide total or partial protection against one or more pathogens.

Figure 3.1. The number of cloned resistance genes (up to 2018). The first was the *Hm1* gene in maize in 1992. The Hm1 gene codes for a protein that detoxifies the *Helminthosporium carbonum* toxin, HC toxin. The second was the *Pto* gene in tomato, providing resistance against *P. syringae* pv. *tomato* in 1993. Then, in 1994, the *Cf-9* gene, also from tomatoes, against *Cladosporium fulvum.* Since then, hundreds of resistance genes have been cloned.

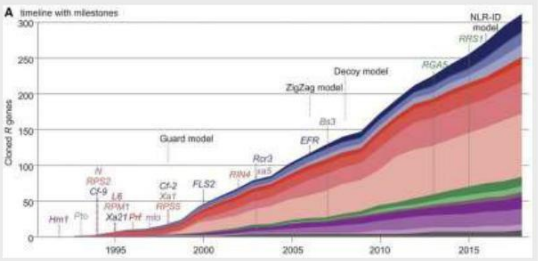
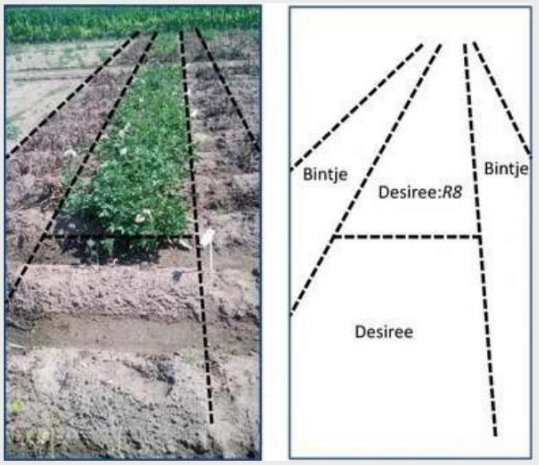


Figure 3.2. Effect of resistance genes on plant-pathogen interaction. The desired potato variety was genetically transformed by introducing the R8 late blight resistance gene (desired:R8) in a field trial. Desiree and Bintje are both susceptible to late blight in their natural state. Désirée transformée (Désirée: R8) is resistant (to the environment, while désirée and bintjé are devastated by the disease (Vossen et al., 2016).



k *Definition*

A resistance gene (*R*) is any gene that determines a difference in susceptibility to a pathogen (Michelmore et al., 2013).

One of the models explaining plant resistance to pathogens is the gene-for-gene model. It states that in a plant-pathogen interaction, resistance will only occur if there is recognition between a gene of

dominant resistance gene (*R*) from the plant and a dominant avirulence gene (*Avr*) from the pathogen. Alternatively, for each resistance gene (*R*) there is an avirulence gene (*Avr*). This model, developed by Flor (1971), is now considered over-simplified.

Now, we know that to recognize a pathogen, sometimes the plant needs more than one resistance gene, in addition to membrane receptors (Pattern Recognition Receptors (PRRs)) which are responsible for detecting pathogens based on the recognition of elicitors (PAMPS and DAMPS) (see chapters: Elicitors and Recognition, in this course, and chapter: Effectors in the "Mechanism of pathogenicity" course). Also, in certain situations, a single resistance gene is responsible for a plant's resistance to several pathogens.

Figure 3.3. Number of cloned resistance genes per host plant (Kourelis & vad der Hoom, 2018*).

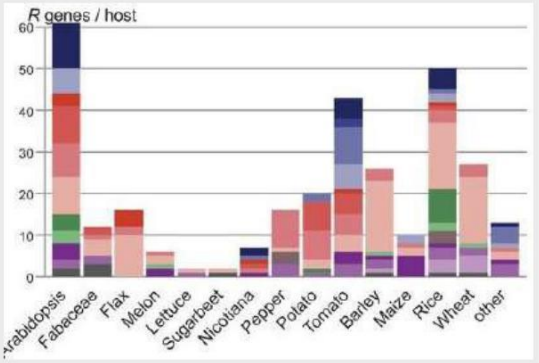


Figure 3.4. Phylogeny of certain plants and gene quantities of resistance that each plant has (Barragan & Weigel, 2021*).

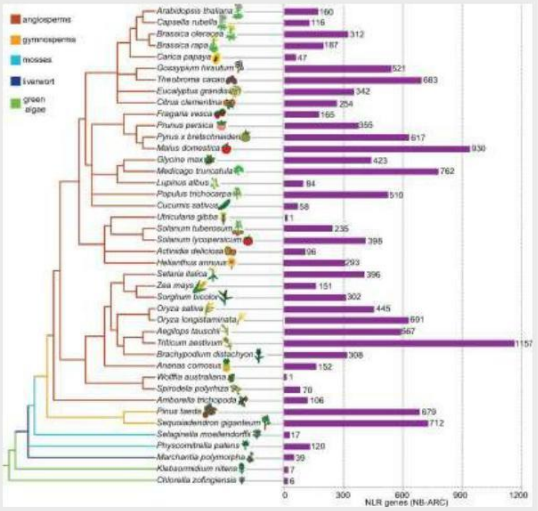


Figure 3.5. The number of resistance genes (NOD-like recepetor, NLR) relative to the genome of selected plant species (Borrelli et al., 2018*).

Figure 3.6. The number of cloned resistance genes (Kourelis & vad der Hoom, 2018*).

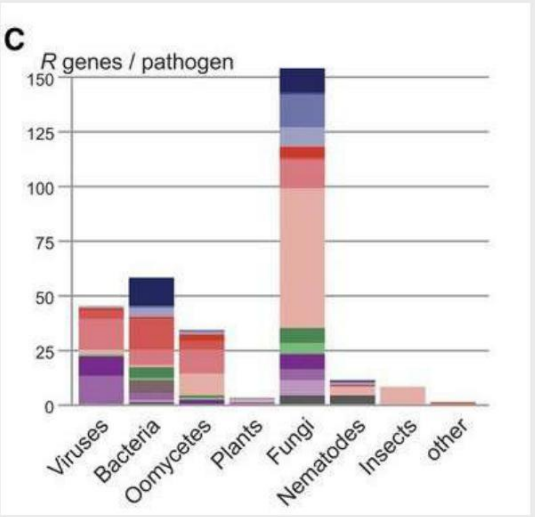


Figure 3.7. Schematic diagram of the structure of a typical NB-LRR resistance protein (Lukasik & Takken, 2009).
Legend: Orange: CC-TIR domain, Red: NB, Pink: ARC1, Blue: ARC2, Green: LRR; retained patterns are represented as lines, with the sequence displayed next to each.

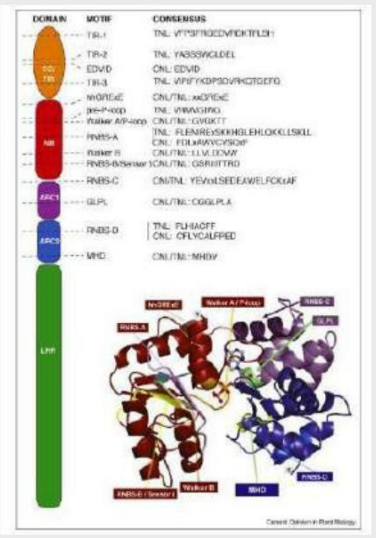
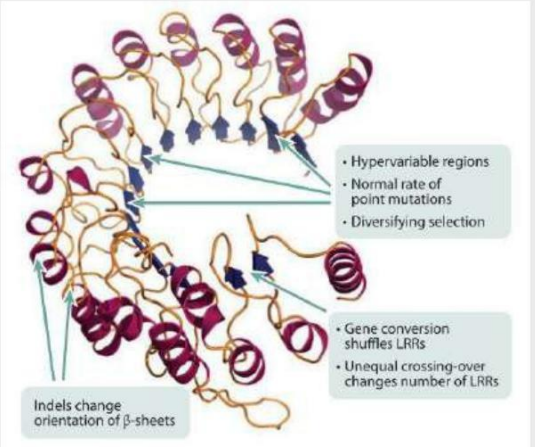


Figure 3.8. Schematic of the C-terminal portion of the Leucine Rich Repeat (LRR) fragment of the lettuce Dm3 gene illustrating likely events in the event of a change in binding specificity (Michelmore et al., 2013).



A *Example*

Wheat needs 2 resistance genes: *Lr10* and *RGA2*, to induce resistance against rust,

In tomatoes, the Cf-2 gene confers resistance to *Cladosporium fulvum* and a nematode,

Also in tomatoes, the Mi gene provides resistance against a nematode, aphid and whitefly.

2. Characteristics of Resistance Genes

2.1. Highly preserved sequences

Resistance genes are made up of DNA sequences. Some of these sequences are highly conserved. They do not change even across different plant species and botanical families.

Generally, a gene contains at least one highly conserved sequence. The

most common highly conserved sequences are the following:

Figure 3.9. Amino acid sequence of the highly conserved regions of the *R8* gene, a potato resistance gene against the late blight causal agent *P. infestans* (Vossen et al., 2016*).

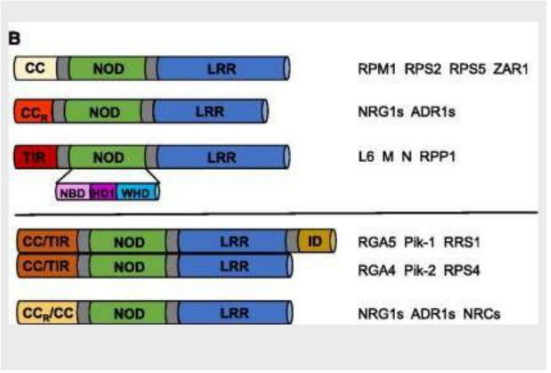


Figure 3.10. Structure of the various resistance genes (Wang & Chai, 2020).

2.1.1. Leucine Riche-Repeat

This sequence is very rich in the amino acid Leucine. This motif is repeated several times. So, it's a DNA fragment rich in Leucine, and this fragment is repeated in several copies in the same gene.

2.1.2. Nucleotide Binding Site

This is the domain to which an ATP/ADP molecule is attached for activation of the resistance protein.

2.1.3. Coiled Coil (Leucine Zipper)

This is a helical tail.

2.1.4. TIR Domain

This domain resembles the *Toll* gene, which controls the gigantism phenotype in Drosophila. It is also involved in Drosophila immunity, as well as the *Interleukin* domain in mammals.

2.1.5. Protein Kinases

This is the area where the phosophore attaches. It is involved in signaling.

3. The role of resistance genes

Resistance genes code for resistance proteins.

These proteins, known as resistance proteins, have the role of detecting and recognizing the pathogen.

Figure 3.11. Interaction of avirulence (virulence) proteins with resistance proteins (Ali et al., 2013*), according to the gene-for-gene theory.

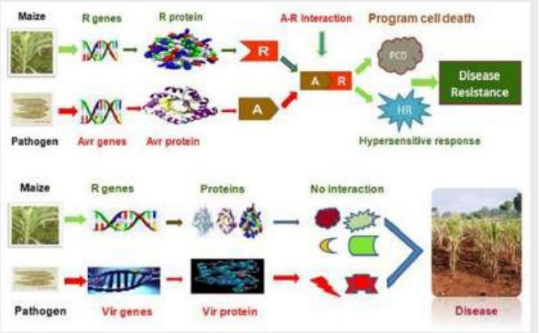
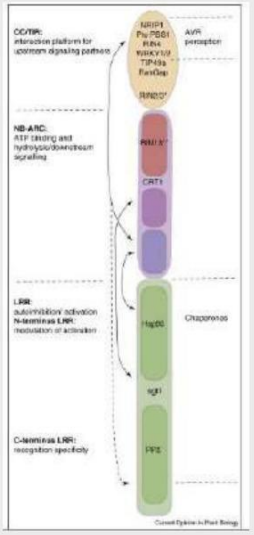


Figure 3.12. Roles of some NB-LRR resistance protein domains (Lukasik & Takken, 2009).



Fundamental

Resistance genes (resistance proteins) have no role in the process of "killing" the pathogen. Their role is to detect the pathogen.

4. Classification of Resistance Genes

Resistance genes have been classified according to their highly conserved DNA sequences. Several classifications have been made, but all have been based on the similarity of highly conserved sequences in these genes.

Attention

The classification of resistance genes varies considerably from one author to another.

4.1. Classification according to retained sequences

4.1.1. Class I

This class is characterized by the presence o f a variable N-terminal domain, and a highly conserved *Nucleotide-Binding Site* (NBS) domain, involved in signal transduction following interaction between the plant and a pathogen. In addition, there is a "Leucine-Rich Repeat" domain (LRR=domain rich in repeated leucine) containing a variable number of repeats in the C-terminus. This is responsible for pathogen recognition.

Fundamental

Genes in this class are known as NB-LRR, NB-ARC or NBS-LRR. This is the most widespread class of resistance genes. It contains more than half of all plant resistance genes (61% of cloned genes are of this type).

Genes in this class code for large cytoplasmic proteins (860-1900 amino acids). This class is divided into 2 subclasses:

Note

In current scientific literature, resistance genes belonging to this class are referred to as NLRs and NODs.

a) TNL subclass

Attention

TNL= Toll Interleukin Receptor (TIR) Nucleotide-Binding Site (NBS) Leucine-Rich Repeat (LRR)

Complement : The Toll gene

The Toll gene is a Drosophila gene. The name *Toll* comes from the German vernacular, and means super or fantastic. It was used in the early 1980s by C. Nu¨sslein-Volhard to qualify the phenotype of a new mutant discovered in her mutagenesis screen to dissect the genetic pathways controlling embryonic development in the fruit fly *Drosophila melanogaster*.

It took several years for researchers to realize that the Toll-like receptor (encoded by this gene) also has immune functions in adult Drosophila, and that these mammalian orthologs play a key role in innate immunity.

Complement: Interleukin

The term interleukin derives from "inter": as a means of communication", and "leukin": deriving from the fact that many of these proteins are produced by leukocytes and act on leukocytes".

Proteins of this type were first observed in white blood cells (leukocytes).

Proteins in the TNL subclass have an intracellular Toll-like signaling domain in the N-terminal region and at the mammalian Interleukin-1 (IL-1) receptor.

b) CNL subclass

Attention

CNL= Coiled-Coil (CC) Nucleotide-Binding Site (NBS) Leucine-Rich Repeat (LRR)

Proteins in this subclass contain at least one Coiled-Coil domain in the N-terminal region.

4.1.2. Class II

Genes in this class code for proteins with a transmembrane (TM) domain associated with an extracellular LRR domain, with a short cytoplasmic t motif in the C-terminal region, and no kinase domain.

A *Example*

Tomato resistance genes against *Cladosporium furvum*: *Cf-2*, *Cf-4*, *Cf-5* and *Cf-9*.

4.1.3. Class III

Class III genes are characterized by an extracellular LRR domain, a TM region and a Serine receptor. /Threonine Kinase (STK).

A *Example*

Genes of this type are found in *Arabidopsis thaliana* (600 genes) and *Oryza sativa* (1100 genes), ensuring the resistance of these 2 species to different races of *Xanthomonas* bacteria.

4.1.4. Class IV

Genes in this class have an STK (Serine/Threonine Kinase) domain. Also known as kinases. The proteins encoded by genes in this class are cytoplasmic proteins. They are characterized by an active cytoplasmic kinase which phosphorylates serine and threonine residues.

A *Example*

Tomato *Pto* resistance genes against the bacterium *Pseudomonas syringae* pv. *tomato*.

4.1.5. Class V

Genes in this class code for proteins with a spiral tail (CC) domain, which are attached to the cell membrane. Some proteins have both a TM and a CC domain.

A *Example*

The corn *Hm1* gene against *Cochliobolus carbonum*,

The barley *Mlo* gene against powdery mildew, and the *RPW8* gene (*A. thaliana*) against powdery mildew.

5. Evolution of resistance genes

In natural ecosystems, resistance genes are constantly evolving as pathogen effectors evolve.

Figure 3.13. Evolution of resistance genes interacting with pathogen effectors. Arms race-type co-evolution between plant resistance genes (here NLRs) and pathogen effectors. Direct recognition of effectors by resistance proteins can lead to diversification and expansion of the pathogen's effector pool and resistance genes in a host and pathogen population respectively. In an iterative process, the pathogen is forced to diversify and expand its effector r e p e r t o i r e to avoid recognition by existing resistance proteins in the host plant (steps 1 and 4). On the other hand, the plant will diversify and expand its range of resistance genes in response to the expansion of the pathogen's effector repertoire (steps 2 and 5) and, as a result, alleles encoding resistance protein variants effective in detecting and recognizing pathogen effectors will be selected and maintained in the population (steps 3 and 6) (Saur et al., 2020*).

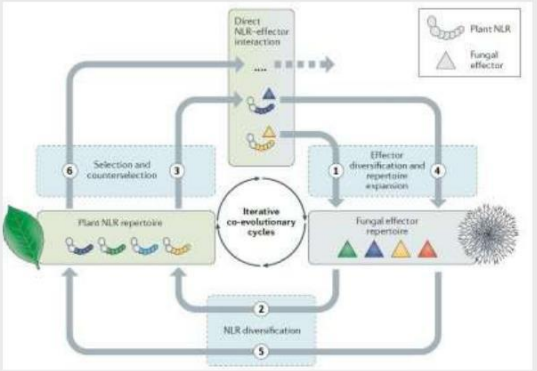
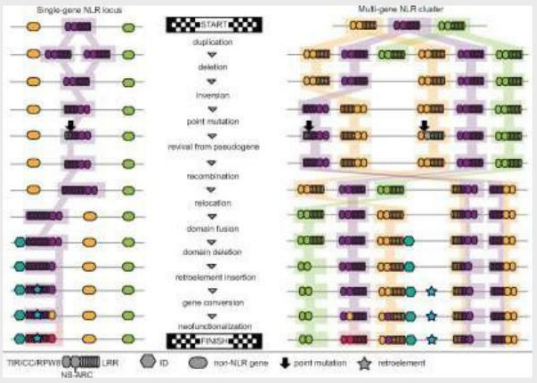


Figure 3.14. Examples of possible changes in genomic sequence and organization in the case of individual and clustered genes during evolution (Barragan & Weigel, 2021).



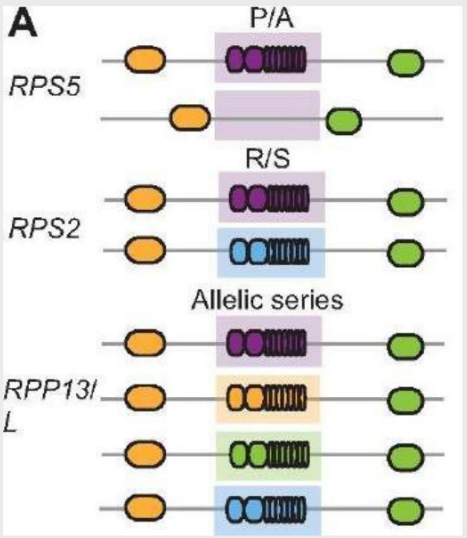


Figure 3.15. Examples of allelic variation at individual resistance gene loci. P/A: Presence/Absence; R/S: Resistant/Sensitive (Barragan & Weigel, 2021).

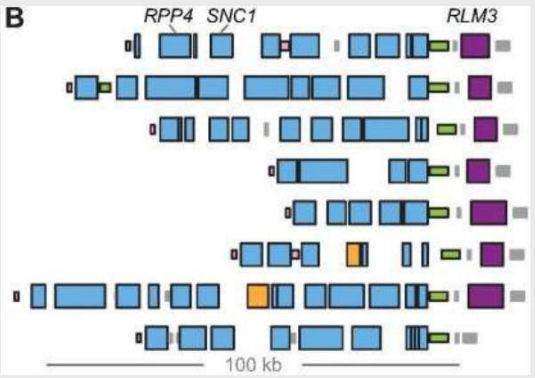


Figure 3.16. The RPP4/RPP5 cluster of resistance genes in 8 different *Arabidopsis thaliana* accessions. Long rectangles represent R genes and short rectangles are not R genes. Colours indicate sequence similarity. There is significant diversity in the number of blue rectangles of R genes in the cluster. In the adjacent RLM3 gene (purple), there is a presence/absence polymorphism. For the RPP4 cluster
/RPP5 has been invaded by other R genes that have no relation to the genes in the cluster (yellow). other genes (green, pink) that are not R genes have become entangled and duplicated in the cluster (Barragan & Weigel, 2021).

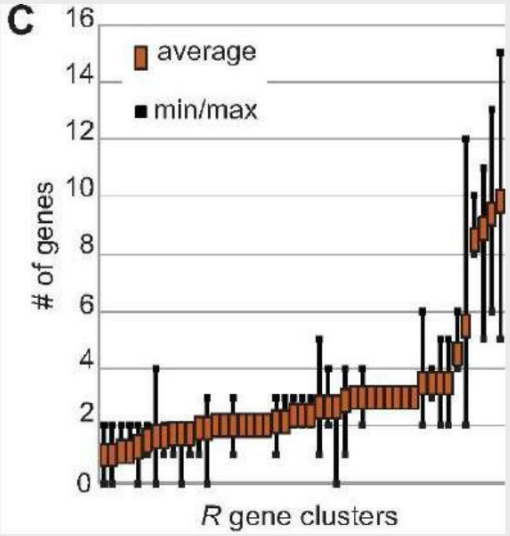


Figure 3.17. The number of R gene clusters per genotype in 8 *Arabidopsis thaliana* accessions. The average is 3, but some accessions have more than 10 R gene clusters (Barragan & Weigel, 2021).

IV The Susceptibility Genes

1. Introduction

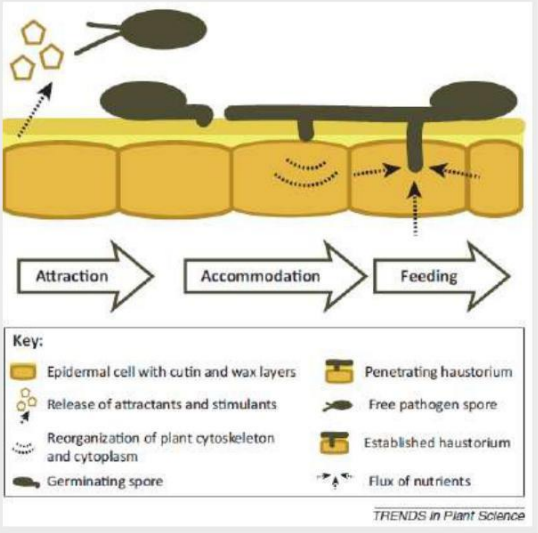
Susceptibility Genes

k *Definition*

Susceptibility genes are genes encoding proteins/molecules that facilitate infection and/or colonization and survival of the pathogen in a host plant.

Figure 4.1. The *PMR6* gene is associated with powdery mildew susceptibility in Arabidopsis. Arabidopsis seedlings inoculated with *Erysiphe cichoracearum*. The wild type (right) shows characteristic powdery mildew symptoms. The *pmr6* mutant (left) is completely resistant to infection and does not develop symptoms of the disease, although it does not show any of the well-defined characteristics of plant defense (such as host cell death), suggesting that *PMR6* encodes a host susceptibility factor.

Figure 4.2. The host plant promotes pathogen infection and development. The host plant with susceptibility genes attracts, promotes infection and installation of the pathogen and thus its fitness ((Lapin & Van den Ackerveken, 2013*).

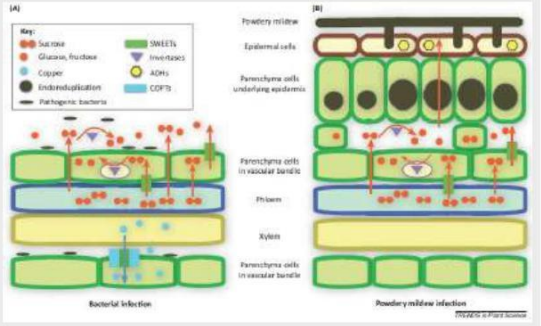


2. The Mode of Action of Susceptibility Genes

Susceptibility genes play an important role in plant life. Otherwise, plants will have eliminated them during evolution.

Pathogens use the plant's need for these genes to facilitate infection.

Figure 4.3. Mode of action of susceptibility genes in the case of bacterial, fungal or oomycete pathogens.



2.1. Pathogen installation

Successful infection and subsequent disease development require pathogens to be welcomed by the host plant, creating favorable niches for growth and spread.

Pathogens use genes that facilitate the installation of symbiotic microorganisms to their advantage. In fact, they take advantage of these mechanisms. Common symbiotic genes can act as susceptibility genes.

In Arabidopsis, mutation of several orthologs of legume symbiosis genes led to reduced susceptibility to mildew.

Mutation of the Medicago *API* and *RAD1* genes also disrupted susceptibility to *Phytophthora palmivora* root infection.

2.2. Creating a favorable environment for Pathogens

Several bacterial pathogens create an aqueous environment in their host. *Xanthomonas gardneri* indirectly activates a pectate lyase in tomato and *Xanthomonas translucens* stimulates the ABA biosynthetic pathway in wheat, both resulting in *watersoaking*, which is suggested to promote bacterial multiplication and/or spread. The activation of these pathways by TALes is illustrated in figure 1.

2.3. Maintaining the pathogen

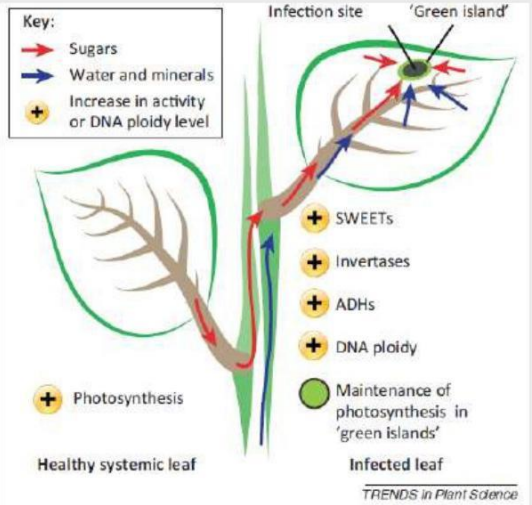
Once infections are established, pathogens need a continuous supply of nutrients and host cellular factors to support host colonization.

Sugar transporters contribute to pathogen proliferation. Several bacterial species hijack host nutrient secretion systems for efficient pathogen reproduction in planta, a s illustrated by t h e sucrose efflux exporters SWEET in rice. Their transcriptional induction by Xanthomonas TAL effectors is crucial for disease development.

The role of SWEET sugar transporters in susceptibility appears to be conserved in other hosts, such as cotton and cassava, and in infections by pathogens lacking TALe, for example, *Pseudomonas syringae*.

Ralstonia solanacearum hijacks the plant host's metabolism for the biosynthesis o f gamma-aminobutyric acid (GABA) to support its growth.

Figure 4. 4: Nutrient flow in an attacked plant. The nutrients are transported to the "Green Islands" (Lapin & Van den Ackerveken, 2013*). Changes affecting plant physiology during infection by an obligate pathogen (biotroph)



2.4. Negative regulation of the immune system

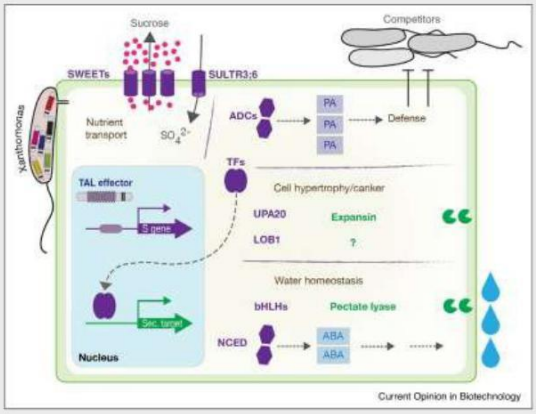
Given the antagonism between plant productivity and immune system activation, plants regulate the latter very carefully (see chapter on HR). An important group of *S* genes code for negative immune regulators, which plants use to fine-tune defense responses and limit trade-offs (between resistance and productivity). Mutants of these *S* genes show increased resistance, often to a wider range of pathogens.

Some negative regulators are targeted by pathogen effectors to stimulate their suppressive effect on plant immunity.

3. Gene expression

The expression of susceptibility genes is stimulated by the pathogen. The pathogen uses effectors to manipulate the expression of these genes.

Figure 4. 5: Role of S genes targeted by TAL effectors (*Transcription Activator-Like effectors*). Our in-depth understanding of the molecular mechanisms underlying the action of TAL effectors (TALe) has revolutionized the search for their targets in planta. Because TALes act as true eukaryotic transcription factors whose DNA-binding sites are highly predictable, transcriptomic approaches combined with in silico target promoter research enable rapid identification of their candidate target genes. So much so that almost 10 classes of S genes have been discovered since the TAL code was elucidated in 2009 [49,50]. Their function is quite diverse, ranging from sucrose (SWEET) and sulfate transporters, to enzymes involved in the biosynthetic pathway of



various compounds such as polyamines (arginine decarboxylases), ABA (9-cis-epoxycarotenoid dioxygenase) or even small RNAs (Hen1 methyltransferase), to different types of transcription factors (LOB, bHLH, bZIP, ERF) involved in the control of various phenotypes such as host cell enlargement, pustule formation, water, etc. Other classes of S genes are expected to be discovered as new TAL effectors with major or even moderate virulence functions are characterized. The potential is high, as the majority of Xanthomonas species depend on TALs to infect their hosts, and only S genes corresponding to 7 pathosystems have been studied to date, whereas there are at least fifty Xanthomonas species or pathovars with unique characteristics yet to be studied. This figure gives an overview of the most relevant S gene categories targeted by TALes and for which a function is described. Text in brown refers to the types o f activity conferred by the S genes. Primary and secondary targets are shown in purple and green (text, shape), respectively. Abbreviations: SWEET, Sugars Will Eventually Be Exported Transporter; SULTR, sulfate transporter; ADC, arginine decarboxylases; PA, polyamines; TF, transcription factors; UPA, up-regulated by AvrBs3; LOB1, lateral organ boundaries 1; ABA, abscisic acid; bHLH, basic helix-loop-helix; NCED, 9-cis-epoxycarotenoid dioxygenase. Shapes: cylinder, nutrient transporter; hexagon, biosynthesis pathway enzyme; two-ovoid transcription factor; Pacman-like, cell wall-modifying proteins (Garcia-Ruiz et al.,

2021*).

V The Phenomena of Recognition

1. Introduction

As in the case of animals, plants have developed an immune system capable of neutralizing the various pathogens and containing infection. To protect themselves against invasion by pathogens, plants monitor all signs of invasion, whether extra- or intracellular.

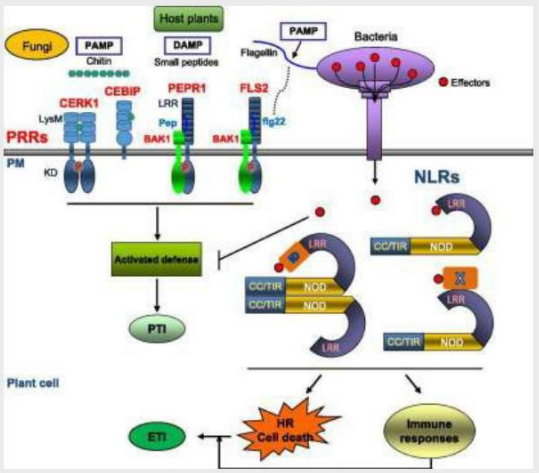
For defense mechanisms to be activated, the plant must detect and recognize the pathogen in question. Over the course of evolution, plants and their pathogens have developed systems to counteract each other's "weapons". Pathogens evolve rapidly, some more rapidly than others, to overcome plant defense mechanisms and even to resist various chemical pesticides. Plants, in turn, evolve to resist their pathogens.

The prerequisite for defense is pathogen identification. Plants have evolved two systems for detecting and recognizing pathogens. Other authors speak of a single system, but with two levels of perception.

The first level is the recognition of elicitors (MAMPs, PAMPs and DAMPs). This detection is carried out by extracellular membrane receptors known as *Pattern-Recognition Receptors* (PRRs). This type of recognition induces what is known as PTI (*PAMPs Triggered Immunity*).

The second level is responsible for effector detection and recognition. This function is performed by resistance proteins. Pathogen detection and recognition via effectors leads to the triggering of defense reactions, known as ETI (*Effector* Triggered Immunity).

Figure 5.1. Pathogen recognition is based on the recognition of elicitors (MAMPs, PAMPs & DAMPs) and effectors by *Pattern* Recognition *Receptors* (PRRs) and resistance proteins respectively (Wang & Chai, 2020*).



2. PRR

PRRs are extracellular membrane receptors whose role is to interact with various elicitors originating from any organism foreign to the plant (MAMPs, PAMPs), and elicitors resulting from plant-pathogen interaction (DAMPs). Perception of these elicitors induces different plant immune responses (PTI), which vary in intensity according to the concentration and nature of the elicitor.

2.1. Structure

A very wide range of plant immune receptors have been identified as Receptor-Like Kinases (RLK) and Receptor-Like *Protein* (RLP). They share the same structure, except that receptor kinases have an intracellular kinase domain.

Figure 5.2. Representative diagram of the structure of the various PRRs proteins, with examples on the right.

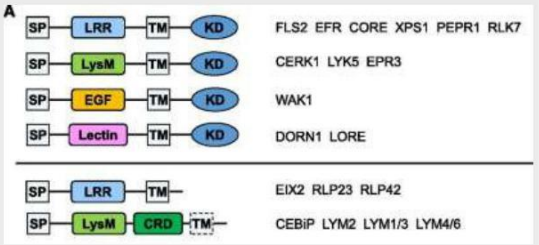
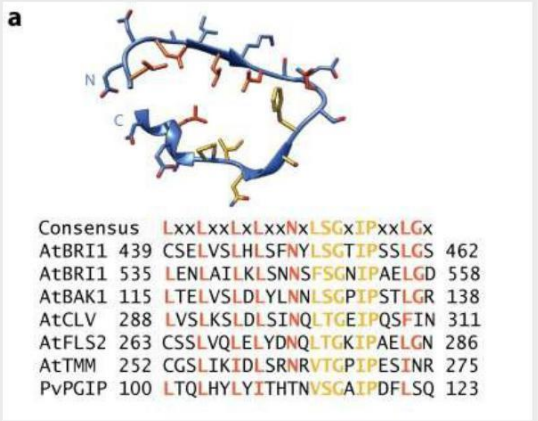


Figure 5.3. Sequences conserved in PRR receptors. The sequences (in amino acids) making up the Leucine-Rich Repeat (LRR) ectodomain of kinase-type receptors. They may vary in size and shape, but are made up of similar repeating units (in red, yellow, etc.). We observe the amino acid leucine (letter "L" in the sequence) repeating several times and in several receptors of this type (having an LRR ectodomain), hence the name Leucine-Rich Repeat (leucine-rich, repetitive domain) (Hohmann et al., 2017).



Fundamental: PRR structure

PRR receptors contain the following domains:

1. Ligand-binding ectodomain
2. Single-pass transmembrane domain
3. Cytoplastic kinase domain: divided into 3 subclasses
    - RD (arginine-aspartate) kinase domain
    - Non-RD kinase domain
    - Pseudokinase domain

Figure 5.4. PRR structure

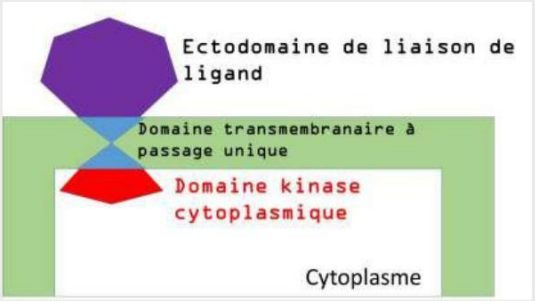
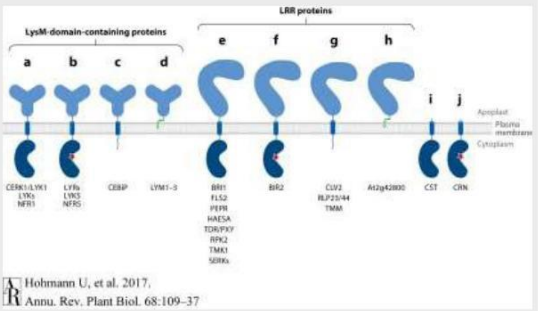


Figure 5.5. PRR architecture in plants. Receptor kinases (RKs) (a, b, e, and f) consist of an extracellular ligand-receptor domain (light blue), a transmembrane helix domain (blue cylinder), and a cytoplasmic kinase domain (dark blue), which may be a pseudokinase with weakened catalytic capabilities (dark blue with red stars). Loop regions (blue lines) link the different domains. *Receptor-like* proteins (RLPs) (c, d, g and h) lack the cytoplasmic kinase domain. The majority of *receptor-like proteins* have a single helical transmembrane domain and may have a long, unstructured Loop region (c and g), while others may lack any transmembrane element and attach to the cytoplasmic membrane via a anchor glycosylphosphatidylinositol (GPI) anchor (green) (d and h). *Receptor-Like Cytoplamic Kinases* (RLCKs) (i and j) are composed o f a helical transmembrane domain and a cytoplasmic kinase or pseudokinase domain, and lack extracellular domains. Although the architecture of RKs and RLPs is almost identical, the nature of the extracellular domain varies across families of different RKs and RLPs. Figures a-d show receptors with an extracellular domain consisting of 3 lysine motifs (LysM). Diagrams e-h

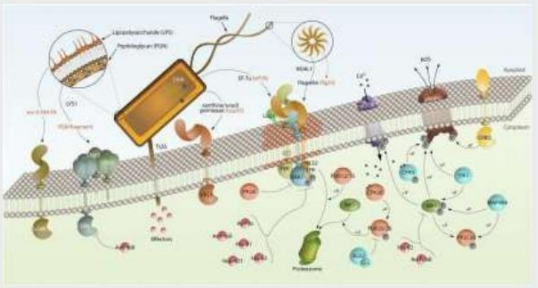


represent receptors with a *Leucine-Rich-Repeat* (LRR) domain. The diagrams show the different possible combinations of extracellular domains, transmembrane helix, cytoplasmic kinases and GPI anchors in these proteins (Hohmann et al., 2017*).

2.2. Location

PRRs are located at the level of the cytoplasmic membrane

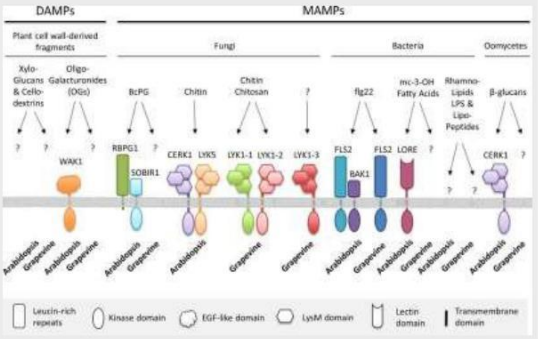
Figure 5.6: Interaction between the bacterium *Pseudomonas syringae* and its host plant. Recognition of the P. syringae bacterium by PRRs and the role of the bacterium's effectors in preventing recognition (Albert et al., 2020*).



2.3. Roles

The role of PRRs is to detect and recognize pathogens and initiate the signaling cascade that transmits information to the nucleus to trigger defense mechanisms.

Figure 5.7. Elicitors of different types (DAMPs, MAMPs) are recognized by PRRs. Here in the case of grapevine and arabidopsis (Héloir et al., 2019*).



2.4. PRR types

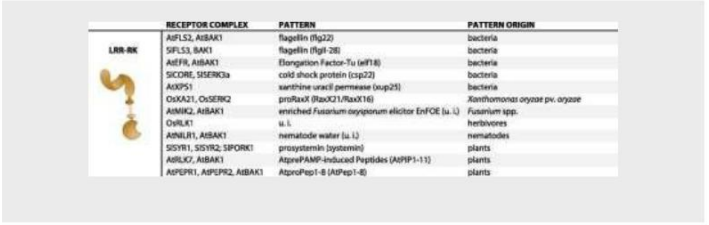
2.4.1. Receptors with a Leucine-Rich Repeat Ectodomain

These are receptors with a fragment rich in repeated leucine amino acid residues. This group includes 2 subclasses of membrane receptors:

a) LRR-RK-type receptors

These receptors are menus of an intracellular kinase fragment

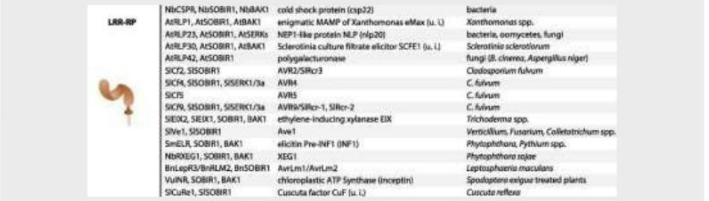
Figure 5.8. LRR-RK-type receptors



i LRR PK-type receptors

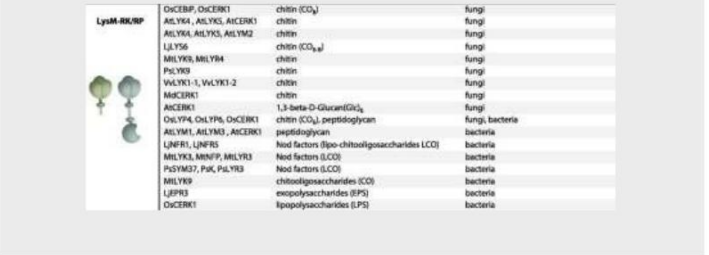
These receptors lack the intracellular kinase fragment.

Figure 5.9. LRR PK-type receivers



2.4.2. Receptors with a Lysine Motif (LysM)

Figure 5.10. LysM-type receptors



2.4.3. Other PRR receptor types

Figure 5.11. PRR receptors of other types

2.5. Mode of action of PRRs

PRR receptors recognize different elicitors from different pathogens, microorganisms, and also from the plant itself. In general, elicitors (PAMPs, DAMPs, etc.) are not species-specific. On the contrary, they are conserved in several groups of microorganisms. For example, chitin is common to all fungi. Elicitors are detected by a ligand-receptor relationship.

Figure 5.12. The 4 stages of signaling by plant receptor kinases. (1): Receptor kinases detect the ligand using their extracellular domain. Among the many potential ligand molecules present in the extracellular space, receptor kinases specifically detect foreign or native small molecules, peptides and/or proteins. (2): Attachment of the ligand to the extracellular domain activates the receptor by inducing a change in its cytoplasmic kinase domain. (3): Subsequently, the kinase domain activates (blue arrows) or deactivates (red bars) cytoplasmic components to generate an outgoing signal, which ultimately influences the activity of transcription factors (TFS). (4): Receptor kinase activity is regulated at several levels. For example, protein interaction sites can be regulated by inhibitory proteins (orange), the kinase domain can be inactivated by protein phosphatases (PP2), and the position of receptor kinases can be altered by endocytosis, leading to their recycling or degradation. Legend: light blue: extracellular domain, Blue cylinder (middle): transmembrane domain (helix), Dark blue (bean-shaped, bottom): cytoplasmic kinase domain, Blue lines connecting the different parts: Loop domain, Potential ligands, in red pentagons, green stars, yellow sticks, Red circles: protein phosphorylation sites (Hohmann et al., 2017*).

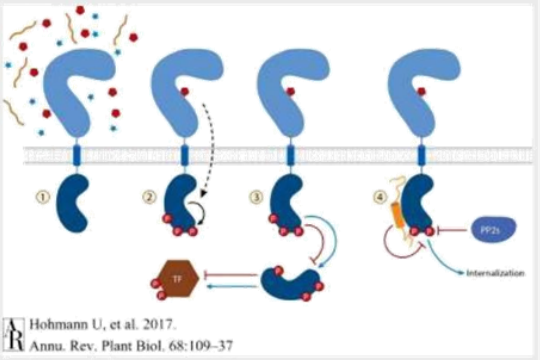
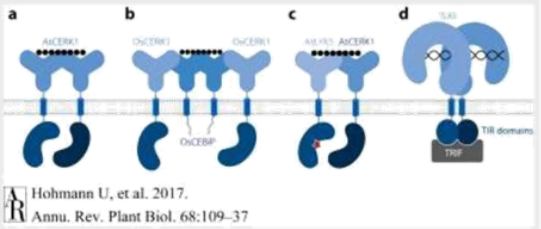


Figure 5.13. Activation model for RK-type receptors containing a lysine motif (LysM). (a) A chitin oligomer of at least 7 N-acetyl-d-glucosamine (NAG) units (hexagons in black) induces dimerization of two AtCERK1 receptor kinases. Both proteins bind the ligand (chitin) with their LysM2 domains. Each receptor attaches to different NAG units, so the chitin molecule binds the two receptors together. (b): Like AtCERK1, OsCEBiP (an RLP-type receptor) associates with another receptor of the same type (dimerization) by attaching via a chitin molecule. The two CEBiPs bind to opposite sides of the same chitin oligomer (in a manner opposite to the binding of AtCERK1s). Because studies have suggested that OsCERK1 binds OsCEBiP (heterodimerization), ligand binding could lead to the formation of a tetrameric (4 molecules) or larger signaling complex (c) : After ligand binding, heterodimerization of AtCERK1 with another chitin-attaching receptor of type



LysM-RK, e.g. AtLYK5, can initiate downstream signaling. (d): chitin-induced homodimerization of AtCERK1 may resemble double-stranded RNA TLR3 homodimerization in the animal immune system. In both cases, a polymeric ligand causes homodimerization of the extracellular domains, which in turn brings the intracellular parts of the receptors to initiate the signaling at downstream(Hohmann et al., 2017*).

Figure 5.14. Recognition of elicitors by PRR receptors (Hohmann et al., 2017*).

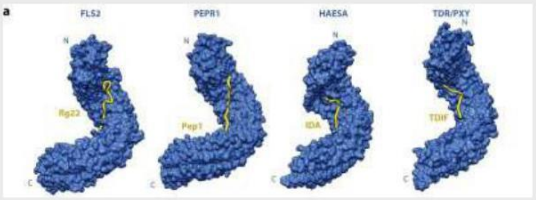
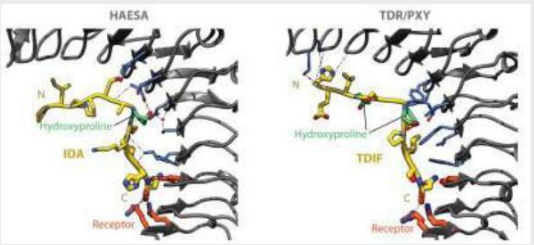


Figure 5.15. Recognition of elicitors by PRR receptors (Hohmann et al., 2017*).



To improve the efficiency and specificity of the immune response, plants use well-defined strategies.

Figure 5.16. PRRs also use co-receptors. SERK proteins are essential co-receptor proteins for receptor kinases. (Hohmann et al., 2017*).

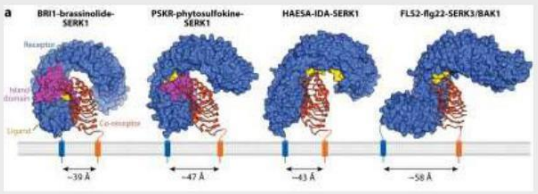


Figure 5.17. PRR receptors also use co-receptors. SERK proteins are essential co-receptor proteins for receptor kinases. The kinase domains of these receptors can form a dimer in the cytosol with the co-receptors upon activation following elicitor detection by PRR receptors. The LRR ectodomain (extracellular) (Hohmann et al., 2017*).

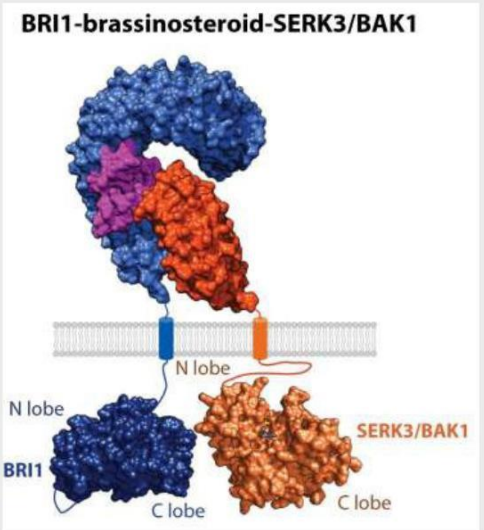


Figure 5.18. PRRs also use co-receptors. SERK proteins are essential co-receptor proteins for receptor kinases. (Hohmann et al., 2017*).

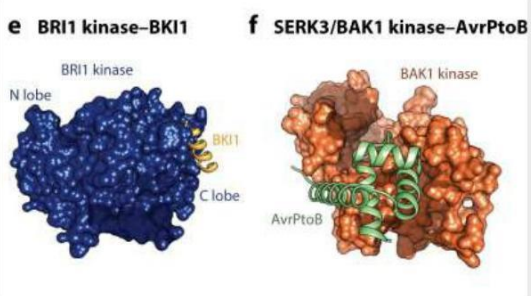
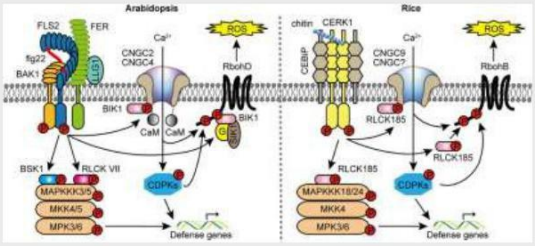


Figure 5.19. Mode of action of PRRs in detecting elicitors from from pathogens. The receptors receptors (PRRs) detect elicitors (PAMPs) and activate a series of immune responses (PTIs). For example receivers *leucine-rich repeat receptor-like kinase* (LRR-RLK) *Flagellin Sensing 2* (FLS2) in *Arabidopsis*, involves (hiring) an *LRR-RLK* co-receptor *Brassinosteroid Receptor-Associated Kinase 1* (BAK1) to form a complex at the moment of flg22 attachment and initiates (the complex) PTI by auto- and trans-phosphorylation (red circles) of their kinase domains. Another LRR-RLK receptor FER and its co-receptor LLG1 act as a scaffold for the formation of the FLS2- BAK1 complex induced by the flg22 elicitor. The activated FLS2-BAK1 complex phosphorylates and releases, downstream, *receptor-like cytoplasmic kinases* (RLCKs), such as *Botrytis-Induced Kinase* (BIK1) *BR-Signaling Kinase 1* (BSK1), to activate the and downstream signaling (via MAPK and other signaling pathways, ***see signal transduction chapter***). BSK1 and group VII members of the RLCKs directly phosphorylate MAPKKK5 and activate the Mitogen- Activated Protein Kinase (MAPK) signaling cascade, followed by overexpression of defense genes. BIK1 positively regulates the activation of RbohD and heteromeric G protein to induce reactive oxygen species (ROS).



3. Resistance proteins

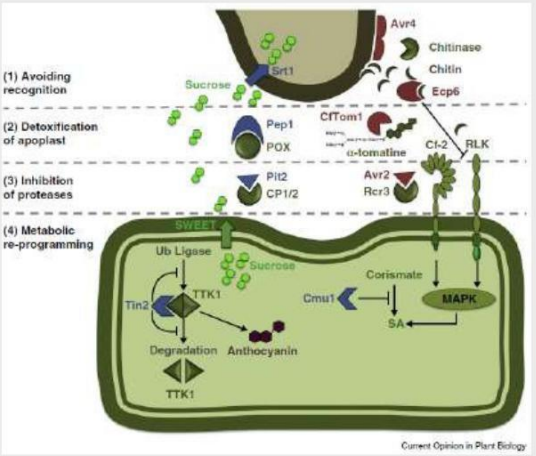
Resistance proteins are encoded by resistance genes. There is enormous variability in resistance genes, and consequently in resistance proteins.

Complement : Effectors

Effectors are molecules used by the pathogen to manipulate the host plant and modify its physiology in favor of the pathogen. If effectors are recognized by resistance proteins, they induce resistance.

For more details on effectors, please consult the course "Mechanisms of Pathogenicity of Plant Pathogenic Fungi".

Fig 5.20. The roles of effectors in the infectious process (Okmen & Doehlemann, 2014*)



3.1. Structure of Resistance proteins

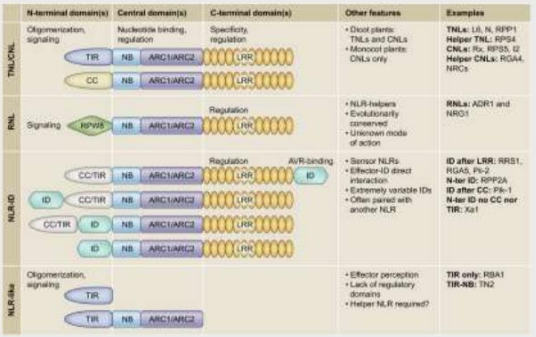
As in the case of PRRs, resistance proteins have well-defined structures (see Resistance genes). They generally have 3 highly conserved domains:

N-Terminal

Central domain (usually an NBS domain) C-

Terminal

Figure 5.21. Structures and characteristics of resistance proteins. The functions and properties of the individual domains, either predicted or well proven experimentally, are shown, together with particular features and also examples. Scale is not respected in domain drawings. CC: Coiled Coil, TIR: Toll/Interleukin-1 Receptor /Interleukin-1), NB: Nucleotide-Binding Site, LRR: Leucine-Rich Repeat, ID: Integrated-Domain, RPW8: Resistance to Powdery Mildews 8, NLR: Nucleotide-binding and Leucine-rich repeat-containing protein of resistance TNL: Toll/Interleukin-NLR, ...(Cesari, 2018).



3.1.1. Le Domaine Central

In this part of the protein we find highly conserved domains called: *Nucleotide-Binding Site* (NBS (nucleotide attachment site).

3.2. Roles and Functions of Resistance Proteins

The main role of resistance proteins is to detect and recognize pathogens attacking the plant, through the various monitoring systems (see ***Recognition model***).

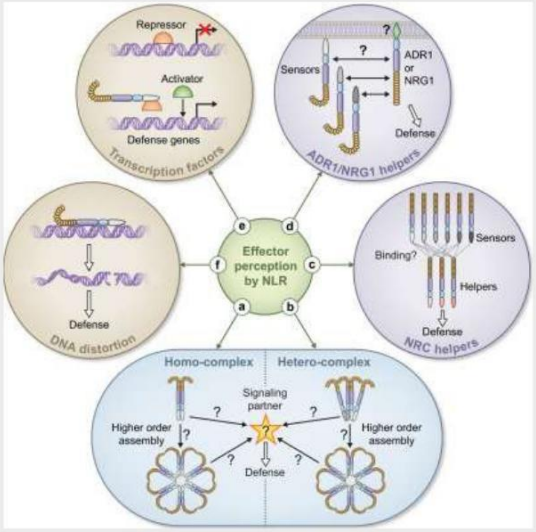


Figure 5.22. Roles and functions of resistance proteins. Resistance proteins are involved in various signalling and activation mechanisms following effector perception. (a, b): Many TNLs and CNLs require oligomerization to function (see ***Resistosome***). Some form heterocomplexes of unknown stoichiometry, while others are likely to homo-multimerize via their CC- or TIR-type terminal domains. N-terminal autoassociation (several R proteins of the same type associate to form an oligomer) is important for triggering programmed cell death (HR), and is thought to enable interaction with as yet undefined signaling "partners" (see ***Signal transduction***). Signaling may involve a higher order of R protein assembly, but this remains experimentally unproven. (c) : Some R proteins need other proteins called ***helpers*** to function. A class of R helper proteins
A class of asteroid-specific R proteins is called NRC. This class is required for downstream R proteins for ***Sensor-type*** R proteins.
(d) : ADR1 and NRG1 are RNL helpers possessing an N-terminal domain that resembles RPW8 membrane-targeting protein. Ceasri (2018) speculates that RNLs may have a function at the membrane level, but their defense-related signaling mechanism remains to be determined. (e, f): Some R proteins induce resistance through interaction with transcription factors (activators or suppressors) in host cell nuclei, while others physically bind t o DNA through their NB domain and induce DNA distortion and potentially cleavage (Cesari, 2018).

3.2.1. NLRs

k *Definition*

These are NLRs with an N-terminus homologous to the RPW8 signaling domain.

k *Definition: RPW8: Arabidopsis broad-spectrum mildew resistance protein*

This is a family of *Arabidopsis thaliana* powdery mildew resistance proteins with a broad spectrum of action.

3.2.2. The Sensors

k *Definition*

These are NLRs responsible for binding an effector or recognizing its activity. These proteins act as effector detectors

3.2.3. The Helpers

k *Definition*

These are NLRs that are activated by another NLR or downstream of the signalling cascade following recognition of effectors.

These proteins play an important role in ETI-related signalling pathways. They work in close conjunction with *sensor-type* resistance proteins. They are responsible for signal transduction and induction of defense reactions.

Note

The resistance proteins *sonsors* and *helpers* are used by the plant as a pair, whose role is to recognize the pathogen's effectors and activate immune responses.

3.2.4. The Resistosome

k *Definition*

The resistosome is a wheel-shaped oligomeric structure composed of several NLRs which are assembled after activation.

Figure 5.23. Resistosome formation in response to pathogen invasion. The uridylylation of PBL2 by the AvrAC effector leads to changes in the interactions between PBL2 and ZAR1-bound RKS1. This in turn alters the level of exposure of the ZAR1 nucleotide-binding domain, allowing the ZAR1 CC domains to oligomerize. The resulting pentamer has been called the plant "resistosome" (Wersch et al., 2020*).

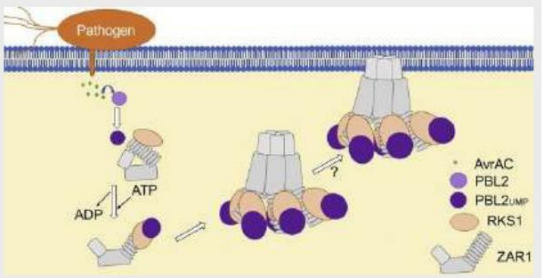
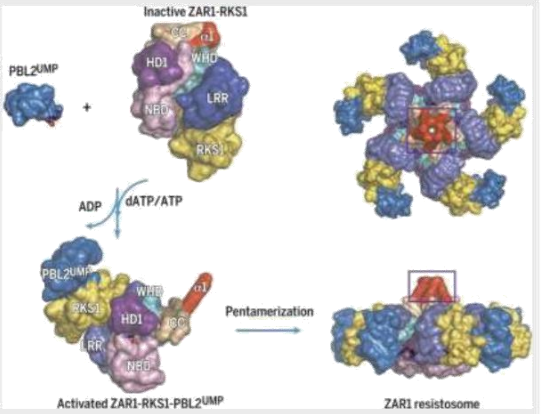


Figure 5.24. Resistosome formation in response to pathogen invasion. Interaction of PBL2UMP (blue) with the preformed ZAR1-RKS1 complex (inactive ZAR1-RKS1) triggers conformational changes in the release of ZAR1NBD and adenosine diphosphate (ADP), enabling the complex to bind dATP or ATP. Binding to either dATP or ATP induces structural remodeling and fold switching of ZAR1, leading to

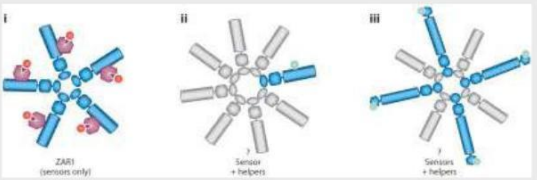
complete activation of ZAR1 (activated ZAR1-RKS1-PBL2UMP) and formation of the pentameric ZAR1 resistosome (shown in two orientations). The very N-terminal (α1) helix (red) of ZAR1 buried in the inactive ZAR1-RKS1 complex becomes exposed to solvent in the activated ZAR1-RKS1-PBL2UMP complex and forms a funnel-like structure (highlighted in the purple frame) in the ZAR1 resistosome that is required for ZAR1 PM association, cell death initiation and disease resistance (Wang et al., 2019*).



k *Definition: Uridylation*

Urylylation is the post-translational addition of a uridylyl group to a protein, RNA or sugar phosphate.

Figure 5.25. The different possible resistosome formations. The formation of a multimeric complex of activated R proteins is conserved (common) across the kingdoms. (i) : The known resistosome , as demonstrated by Wang et al. (2019) several hyptothetical combinations are possible (ii = one sensor protein with several helper proteins and iii= several sensor proteins and other helpers) which may be analogues to the diversity of inflammasomes found in mammalian R proteins (Tamborski & Krasileva, 2020*).

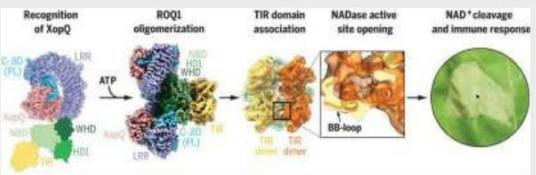


Attention

The discovery of the resistosome is very recent in plants (Wang et al., 2019). Neither the mode of operation nor signaling is known.

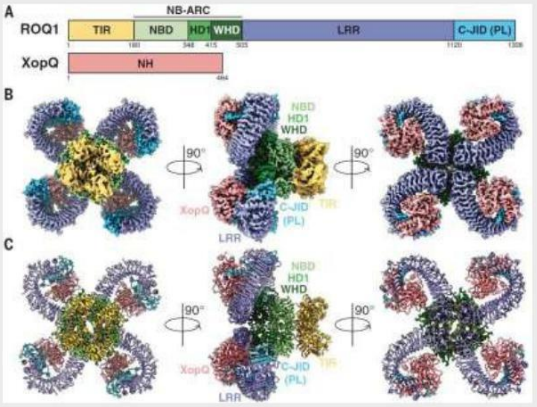
We know that it is involved in autoimmune reactions and induces the HR reaction, but we don't know the mechanism of action.

Figure 5.26. Proposed mechanism o f ROQ1 activation. The LRR and C-JID domains of the ROQ1 protein recognize the pathogen's XopQ effector. ROQ1 becomes an oligomer (several ROQ1 molecules associate together) via the NB-ARC domain (NBD, HD1, WHD) in an ATP-bound state. The association of the TIR domain induces a conformational rearrangement of the BB-loop domain, opening up the active site of



NADase. TIR domain catalytic activity The catalytic activity of TIR domains also signals the immune response, leading to cell death (Martin et al., 2020*).

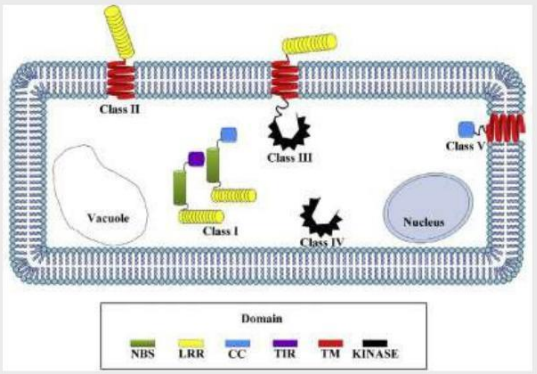
Figure 5 . 27. Formation of the ROQ1 resistosome. (A): Schematic representation of the ROQ1 resistance gene and XopQ effector, with color-coded domains: TIR, yellow; NB-ARC NDB, HD1, and WHD, light, green and dark green respectively; LRR, purple; C-JID,(or PL domain), light blue and XopQ, salmon pink. (B) and (C): Composite density map of the ROQ1-XopQ complex from 3 cryo-EM reconstructions (B) and corresponding atomic model (C) shown in 3 orthogonal views (Martin et al., 2020*).



3.3. Location

The majority of resistance proteins are located in the cytoplasm. A small proportion are located in the cytoplasmic membrane.

Figure 5.28. Location of the different resistance protein families. Some proteins are found in the cytoplasmic membrane: proteins belonging to classes II (TM-LRR), III (RLK: Receptor-Like Kinase) and V (CC: Coiled Coil); proteins belonging to classes I (NBS-LRR) and IV (STK: Serine /Threonine Kinase) are located in the cytoplasm. Abbreviations: NBS-Nucleotide Binding Site; LRR-Leucine-Rich Repeats; CC-coiled-coil; TIR-Toll-Interleukin Receptor; TM- Transmembrane Domain (Bezerra-Neto et al., 2020*).



3.4. The Recognition Phenomenon

Pathogen recognition by resistance proteins results from the interaction between the R protein and a pathogen effector molecule. Given the diversity of pathogens and the multitude of plants interacting with pathogens, as well as the complexity of these interactions, several models have been proposed:

- Direct interaction
- The "Guard" model
- The "Decoy" model
- The "Integrated Decoy" (ID) model
- The NLR-Like model, and the "Bait" model

It should be noted that each model explains the recognition of the pathogen by its host plant. Some pathosystems follow one model, others another. Each model is valid for certain interactions and not others.

Fig. 5.29. Activation of a TIR-NB-LRR gene (Bernoux et al., 2011).

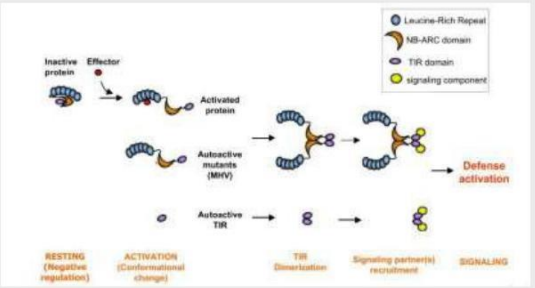
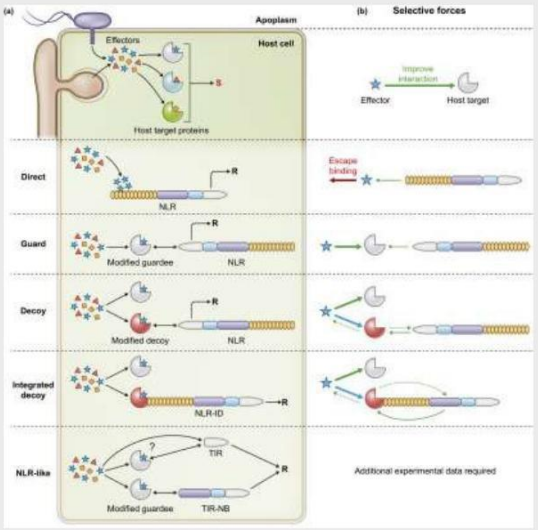


Figure 5.30. The different models explaining pathogen recognition by resistance proteins. Resistance proteins detect and recognize pathogens specifically, using different mechanisms with different evolutionary constraints. (a): o n infection, pathogen effectors target and modulate host proteins to promote (host plant) susceptibility (S). Direct interaction between effectors and resistance proteins induces resistance (R). Indirect recognition occurs when the effector targets a protein "guarded" by an R protein (the guarded protein is called a *guardee*), or when a copy of the target protein gene evolves and codes for a *decoy* protein, which is also guarded by R proteins. In the latter two situations (indirect recognition), the resistance proteins detect changes in the target/guarded proteins caused by the effector. *Decoy* molecules are often integrated into the R protein. Some NLR-like proteins (which resemble NBS- LRR resistance genes) but lack regulatory domains such as NBS and LRR, or only LRR, also function as effector detectors, but their mode o f action is unclear. (b): Effectors evolve to interact with host target proteins (including *guardee* proteins), while their targets (the target proteins) do not show evidence of oriented positive selection, probably because variations (mutations in the genes encoding these proteins) can be detrimental.

/In the direct recognition model, the effector genes mutate to avoid binding (recognition), and the R genes are under pressure to maintain or restore binding (recognition). In the direct recognition model (effector-R protein), effector genes mutate to avoid binding (recognition) and R genes are pressured to maintain or restore binding (recognition).



The *guard*, *decoy* and *integrated decoy* models offer more effective detection/recognition than the direct recognition model. Through their functions, effectors are forced to evolve towards greater recognition/detectability (the more they evolve, the more likely they are to be detected) and they cannot easily avoid detection without modifying their functions (physiology, role). In the *integrated decoy* (ID) model, the proximity of R proteins to effector targets (target proteins=effectors) is maintained by a physical attachment (connection). The ID and other R protein domains can still evolve to maintain interoperability.

(Cesari, 2018**).

Attention

Regardless of the pathogen, host plant and/or model, recognition based on R proteins (NLRs) is highly specific.

Fundamental

A host molecule (protein) may be the target of several different (unrelated) effectors. Generally, this molecule is monitored by one or a limited number of resistance proteins.

3.4.1. Direct Recognition

In this situation, the effector molecule is detected after direct interaction with the R protein. This interaction is of the receptor-ligand type. The resistance protein enters into direct physical interaction with the effector

Note

To date, research has shown that this type of detection is rare in nature. In the majority of cases (so far), detection is indirect. This is rarely where there is direct physical contact between the R protein and the effector.

3.4.2. The Guard Model

In this case, the resistance protein detects the modifications undergone by the target molecule (protein) called " *guardee*" (guarded), caused by the effector.

3.4.3. The Decoy Model

In this case, the R protein detects changes in a decoy molecule (protein) that mimics the target molecule.

Fundamental

The *decoy* molecules (proteins) have no function apart from catching the effector.

3.4.4. The Integrated-Decoy Model

In this model, the *decoy* protein is integrated (physically linked) with the resistance protein.

Note

It is estimated that 10% of resistance proteins in each species function according to this model.

Note

The decoy molecule can integrate at any position in the R protein.

4. The mechanisms of Recognition

Plants have a two-level pathogen recognition system. The first level consists of membrane receptors: PRRs, which are responsible for detecting PAMPs and DAMPs. The second, more complicated level consists of resistance proteins. Resistance proteins are located either in the plasma membrane or in the cytoplasm. Cytoplasmic resistance proteins predominate over membrane resistance proteins. In addition, recognition is direct in the case of PRRs. In the case of resistance proteins, the majority detect and recognize pathogen effectors indirectly.

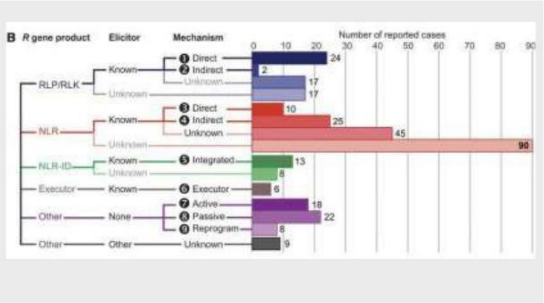


Figure 5.31.The different mechanisms explaining pathogen detection and recognition by the host plant (Kourelis & vad der Hoom, 2018*).

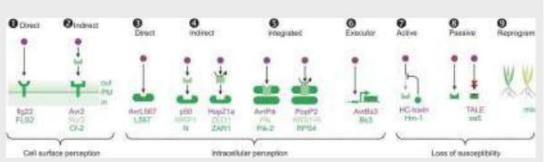


Figure 5.32. Recognition mechanisms explaining the functions of resistance proteins. (1): Direct recognition,
(2): indirect recognition, in which case the R proteins are on the cell surface (cytoplasmic membrace, In the case of R proteins active in the cytoplasm, there are 4 mechanisms of action (3-6), plus 3 loss-of-susceptibility mechanisms (7-9). PAMPs and

effectors are colored in pink, receptors à direct recognition (in green) clear) and receivers à recognition indirect (in green green) (Kourelis & vad der Hoom, 2018*).

4.1. Extracellular Perception

Extracellular perception occurs when the plant detects and recognizes the pathogen before it can penetrate the cell. The plant has two mechanisms that ensure this ability:

- PRRs
- Membrane R proteins

4.1.1. Direct Perception at the Cell Surface

In this situation, the plant recognizes the pathogen via PRRs. The PRRs interact directly (receptor-ligand relationship). The plant detects PAMPs, DAMPs and certain types of effectors (but this recognition is consistent with PRR-PAMP recognition: the plant recognizes a fragment of the protein and not the activity of the effector molecule).

A *Example: The FLS2 receiver*

The best example is flagellin, a bacterial protein. There is a fragment called flg22 which is recognized by the FLS2 (Flagellin-Sensitive *2*) receptor in *Arabidopsis*. The flg22 peptide binds directly to FLS2, attracting BRI1-Associated *Receptor Kinase* (BAK1).

Several other PAMPs are perceived in the same way as flagellin, e.g. chitin, lipopolysaccharide, peptidoglycan, RNA, etc.

Some effectors are also perceived directly extracellularly by membrane receptors.

A *Example: The RLP23 receiver*

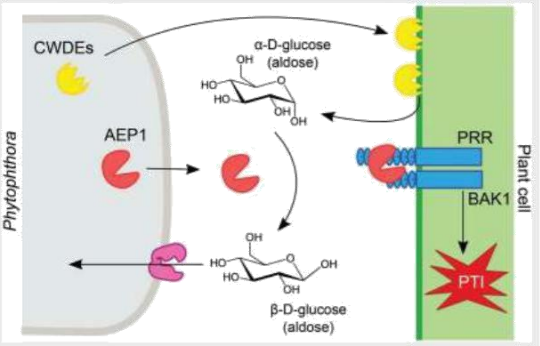
Other effectors are also recognized by PRRs, for example nlp20, which is a fragment of the NEP1 (*Necrosis and Ithylene-inducing Peptide 1*) effector by the RLP23 receptor. NLPs (NEP-Like proteins) are secreted by bacteria, oomycetes and phytopathogenic fungi.

Complement: Case of AEP1 end effector

AEP1 (Aldose-1 Epimerase enzyme) is an effector whose role is to modify sugars such as glucose from its α- to β-form, to facilitate their absorption by the pathogen (*Phytophthora sojae*).

Figure 5.34. The AEP1 effector induces PTI after detection by PRRs. Upon infection, the *P. sojae* pathogen secretes CWDEs to degrade the cell walls of its host plant (soybean). CWDEs release various cell wall degradation monomers (sugars, fatty acids, etc.) into the applasm. Pathogens generally make use of these degradation products

as carbon sources (see ***Mechanisms of pathogenicity of phytopathogenic fungi*** course). In the case of P. sojae, and its host plant, soybean, the CWDEs release aldoses (α-glucose) into the applasm, the problem is that the pathogen is unable to absorb this form, the pathogen also secretes with the CWDEs an effector called AEP1 whose role is to convert α-glucose into β-glucose, which is easily absorbed. The plant (soybean) recognizes the pathogen through the detection of AEP1 via PRRs and induces resistance. The plant here does not recognize AEP1 as an effector (effect of the effector on the target protein/molecule) but recognizes its secondary or primary form (as PAMPs) (Copeland, 2021*).



4.1.2. Extracellular perception Indirect

Membrane R proteins recognize the pathogen's effectors, but not directly (no receptor-ligand type effector-R protein director interaction). R proteins monitor the target proteins of the pathogen's effectors and detect any modifications the protein has undergone.

A *Example: The Cf-2 protein*

Tomato Cf-2 resistance protein against *Cladosporium fulvum*. This protein does not directly recognize the Avr2 protein. In fact, Avr2, which is a protease inhibitor, targets the Rcr3 protein (a cysteine-type protease). Cf-2 monitors Rcr3 and recognizes any modifications it undergoes.

4.2. Intracellular Perception

Intracellular perception is ensured by intracellular resistance proteins.

4.2.1. Direct Perception of Effectors

In this situation, the R protein recognizes the pathogen through direct receptor-ligand physical contact with the effector.

A *Example: The RPP protein*

ATR1 (*Arabidopsis thaliana Recognition 1*) is an effector secreted by the oomycete *Hyalperonospora arabidopsidis* and interacts directly with the RPP (*NLR Recognition of Perenospora parasitica1*) protein. This interaction leads to pathogen recognition and the induction of defense responses.

A *Example: Proteins L5, L6, and L7*

Another effector, AvrL567, produced by *Melampsora* liniqui, is recognized by resistance proteins L5, L6 and L7. The interaction between the effector and the resistance protein is direct.

4.2.2. Indirect Perception of Effectors: Decoys & Guardees

Many effectors are recognized indirectly by resistance proteins. In this situation, the resistance protein and the effector do not enter into a receptor-ligand interaction. In the *guard* case, the resistance protein monitors a target protein for effector activity. In the *decoy case*, the plant produces decoy proteins that mimic the target protein.

In this situation, R proteins trigger defense reactions by monitoring :

- Effector interaction with host proteins
- Enzymatic modifications of host proteins
- host cell homeostasis

A *Example: The N*

Tobacco mosaic virus produces a p50 effector, a 50 kD helicase. The resistance protein is called the N protein. The N protein does not recognize p50 directly. It only recognizes it if it interacts with the NRIP1 protein (a rhodanese sulfurtransferase at chloroplase level). The N protein does not interact with NRIP1 when it is free. Protein N recognizes NRIP1 only in the presence of the p50 effector.

A *Example: The RPM1 protein*

The *RPM1-interacting* protein4 (RIN4) is monitored together with the RPM1 resistance protein. *Pseudomonas syringae* effectors AvrB and AvrRPM1 induce phosphorylation of RIN4, reducing its ability to interact with *prolypeptidyl isomerase Rotamase CYP1* (ROC1), thus altering the conformation of RIN4. This altered conformation alerts the RPM1 resistance protein, subsequently triggering defense reactions.

The bacterium uses another effector, AvrRpt2, which breaks down RIN4, preventing RPM1 from triggering defenses (no longer recognized by RPM1). The plant has another R protein monitoring RIN4: RPS2. RPS2 recognizes RIN4 divisions and induces defenses.

A *Example: Arabidopsis SUMM2 protein*

The *Arabidopsis* R protein SUMM2 (*Suppressor of MKK1 MKK2 2*) monitors the phosphorylation status of CRCK3 (*Calmodulin binding RLCK*). CRCK3 is phosphorylated by the MAP kinase signaling cascade, involving the MEKK1, MKK1 and MKK2 kinases, as well as MPK4. The phosphorylated state of CRCK3 in the absence of MPK4, MKK1/MKK2 or MEKK1 kinase activity induces a SUMM2-dependent immune response. The bacterium *P. syringae* produces an effector, HopAl1, which blocks MPK4 activity. The SUMM2 protein recognizes the disruption of cellular phosphorylation homeostasis and detects the presence of the pathogen.

4.2.3. Indrecte collection: Integrated domains

Some R proteins have an additional domain (*decoy)* which is necessary for pathogen recognition and which is integrated with the resistance protein. The 3 best-known resistance genes of this type are : *RRS1* d

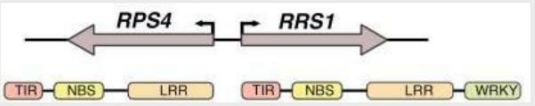
Arabidopsis and *RGA5* and *Pik* in rice. These genes are expressed from the same promoter and in the opposite direction to a resistance gene that has no integrated domain.

The domains most frequently identified as being integrated with resistance proteins are :

- The kinase domain: involved in protein phosphorylation
- The WRKY domain: involved in DNA binding and transcription
- The BED domain: involved in DNA binding There are other domains, but they are less frequent.

Resistance proteins with an integrated domain (ID) function like sensor-type resistance proteins, and generally work in conjunction with other resistance proteins. For example, the *Arabidopsis* resistance protein RRS 1 contains an integrated domain in the C-terminal region: *WRKY transcription factor domain* (WRKY domain), which works with the protein of the RPS4 resistance to induce effector recognition.

Figure 5.35. The resistance protein pair RPS4 and RRS1 from *Arabidopsis.* The latter protein (RRS1) contains an integrated WRKY domain towards the C-terminal region. Both genes have the same promoter and are transcribed in two opposite directions. (Lolle et al., 2020*).



A *Example: The Arabidopsis RRS1 resistance protein*

The WRKY domain of RRS1 interacts with the *P. syringae* effector AvrRps1, inducing immune responses. The *Ralstonia solanacearum* effector PopP2 acts as an acetyltransferase, acting by acetylation of certain key lysine amino acid residues in the WRKY domain of RRS1. In genotypes with the Col-0 allele of the RRS1 gene (RRS1-S), acetylation blocks recognition of AvrRps4. In genotypes with the Nd-1 and Ws-2 alleles (RRS1-R), acetylation is signaled (pathogen recognition), and also recognizes the AvrRps1 effector.

A *Example: The RGA5 protein in rice*

The rice RGA5 protein contains an integrated domain called RATW1 or HMA, which is located in the C-terminal region. RGA5 interacts with Avr-Pia and Avr-CO39 effectors using the HMA domain, triggering immune responses.

Fundamental

All resistance proteins with an integrated domain need a genetically linked protein to trigger signalling (see figure above).

A *Example*

The RRS1 and RPS4 protein pair. The RRS1 protein is able to detect various pathogens, but to signal them it needs the RPS4 protein. The genes of these two proteins are linked, having the same promoters (see figure above).

4.2.4. Executive Genes

k *Definition : Executor gene*

Executor genes are resistance genes whose transcription is activated by TALE effectors (*Transcription* Activator-Like Effectors) produced by bacteria of the *Xanthomonas* genus and which confer resistance against bacteria of this genus producing these effectors.

TALE effectors bind to specific DNA sequences, and modify the transcription of host plant susceptibility factors. Executor gene promoters act as traps for these effectors, forcing them to promote transcription of the genes involved in resistance.

The promoters of the executor genes act as a "*decoy"* for these effectors, mimicking the promoter regions of the susceptibility factors. As a result, these effectors will induce expression of genes involved in defense instead of over-expressing genes encoding host susceptibility factors.

A *Example*

To date, 6 executor genes have been cloned:

Rice: *Xa27*, *Xa10*, and *Xa23*: code for proteins with multiple hypothetical transmembrane domains (*Bs4C-R* codes for a protein of this type).

Peppers: *Bs3/Bs3-E* and *Bs4C-R*: code for proteins with a catalytic function. *Bs3* and *Bs3-E* code for a hypothetical flavin mono-oxygenase.

Complement

A better understanding of the specificity of TALE binding to DNA has enabled the development of synthetic effector genes inducing immunity against multiple races of *Xanthomonas*. This strategy can be used to engineer resistance against the bacterium *R. solanacearum*, which also produces TALE-like effectors known as RipTALs.

4.3. Loss of Susceptibility

Loss of susceptibility is the means of resistance in the case of plants carrying susceptibility genes (*S*) (see Chapter: Susceptibility genes). Loss of susceptibility can occur in 3 ways:

- Active
- Passive
- Reprogramming of the host plant (mutations)

Note

Several cases of loss of sensitivity confer lasting resistance.

Complement

When susceptibility losses become fixed in the population, they behave like non-host resistance.

4.3.1. Loss of Active Susceptibility

Resistance (*R*) genes controlling the mechanisms of active loss of susceptibility code for proteins that disarm the pathogen by interrupting a key pathogen process. These active mechanisms are generally constitutively expressed, and in some cases, over-expressed following detection of a pathogen.

In addition, the active mechanisms of loss of sensitivity can lead to the production of elicitors (PAMPs and DAMPs) which are subsequently detected and amplify immune responses.

A *Example: The Hm1 gene*

The *Hm1* gene (barley) codes for an NADPH-dependent reductase, which is specifically involved in the detoxification of the HC toxin produced by the fungus *Cochliobolus carbonum* (*Helminthosporium carbonum*).

A *Example: The TM-1 gene*

The tomato Tm-1 gene codes for a protein that inhibits replication of *Tomato mosaic virus* RNA, by binding to the virus' replication proteins, thus conferring resistance to TMV in tomatoes.

4.3.2. Passive Susceptibilty Loss

The loss of the ability to interact with key host susceptibility factors by the pathogen's effectors is a very common mechanism controlling recessive resistance (controlled by recessive resistance genes).

A *Example*

Half of the resistance genes against viruses confer resistance through loss of interaction with viral effectors. The majority of recessive R genes identified act against Potyviruses. These genes code for translation initiation factors belonging to the 4E or 4G families, which are unable to interact with the viral mRNA head to initiate translation.

A *Example*

A third of rice's resistance genes against *Xanthomonas oryzae* are inherited in a recessive manner.

A recessive mutation in the promoter of the *xa13* gene prevents the TALEAvrXa13 effector from manipulating the promoter of this gene, thus rendering this rice genotype resistant to the bacterium through loss of susceptibility. This rice is no longer susceptible to the bacterium.

4.3.3. Loss of Passive Susceptibility through Host Reprogramming

Host reprogramming by mutations in cellular pathway components is a common strategy leading to durable resistance against a wide spectrum of pathogens. It is generally a recessive trait, but can involve dominant-negative alleles in some cases. The genes in this group are

generally known as the *Adult Plant* Resistance gene (APR). The resistance controlled by these genes is generally expressed in the adult plant.

A *Example: The MLO gene*

The senescence associated with the loss-of-sensitivity mechanism is controlled by recessive loss-of-function of the *mlo* (*Mildew Locus O)* gene. The *MLO* gene encodes a membrane protein with unknown functions, which acts as a negative regulator of cell death under biotic and abiotic stresses. Loss-of-function MLO alleles are associated with spontaneous cell death. In rice and *Arabidopsis*, the *MLO* gene is co-expressed with the *PEN1*, *PEN2* and *PEN3* genes, which are required for an active response against powdery mildew. *MLO* acts as a negative regulator of the *PEN1/PEN2/PEN3* pathways, but these genes are required for *MLO-induced* immunity. Consequently, the loss of a general suppressor of cell death may confer resistance to powdery mildews.

A *Example: The Lr67 gene*

The Lr67 gene is a dominant wheat gene conferring partial resistance to rusts and powdery mildews and caused by a mutation in a sugar transporter that differs by 2 amino acids from the susceptible alleles. The Lr67 protein shows a dominant-negative effect by heterodimerizing with the protein encoded by the susceptible allele, thus reducing the amount of sugar taken up by the pathogen, and ultimately causing leaf-tip necrosis.

VI Signal Transduction

1. Introduction

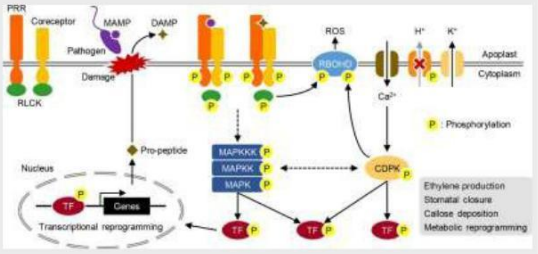
The success of the immune response requires precise control of the timing, amplitude and duration of the induced response. Spontaneous activation or failure to attenuate signalling after immune activation can have a detrimental effect on the host. This explains the complexity of signal transduction processes from immune receptors to the sites responsible for controlling immune responses.

After detecting the presence of a pathogen and recognizing which pathogen it is, the message must be transferred from the recognition systems (PRRs and resistance proteins) to the nucleus and/or other organelles involved in immune responses. Several signalling pathways are involved, depending on the origin of the message: PRRs or R proteins.

- *Receptor-Like Cytoplasmic Kinases* (RLCKs)
- Protein G
- The *Mitogen-Activated Protein Kinase* (MAPKinase) pathway
- Calcium
- *Reactive Oxygen Species* (ROS)
- Nitric Oxide
- Resistance proteins Helpers

and other molecules...

Figure 6.1. Perception of PAMPs and DAMPs by PRR receptors involves dynamic association/dissociation with coreceptors and cytoplasmic receptor-like kinases (RLCKs), and transphosphorylation within PRR complexes to initiate downstream signaling. PRR-derived signals are transmitted via other phosphorylation cascades, including mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs) to downstream targets such as NAPDH oxidase RBOHD, plasma membrane H+-ATPases (PMs) and transcriptional factors (TFs) during PTI.



Note

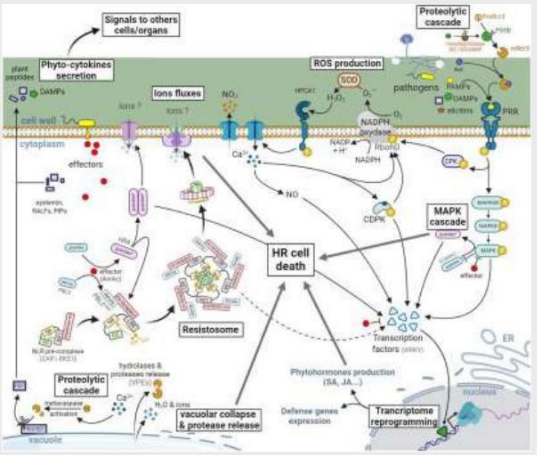
The signalling system in plants operates in a networked, redundant fashion to prevent successful actions by effectors to manipulate plant physiology and block the plant immune system, and also to amplify defense reactions to ensure pathogen elimination and disease limitation.

Signalling (signal transduction) is the key step between pathogen detection and recognition, and the plant's immune response. The most adapted pathogens can interfere with this stage by using effectors. In this case, despite having detected and recognized the pathogen, the plant is unable to trigger immune responses. The message carrying this information is blocked somewhere between detection and response. To overcome this problem, plants use several signalling pathways. Some are specific to PTI, others to ETI, and there are other pathways common to both immune levels.

The plant actually uses a network to transmit the message indicating the presence of the pathogen. Several pathways can transmit the same message. This is known as redundancy in signalling. Despite the additional cost of this redundancy, it is retained for a number of reasons, mainly to :

- Boosting immune responses
- Avoid pathogen effectors bypassing the signaling system.

Figure 6.2. Signal transduction from pathogen detection and recognition to the triggering of t h e hypersensitivity reaction. PRRs are activated by the recognition of elicitor molecules resulting from plant cell degradation (DAMPs) or released by the pathogen (PAMPs: elicitins, apoplastic avirulence factors (Avrs)). The signal is then transmitted by a cascade (series) of phosphorylation events involving MAPKs, cytoplasmic protein kinases (CPKs) and transcription factors, principally the WRKY family. This phosphorylation can also activate the RhohD NAPDH oxidase, leading to the production of ROSs ($^{O2-}$ transformed into hydrogen peroxide ($H2O2$) by superoxide dismutase (SOD)). A flow of intracellular Ca^{2} + is triggered rapidly after $H2O2$ perception by HPCA1, leading to the production o f nitric oxide (NO), as well as activation of transcription factors via calcium-dependent protein kinases (CDPKs). Subsequently, transcriptional activity is reprogrammed to express defense genes involved in the synthesis of phytohormones (SA, Ja,...), antimicrobial phytoalexins or even the release of lytic enzymes (glucanases, chitinases,...) which are part of the *Pathogenesis-Related Proteins* (*PR proteins*). At the same time, effectors secreted by the pathogen to overcome the plant's defenses can also be detected from



either directly or indirectly (through recognition of a modified host protein), recognized by resistance proteins. This recognition generally induces a conformational change in the protein (noted here by an asterix and a change in color), allowing t h e exchange of an ADP for an ATP and consequent activation of the resistance protein, resulting in some cases in a macromolecular complex such as the resistosome or activation of transcription factors. These large molecular complexes are thought to work b y e n g a g i n g other signalling pathways, leading to the reinforcement of existing defences, or by the formation of pores in the plasma membrane. HR-type cell death is then observed, localized at the point of penetration, to block pathogen dissemination. This HR will also be associated with the release of DAMPs, phytohormones and phyto-cytokines, which will transmit information to neighboring cells and organs to prevent future infection of healthy tissue. Certain plant peptides (e.g. PEPs) can be processed by metacaspase and released into the apoplast to initiate immune responses in neighboring cells, thereby establishing local resistance (Roudaire et al., 2021*).

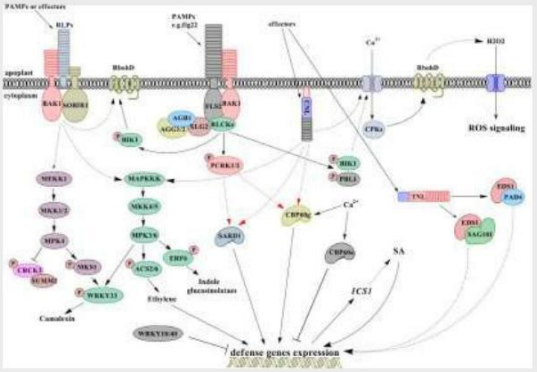


Figure 6.3. Overview of signaling pathways leading to defense responses and triggered by plant immune receptors. Perception of PAMPs or effectors causes activation of membrane receptors in the *receptor-like kinase* or *receptor-like proteins* and families of calcium-inducing resistance proteins, activation of *mitogen-activated protein kinases* (MAPKs or MPKs) and the production of reactive oxygen species (ROS). Several *Receptor-like Cytoplasmic Kinases* (RLCKs) associate with PRRs, such as FLS2. Among these, BIK1 and PBL1 contribute to the activation of calcium fluxes. BIK1 also contributes to the induction of ROS production by phosphorylating Rbohd. Two RLCKs (PCRK1 and PCRK2) contribute to the activation of SARD1 and CBP60g expression. Calcium flux contributes to RbohD activation and ROS production via phosphorylation of RbohD by calcium-dependent protein kinases (CPKs). Activation of MPKs induces synthesis of ethylene, camalexin and indole glucosinolate. Activation of defense responses by

R proteins (TNLs) is facilitated by EDS1/PAD4 and EDS1/SAG101. BAK1 = *BRI1-associated receptor kinase1*, SOBIR1 = *suppressor of bir1 1*, MKK = *MAPK kinase*, MEKK = *MAPK/ERK kinase kinase*, MAPKKK = *MAPK kinase kinase*, CRCK3 = *calmodulin-binding RLCK3*, SUMM2 = *Suppressor of mkk1 mkk2 2*, MKS1 = *MAP kinase substrate 1*, WRKY = *WRKY DNA-binding protein*, BIK1 = *Botrytis-induced kinase 1*, PBL1 =

PBS1-like 1, ACS = *1-AMINO-CYCLOPROPANE-1-CARBOXYLATE SYNTHASE*, ERF6 = *ethylene response factor 6*, FLS2 = *flagellin-sensitive2*, AGB1 = *Arabidopsis G protein b-subunit 1*, AGG1/2 = *Arabidopsis G protein g-subunits 1 and 2,* XLG2 = *extra-large GTP-binding protein 2*, PCRK1/2 = *pattern- triggered immunity compromised receptor-like cytoplasmic kinase 1*
and 2, TNL = *Toll-interleukin 1-like receptor-nucleotide binding-leucine rich repeat*, SARD1 = *SAR deficient 1*, CBP60 = *calmodulin-binding protein 60*, RhohD = *respiratory burst oxidase protein D*, SA = *salicylic acid*, ICS1 = *isochorismate synthase 1*, EDS1 = *enhanced disease susceptibility 1*, PAD4 = *phytoalexin deficient 4*, SAG101 = *senescence-associated gene 101*. Red lines indicate regulation by transcriptional control (Peng et al., 2018*).

2. Receptor-Like Cytoplasmic Kinases (RLCKs)

*Receptor-Like Cytoplasmic Kinases (*RLCKs) are molecules that contain a special cytoplasmic kinase domain containing a Ser/Thr motif, but no extracellular or transmembrane domain. Upon detection of elicitors (MAMPs, PAMPs, DAMPs), PRRs interact immediately and directly with RLCKs.

A *Example: BIK1*

In *Arabidopsis*, BIK1 interacts with the FL2 receptor (a PRR) and is rapidly phosphorylated in a manner dependent on perception of the bacterial flagellum peptide (flg2) by the FLS2 receptor. BIK1 is required for ROS production, following detection of the various PAMPs.

PBL1 (another RLCK) and in association with BIK1 are both required for the increase in Ca^{2} + concentration induced by the perception of PAMPs, suggesting that they are involved in $^{Ca2+}$ signal activation in ITP.

A *Example: PCRK1 and PCRK2*

PCRK1 and PCRK2 also interact with FL2 and are rapidly phosphorylated following flg2 perception. PCRK1 and PCRK2 function redundantly to stimulate pathogen-induced salicylic acid production. Loss of PCRK1 and PCRK2 results in compromised ITP and low resistance to pathogens.

A *Example: OsRLK185*

In rice, OsRLK185 associates with and is phosphorylated by OsCERK1 (chitin PRR receptor) following chitin perception. OSRLK185 and its *Arabidopsis* counterpart PBL27 are involved in chitin perception-related induction of ROS production and activation of the MAPK pathway.

3. Protein G

In fungi, the G protein is composed of 3 subunits: Gα, Gβ, and Gγ serving as couplers to connect the protein to other enzymes in the signal transduction process. In plants, the G protein functions as a convergent point in immune signaling processes via RLKs. Loss of the Gβ subunit (AGB1) or Gγ subunits (AGG1 and AGG2) results in reduced production of ROSs induced by the various elicitors, which will compromise ITP.

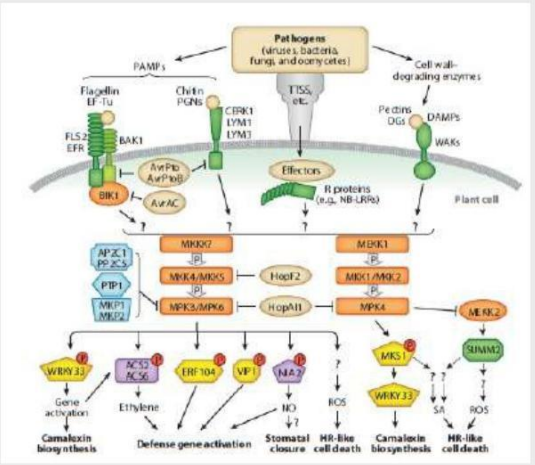
4. The Mitogen-Activated Protein Kinase (MAPKinase) pathway

This is a cascade with several MAPKs, and is highly conserved in eukaryotes. Perception of elicitors (MAMPS, PAMPs, or DAMPs) by PRRs induces rapid activation of the MAPK pathway. In *Arabidopsis,* at least 6 kinase enzymes: MPK1, MPK3, MPK4, MPK6, MPK11 and MPK13 are activated by the bacterial elicitor flg22 (a protein fragment of bacterial flagella).

MAPKs phosphorylate a wide range of target proteins, with different roles in plant immune responses, thus serving as a divergent signalling point.

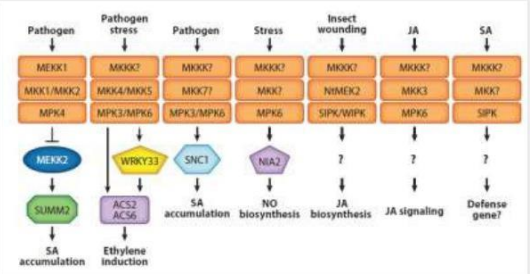
Activation of MPK3 and MPK6 is dependent on upstream MKK4 and MKK5. Activation of MPK4 by the flg22 elicitor is dependent on MEKK1 and upstream MKK1 and MKK2.

Figure 6.4. The pathway of MAPKinase signalling (Meng & Zhang, 2013*)



Mapkinase 1

Figure 6.5. The MAPKinase signaling pathway (Meng & Zhang, 2013)



MAPKinase 2

In *Arabidopsis,* MPK3 and MPK6 are involved in the activation of a multitude of immune responses. They stimulate ethylene biosynthesis by phosphorylating ACS2 and ACS6 (2 enzymes involved in ethylene synthesis). This phosphorylation stabilizes these 2 enzymes, thereby enhancing ethylene synthesis.

MPK3 and MPK6 control camalexin synthesis by targeting the WRKY33 protein. Phosphorylation of the latter by MPK3 and MPK6 is necessary to ensure its role in stimulating pathogen-induced camalexin synthesis. These 2 MPKs stimulate the synthesis of indole glucosinolate (involved in immune reactions) via phosphorylation of *Ethylene Response Factor6* (ERF6). They are also involved in stimulating immune responses involving stomata by modulating malate metabolism during infection.

MPK4 is a multifunctional enzyme. It is involved in the positive and negative regulation of immunity in plants. It contributes around 50% to the stimulation of gene expression following detection of the elicitor flg22. The MEKK1-MKK1/MKK2-MPK4 cascade stimulates basal resistance to pathogens. It is monitored by the SUMM2 resistance protein.

In tomato, silencing of MAPKKKα reduces HR intensity following detection of the bacterial effector AvrPto.

Note

MAPK activation in a PTI response l a s t s less than an hour. Activation of MPK3 and MPK6 by the resistance protein (ETI) lasts several hours.

Fundamental

Continuous activation of MPK3 and MPK6 contributes to the activation of gene expression and resistance against pathogens, without the involvement of salicylic acid (a hormone involved in signaling).

Attention

To date, the involvement of the MAPK cascade in signaling following pathogen detection by resistance proteins remains unproven. The method by which the RPS2 resistance protein activates MAPK remains unknown.

5. Calcium

Calcium acts as a secondary messenger in many signaling processes. Detection of elicitors by PRRs immediately induces *Ca2+* influx at the cytoplasmic membrane. Similarly, detection of pathogen effectors by resistance proteins induces Ca^2 + flux. Several proteins involved in plant immunity that respond to changes in cytoplasmic Ca2+ concentrations have been identified. The channels through which Ca2+ crosses the cytoplasmic membrane are still unknown.

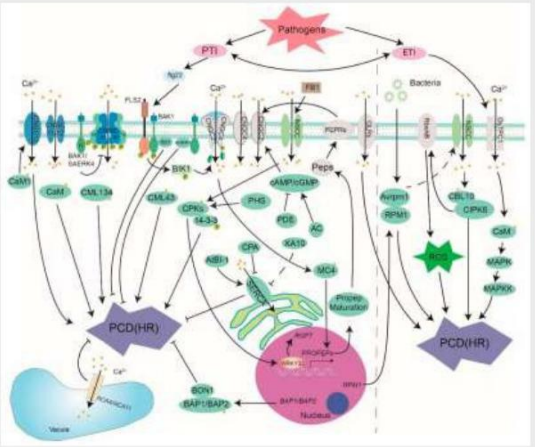


Figure 6.6. Calcium signalling. Calcium chains, the *sensors* and the genes and proteins involved.
shown in the diagram. PTI: PAMP triggered immunity, flg22: a bacterial elicitor (PAMP) composed of 22 amino acids and located in the flagellum, FB1: fumonisins B1, FLS2: Flagellin- sensitive 2, CNGCs: Cyclic nucleotide gated channel, BAK1: Brassinosteroid insensitive 1-associated receptor kinase 1, SERK4 : Somatic embryogenesis receptor kinase 4, BIK1 : Botrytis- induced kinase 1, BIR1 : BIK1-interacting receptor-like kinase1, SOBIR1 : Suppressor of BIR1-1, Peps : Plant elicitor peptide, PERRs : Extracellular Pep receptors, CaM
: Calmodulin, CML: CaM-like protein, CDPK(CPK): Ca^2 +-dependent protein kinase, CBL: Calcineurin B-like protein, CIPK: CBL-interacting protein kinase, cAMP: 3'-5'-cyclic adenosine monophosphate, cGMP: Cyclic guanosine monophosphate, AC: Adenylate cyclase, PDE : phosphodiesterase, PHS : Phytosphingosine, MC4 : Metacaspace 4, 14-3-3 : 14-3-3 proteins, SERCA : Sarco- endoplasmic reticulum Ca^2 +-ATPase, ACA : Autoinhibited Ca2+- ATPase, RPM1 : Resistance to Pseudomonas syringae pv. Maculicola 1, AvrRpm1: Pseudomonas syringae type III effector,
MAPK: Mitogen activated protein kinase
(Ren et al., 2021*).

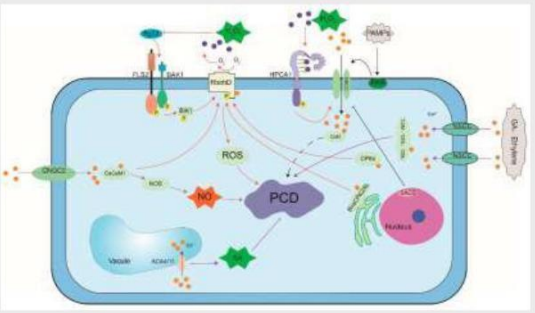


Figure 6.7: Interactions of calcium with other messengers. Many signal molecules can induce programmed cell death, including calcium, ROS, NO and hormones. HPCA1: Hydrogen peroxide sensor, PAMPs: Pathogen- associated molecular pattern, PRR: pattern-recognition receptor, RBOHD: Respiratory burst oxidase homolog protein, SA: salicylic acid, GA: gibberellin (Ren et al., 2021*).

Fundamental

Although calcium is a secondary messenger, it is essential f o r the induction of the hypersensitivity reaction (HR).

A *Example*

In *Arabidopsis,* 4 calcium-dependent *protein* kinases (CPK4, CPK5, CPK6 and CPK11) are involved in stimulating ROS synthesis and reprogramming gene transcription, following detection of the elicitor flg22.

CPK5 and CPK6 are also involved in the up-regulation of ETI. Loss of these two kinases leads to compromised resistance, even if signaled by the resistance proteins RPS2 and RPM1. CPK4, CPK5 and CPK11 can phosphorylate several WRKY transcription factors. This phosphorylation enhances their binding to DNA.

CPK1 and CPK2 stimulate ROS production, following activation of the immune system by the resistance proteins RPS2 and RPM1.

Complement

Several *calmodulin (CaM)-binding* transcription *factors*, such as CAMTA3, CBP60 and CPB60a, have been identified as key defense regulators in *Arabidopsis*. Loss of CAMTA3 causes autoimmunity (partly due to resistance induced by the resistance proteins DSC1 and DSC2). Loss of CPB60a results in enhanced expression of genes involved in basal resistance. On the other hand, loss of CPB60g causes a reduction in t h e accumulation o f salicylic acid induced by the elicitor flg22, and a subsequent increase in susceptibility to the bacterium *Pseudomonas syringae*.

Q *Reminder: Calmodulin (CaM: Calcium-Modulated protein)*

Calcium-modulated protein: This is a ubiquitous intracellular protein receptor for Ca2+ ions. It acts as a multifunctional messenger intermediate.

Complement

Following the perception of various biotic and abiotic stimuli, spatial and temporal changes in free cytosolic Ca^{2+} concentrations ([Ca2+]cyt) are frequently observed as an immediate (perceptual) response.

Fundamental

Calcium is involved in signalling many stimuli, not just stress.

6. Active Oxygen Molecules

ROS production is induced rapidly after elicitor and effector perception. The majority of apoplastic ROSs are produced by RbohD (*Respiratory burst oxidase homolog D*) following its phosphorylation by BIK1 after detection of the elicitor flg22.

The ROSs produced by RbohD contribute to resistance against pathogens. They are also involved in stimulating cell death induced by the resistance protein RPM1.

FIgure 6.8. The C-terminal region of RbohD is phosphorylated, and ubiquitin m o l e c u l e s attached to it by PBL13 and PIRE respectively, in a quiescent state, which will decrease RbohD stability through degradation in the tonoplast. Following detection of the elicitor flg22, RbohD is activated by phosphorylation of the N-terminal region, causing ROS accumulation in the poplasm and s t i m u l a t i n g ROS-induced immunity. PIRE is dynamically phosphorylated during activation of system immune system (Lee et al., 2020*).

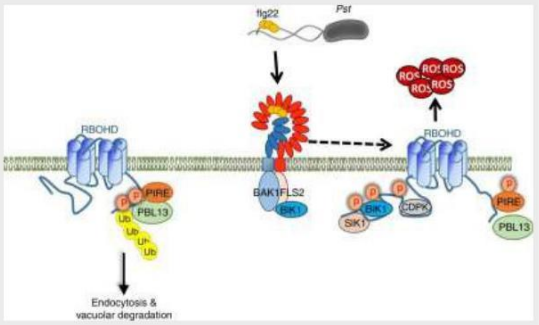
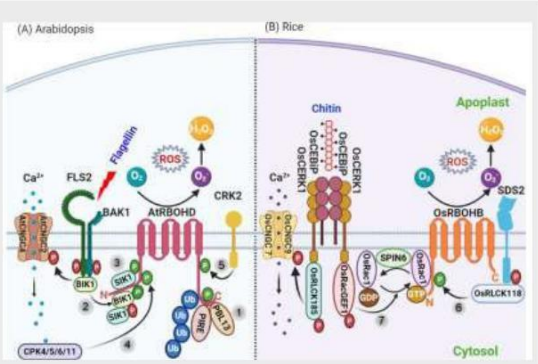


Figure 6.9. Control of the production of ROSs. (Wang et al., 2020*).



7. Resistance proteins: Helpers

Many *sensor-type* resistance proteins use R *helper* proteins in signaling and inducing defense reactions.

To date, all the *herlpers* resistance proteins (discovered) belong to the NLRs family.

Figure 6.10. The signaling network of resistance proteins *sensors* and *helpers*. Experimentally demonstrated dependency links in (resistance protein) signalling are shown as coloured arrows; bold = ADR1-dependent signalling, italic = ADR1-dependent signalling.

NRG1, italic bold = ADR1- and NRG1-dependent signaling, underlined = NRC-dependent signaling, normal = unknown/not analyzed. It is not known whether Solanaceae NRCs require *helpers* for cell death signaling and resistance, but this is very likely, as for example Rx2-induced resistance in the case of *N. benthamiana* requires the involvement of *helpers*. Its Rx paralog requires the involvement of 3 NRCs (NRC2, 3, 4) to induce cell death (Jubic et al., 2019*).

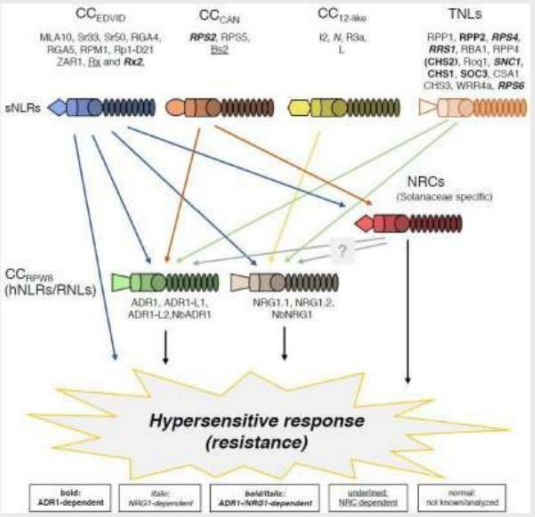
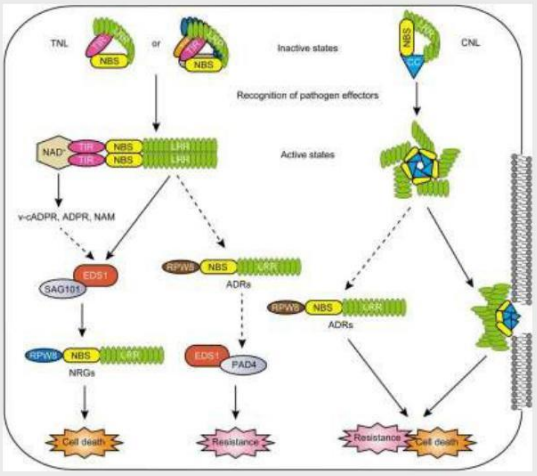


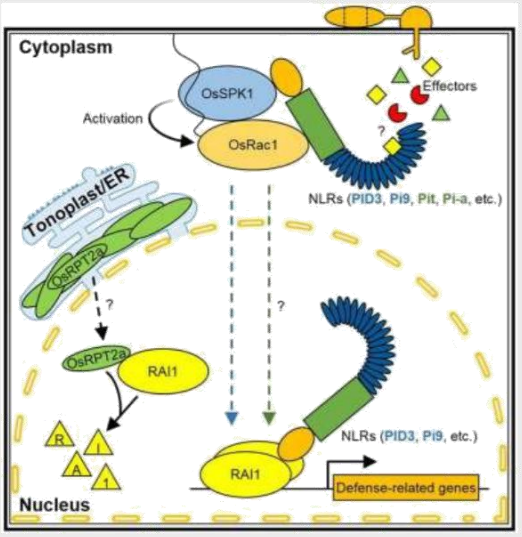
Figure 6.11. Signal transduction after pathogen detection by intracellular resistance proteins. Resistance proteins (NLRs) specifically detect pathogen effectors. In t h e absence o f effectors corresponding to the R proteins available in the plant, the resistance proteins are in a quiescent state. After recognition of the pathogen (via its effectors), NLRs form homo- or heterodimers to activate immune system signalling. In the case of TNLs, self-association (homodimerization) is necessary for the activity of the NADase in their Toll/Interleukin-1 domains, which breaks down NAD+ into v-cADPR, ADPR and NAM. Any of these products o f NADase action (NAD+to v-cADPR, ADPR and NAM) can signal EDS1 to induce cell death. For CNL-type R proteins, effector recognition induces oligomerization of CNLs to form a resistosome. This is necessary to induce resistance. To activate full resistance, Arabidopsis NLRs need NRG1 and ADR1 helper NLRs. All cell death induced by the R proteins of



type TNL spnt dependent on *helpers* from the NRG1 family activating the downstream EDS1-SAG101 complex. Some resistance responses induced by TNLs depend on ADR1 acting upstream of the EDS1-PAD4 complex. Immune responses induced by CNL-type resistance proteins essentially require ADR1 and plasma membrane-associated pore-shaped resistance (Wang et al., 2020*).

Figure 6.12. Signal transduction after pathogen recognition by R proteins in rice. After identification of

Magnaporthe oryzae effectors rice resistance proteins (including PID3, Pi9, Pit, and Pi-a) transmit their signals to the downstream component OsSPK1, a GEF, by binding directly with it. OsSPK1 helps convert the GTPase OsRac1 at the plasma membrane from an inactive GDP-bound state to an active GTP-bound state. Activated OsRac1 induces activation of transcription factor RAI1 at level of nucleus, causing reprogramming the transcription of genes involved in rice defense. It is not yet known whether RAI1 is involved in triggering resistance induced by Pit or Pi-a (marked by a question mark). PID3 and Pi9 both show affinity with RAI1 in the nucleus, which is assumed to protect RAI from degradation by the 26S proteasome. The tonoplast/RE-localized OsRPT2a offers a solution f o r refining RAI accumulation *in-vivo*. It moves to the nucleus (means unknown) where it associates with RAI1, leading to a reduction in RAI1 accumulation in a proteasome-dependent manner (Yu et al., 2021*).



7.1. ADR1 family

ADR1= Activated Disease Resistance 1

7.2. NRG1 family

NRG1= N Required Gene 1

7.3. NRC family

NRC= NB-LRR protein required for HR-associated cell death

VII The Plant Immune System

Plants use two pathogen "surveillance" systems. The first is based on cellular membrane receptors, called *Pattern* Recognition *Receptors* (*PRR*). Its role is to detect the presence of pathogens as early as possible. This detection is based on a physical receptor-ligand bond. It recognizes molecules of generally non-specific microbial origin (*Microbe-Associated Molecular Patterns* (*MAMPs*)), or linked to the various pathogens (*Pathogen-Associated Molecular Patterns* (*PAMPs*)), or resulting f r o m plant-pathogen interaction (*Damage-Associated Molecular Patterns* (*DAMPs)*), e.g. sugar molecules, fatty acids, etc. resulting from CWDE-induced digestion of the plant wall.

The second system is more specific, based on pathogen recognition through the detection of highly specific molecules linked to the pathogen in question. These are known as effectors. Effectors are detected and recognized by resistance proteins.

Physically and temporarily, non-host resistance is ensured by the pathogen's ***maladaptation to the*** plant with which it has come into contact. For example, the plant does not produce molecules that enable pathogens to recognize it and start the infectious process (for more details, see the course ***Mechanisms of pathogenicity of phytopathogenic fungi***).

In the case of a host plant, both of the above-mentioned monitoring systems come into play. The ***adapted*** pathogen manages to initiate the infectious process, or at least is able to recognize that the plant it has come into contact with is a host plant. In this case, we have two scenarios: firstly, the PRRs detect the pathogen and trigger the plant's defensive responses; secondly, the pathogen manages to escape detection by the PRRs (for more details, see the chapter on ***Effectors*** in the course on ***Mechanisms of pathogenicity in phytopathogenic fungi***). In this situation, the plant uses the second detection/recognition system, in this case, the resistance proteins.

1. Different Models of the Plant Immune System

Attention

It should be noted that PTI cannot exceed the level of an immune response that induces HR. Only ETI induces HR.

1.1. Gene-for-Gene theory

The first model (theory) explaining plant-pathogen interactions is the gene-for-gene theory, developed by Flor (1971) when studying flax resistance to rust. This theory states that for every plant resistance gene there is a pathogen avirulence gene. For a plant to be resistant to a given pathogen, it must have a resistance gene whose product (the resistance protein encoded by this gene) recognizes the product of the avirulence gene (avirulence protein). If there is no recognition, the plant is susceptible and disease will result.

Once the pathogen has been recognized (either by PRRs or by R proteins), the plant triggers immune responses to defend itself. These responses may vary in intensity depending on the pathogen, but also on the host plant's genotype.

Table 7.1. Gene-for-gene theory

Pathogen/Plant	***Plant R***	***Plant r***
Avirulence	Resistance	Sensitivity
avirulence	Sensitivity	Sensitivity

The plant is resistant when the plant with a dominant resistance gene interacts with a pathogen with a dominant avirulence gene. In other situations, the plant is not resistant. Legend: R: resistance gene, r: susceptibility gene, avr: virulence gene, Avr: avirulence gene

Table 7.2. The interaction (according to the gene-for-gene theory) between a plant with 2 resistance genes and a pathogen with 2 avirulence genes.

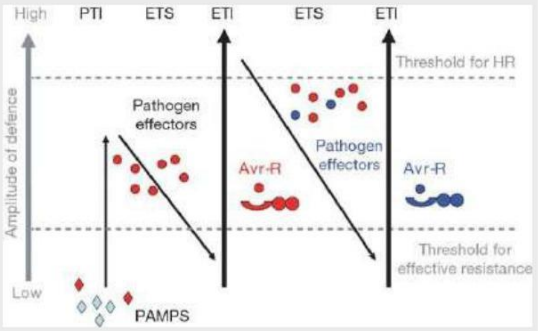
Pathogen/Plant	***R1R2***	***R1r2***	***r1R2***	***r1r2***
Apr1Apr2	Resistance	Resistance	Resistance	Sensitivity
Apr1avr2	Resistance	Resistance	Sensitivity	Sensitivity
avr1Avr2	Resistance	Sensitivity	Resistance	Sensitivity
apr1avr2	Sensitivity	Sensitivity	Sensitivity	Sensitivity

Legend: R: resistance gene, r: susceptibility gene, avr: virulence gene, Avr: avirulence gene

1.2. The Zig-Zag Model

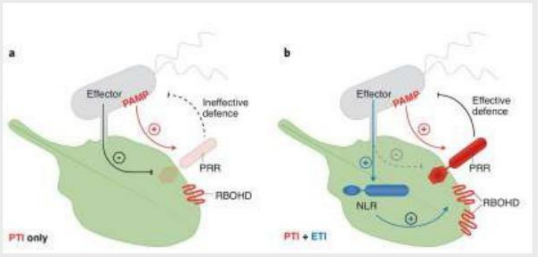
The gene-for-gene theory assumes that there is an interaction between the R protein and the avirulence proteins (direct recognition). We now know that this direct recognition model does not represent the majority of interactions. Other models have been developed to explain plant-pathogen interactions. The most widely used and accepted is the zig-zag model, developed by Jones & Dangle in 2006 (see figure below). This model incorporates the notions of a two-tier plant immune system: PTI and ETI.

Figure 7.1. The zigzag model o f plant-pathogen interaction (Jones & Dangl, 2006*). Elicitors (PAMPs, DAMPs) are detected by the PRR, inducing a PTI-type immune response in the host plant. The adapted pathogen uses effectors to interfere with PTI. It should be noted that PTI never reaches a level at which HR can be triggered. The pathogen uses effectors to block PTI. The plant uses resistance proteins to monitor effector action. The resistance proteins detect and recognize the pathogen, triggering ETI. In turn, the pathogen uses a number of effectors to block ETI, inducing ETS (*Effector Triggered* Susceptibility). The plant continues to monitor the effectors/target molecules and once again manages to detect and recognize the pathogen, inducing ETI once again.



The most recent studies prove that the two branches of the plant immune system (PTI and ETI) are not separate from each other. In fact, there is mutual reinforcement between PTI and ETI. The work of Ngou et al. (2021)* and Yuan et al. (2021)* shows that ETI is dependent on PTI components and functions by enhancing PTI.

Figure 7.2. Mutual reinforcement of PTI and ETI. (a): Bacterial infection triggers plant defensive responses, such as activation o f NADPH oxidase activity (RBOHD) t o combat pathogen infection (PTI, in red). However, adapted pathogens use effectors (in black) to suppress PTI, leading to ineffective defense on the part of the host plant (black lines). (b): With the presence of resistance proteins, effectors are recognized, and ETI is activated (blue). Activation of the resistance proteins leads to stimulation of PTI components such as RBOHD, thus bypassing t h e suppressive effect of bacterial effectors (grey lines). As a result, t h e concerted action of ETI and PTI effectively halts infection (in black). (Pruitt et al., 2021*).



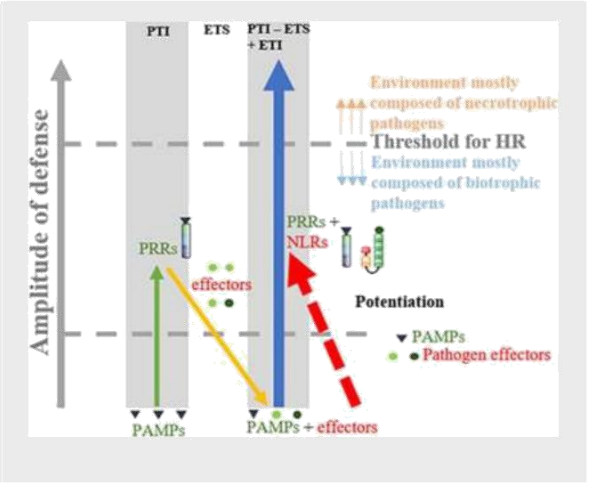


Figure 7.3. The zig-zag model modified by Ngou et al (2020)

1.3. The Invasion Model

In this model, the pathogen will use invasion molecules (broad- or narrow-spectrum (specific)) to invade the plant and bypass its defenses. Plant receptors (PRRs and R proteins) will interact with the pathogen's invasion molecules to induce two types of immunity:

- Cytoplasmic immunity: immunity triggered by pathogen detection in the cytoplasm.
- Induced immunity in the apoplast: this is the immunity triggered by pathogen detection by membrane receptors (membrane R proteins and PRR).

Depending on the mode of activation of the immune system (cytplasmic or membrane (apoplastic)), signal transduction and the triggering of immune responses will follow.

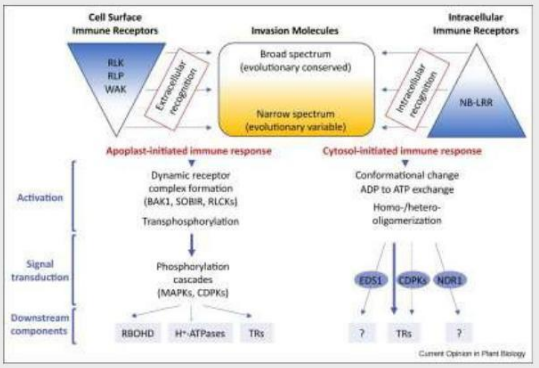
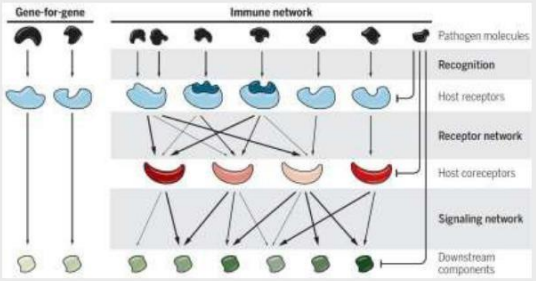


Figure 7.4. The Invasion model (Kanyuka & Rudd, 2019*).

1.4. The Immune Network

The "network" model emphasizes signal transduction redundancy. Following pathogen detection, the plant will use multiple, redundant signals to transmit the message of pathogen detection and recognition. The same message is transmitted by several pathways and reaches the nuclei by different routes and methods. The pathogen uses effectors to try to prevent the signal of its presence from reaching the nucleus. The plant will therefore trigger several immune responses, either simultaneously or consecutively, at very short intervals. The pathogen also uses effectors to try to block/inhibit these immune responses.

Figure 7.5. The networked immune system (Wu et al., 2018*)



2. Elicitor-induced immunity

Elicitor-induced immune responses are triggered by the detection of the pathogen's presence in elicitors (PAMPs and/or DAMPs). In scientific literature, this is referred to as PTI (*PAMPs Triggered Immunity*). After detection, a signaling cascade is triggered to induce plant defense.

Figure 7.6. The plant recognizes the pathogen if the elicitor is compatible with one of the PRR receptors. This recognition leads to the triggering of immune responses. If there is no compatibility between the elicitor and the plant's PRR receptors, the pathogen will go undetected by the plant, and disease will result, making the plant susceptible. Green: receptor, purple: elicitor (Zhang et al., 2013*).

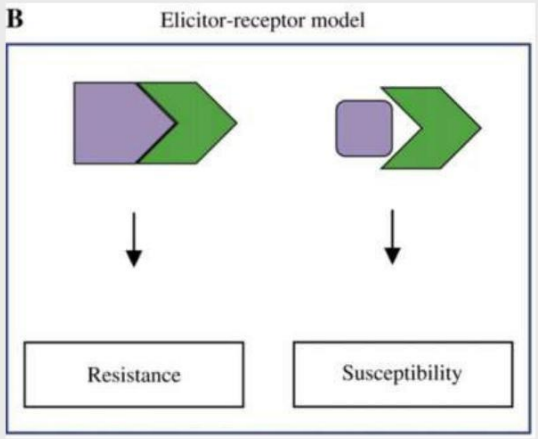


Figure 7.8. After recognition of the pathogen, the message is transmitted to the nucleus to trigger transcription of the genes involved in defense, e.g. PR proteins, secondary metabolites, etc. (Mengiste, 2012*).

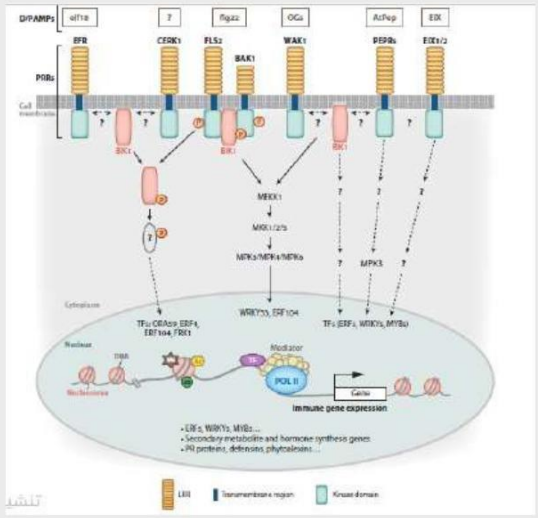
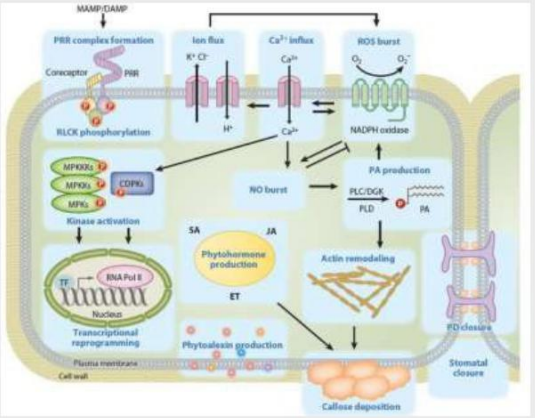


Figure 7.9. After detection and recognition of t h e p a t h o g e n , several defense mechanisms are set in motion (Zhang et al., 2017*).



3. Effector-induced immunity

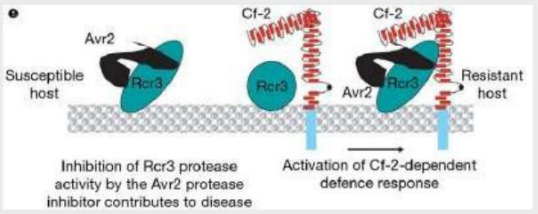
Fundamental: PTI and ETI

In reality, the differentiation between PTI and ETI is only theoretical. In practice, so far we've been unable t o distinguish one from the other. What's more, the defense mechanisms induced are almost identical, regardless of their mode of induction!

Fundamental

The major differences between PTI and ETI are that the former is based on recognition of PAMPs and the latter is based on recognition of effectors, and also that the latter can induce HR.

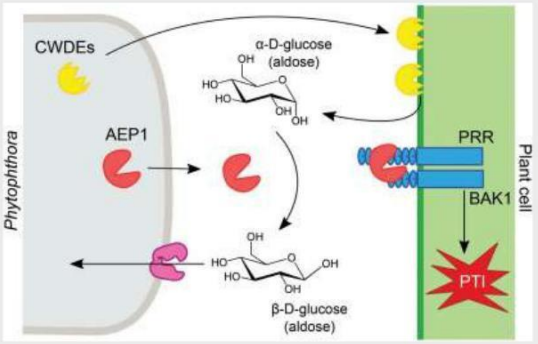
Figure 7.10. The Cf-2 resistance protein does not recognize the Avr2 effector. Instead, it recognizes the Avr2-Rcr3 complex (Rcr3 is a protease linked to pathogenesis). The pathogen targets Rcr3 to inhibit its action. Recognition of the Avr2-Rcr3 complex by the Cf-2 resistance protein activates the plant's immune responses (ETI).



Complement: Case of AEP1 end effector

AEP1 (Aldose-1 Epimerase enzyme) is an effector whose role is to modify sugars such as glucose from its α- to β- form, to facilitate their absorption by the pathogen (*Phytophthora sojae*).

Figure 7.15. The AEP1 effector induces PTI after detection by PRRs. Upon infection, the *P. sojae* pathogen secretes CWDEs to degrade the cell walls of its host plant (soybean). CWDEs release various cell wall degradation monomers (sugars, fatty acids, etc.) into the applasm. Pathogens generally use these degradation products as carbon sources (see ***Mechanisms of pathogenicity of phytopathogenic fungi*** course). In the case of
P. sojae, and its host plant, soybean, the CWDEs release aldoses (α-glucose) into the applasm, the problem is that the pathogen is unable to absorb this form, the pathogen also secretes with the CWDEs an effector called AEP1 whose role is to convert α-glucose into β-glucose, which is easily absorbed. The plant (soybean) recognizes the pathogen through the detection of AEP1 via PRRs and induces resistance. The plant here



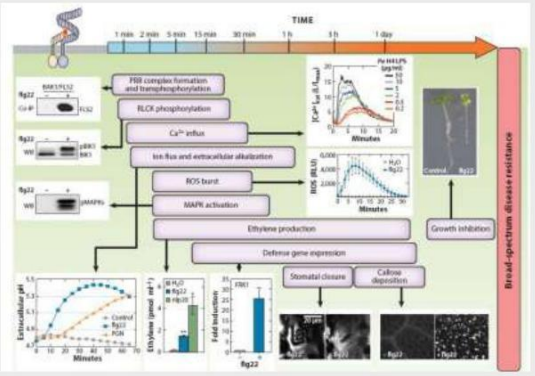
does not recognize AEP1 as an effector (effect of the effector on the target protein/molecule) but does recognize its secondary or primary form (as PAMPs) (Copeland, 2021*).

Other effectors are also recognized by PRRs, for example nlp20, which is a fragment of the NEP1 (*Necrosis and Ithylene-inducing Peptide 1*) effector by the RLP23 receptor. NLPs (NEP-Like proteins) are secreted by bacteria, oomycetes and phytopathogenic fungi.

4. Immune Responses

After pathogen detection by PRRs or R proteins, the signal (of pathogen presence) is transmitted to the nucleus, where various defense responses are triggered.

Figure 7.10. Temporal dynamics of the immune response (Zhang et al., 2017*).



After the signal, indicating the presence of a pathogen (PTI) or in the case where the plant is able to recognize which pathogen (ETI), reaches the nucleus the plant triggers a panoply of responses in order to eliminate the pathogen:

- Wall reinforcement
- Secondary metabolites
- Proteins linked to pathogenesis
- Hypersensitivity r e a c t i o n s

VIII Physical Barriers of Defense

Physical or mechanical barriers are a plant's first line of defense against aggressors (pests and/or pathogens).

1. Constituent Barriers

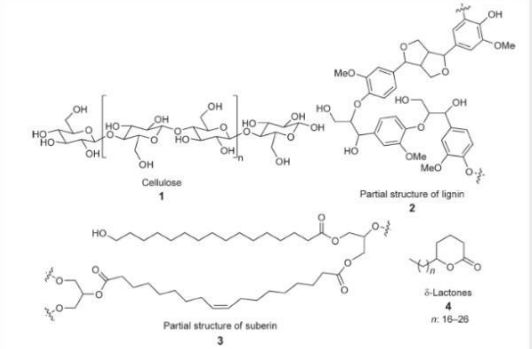
A *Example*

The leaves of some plants, such as Ficus, are covered with wax. This wax makes the leaves very hydrophobic and will prevent water droplets from remaining on the leaves. These water droplets are necessary for successful infection.

Figure 8.1. *Ficus macrophylla* leaf. This leaf is covered with a layer of highly hydrophobic wax, preventing the stagnation of water droplets needed for infection.

Constitutive barriers are structures that exist before the presence of the pathogen is detected. They are part of the plant's normal constitution. The presence of these barriers will prevent most pathogens from infecting the plant.

Figure 8.2. Constitutive physical barriers. The walls of tissues in contact with the exterior are naturally reinforced with other molecules to protect the plant: In addition to cellulose and other wall molecules, we can find wax, lignin, xylan, etc. (Spiteller, 2008*).



Physical barriers

1.1. Wax

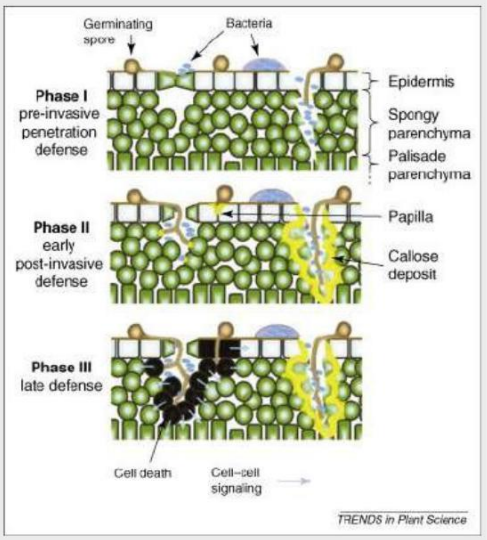
The wax is generally deposited on the outer part of the leaves, making them very hydrophobic and therefore dry. In order to germinate, fungus spores need at least one droplet of water (see course on the pathogenicity of phytopathogenic fungi).

Figure 8.3: Plant leaves are generally covered w i t h a layer of wax. The thickness of this layer varies from species to species (see figure 8.1). The wax's hydrophobic properties prevent water from stagnating on the leaf. On leaves, water is generally in the form of droplets (not spread out) with a fragile equilibrium. All it takes is a slight breeze to cause these droplets to fall. If these droplets remain long enough (a few hours (2-4h)), pathogen spores germinate and penetrate.

2. Induced Barriers

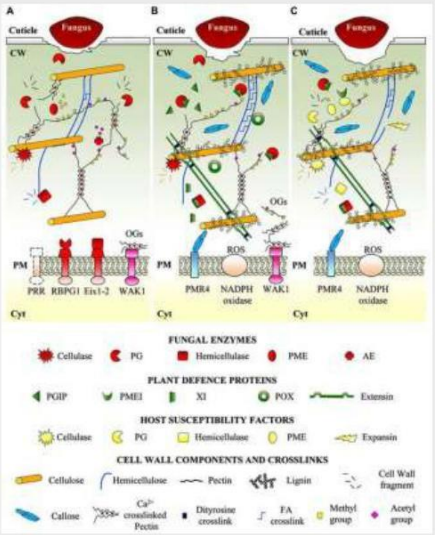
These are the barriers produced after pathogen detection. The plant reinforces the point(s) of pathogen penetration, in order to prevent it. The formation of papillae is one of the first plant defense responses observed.

Figure 8.4. Callose deposition and papilla formation are the first steps in plant defenesis following infection by a pathogen (Ton et al., 2009*).



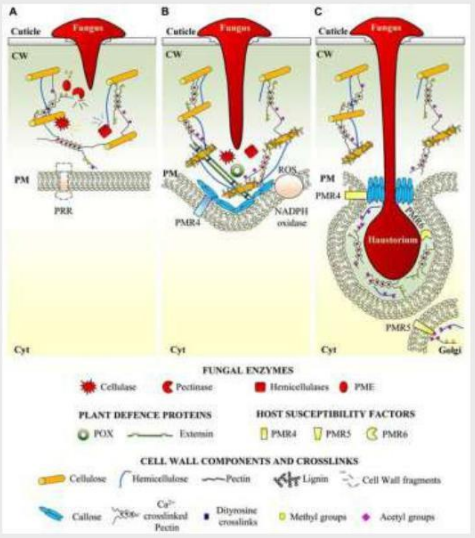
Infection mechanisms differ from biotrophic to necrotrophic pathogens, and so do plant responses:

Figure 8.5. Changes to the plant cell wall following infection by necrotrophs. (A) Necrotrophic fungi secrete a large arsenal of cell wall degrading enzymes (CWDEs) such as PGs, hemicellulases and cellulases, assisted by PMEs and AEs in the apoplastic space to degrade cell wall polymers and facilitate nutrient availability. It has been proposed that PGs and EIXs function as PAMPs recognized by membrane receptors RBPG1 and Eix1 or 2, respectively. (B) As a first line of defense, plants produce a variety of CWDE inhibitors to prevent degradation by microbial CWDEs. For example, inhibition o f PG degradation activity by PGIPs induces the accumulation of elicitor-active pectin (OG) fragments perceived by WAK1 receptors. The presence of other as yet unidentified receptors detecting damage to other cell wall components cannot be ruled out. The perception of cell wall damage triggers specific signaling pathways activating defense responses aimed at reinforcing the cell wall structure. The most obvious defense strategies are callose and lignin deposition, induction o f peroxidases/ROS mediated by cross-links between cell wall structural proteins and polysaccharides. (C) Necrotrophs force plants to "cooperate" in disease by exploiting plant cellulases, expansins, PGs and PMEs as susceptibility factors. Legend : PM, plasma membrane; CW, cell wall; Cyt, cytoplasm; OGs, oligogalacturonides; WAK1, wall associated kinase 1; AEs, acetyl esterases; PGs, polygalacturonases; EIXs, ethylen induced xylanases; PME, pectin methylesterases; PMEI, pectin methylesterase inhibitor; FA, ferulic acid; Eix1-2, receptors of ethylene induced xylanases; RBPG1, Responsiveness to Botrytis PolyGalacturonase 1; Ca2+, calcium



ions; XI, xylanase inhibitor; PRR, pattern recognition receptor; POX, peroxidase; ROS, reactive oxygen species (Bellincamp et al., 2014*).

Figure 8.6. Changes to the plant cell wall following infection by biotrophs. (A) Biotrophic fungi use appressorial mechanical pressure and secrete cell wall-degrading enzymes to penetrate the plant cell wall. (B) Plants sense the penetration of fungal biotrophs with as yet unidentified receptors (PRRs) and respond by appositioning "papillae" between the cell wall and plasma membrane. The papillae, in addition to the new cell wall material, are also sites o f ROS accumulation possibly involved in cell wall reinforcement. (C) If wall reinforcement (papillae) is not effective in stopping infection, the fungus penetrates and then forms the haustorium feeding organ invaginated in the host membranes and plant cell wall. Biotrophs locally affect cell wall metabolism by inducing susceptibility factors (callose synthase PMR4, O-acetyltransferase PMR5 and pectate lyase PMR6) to modify the extra-haustorial matrix t o i m p r o v e nutrient accessibility or ensure the mechanical stability of the haustorium. Legends: PM, plasma membrane; CW, cell wall; Cyt, cytoplasm; PG, polygalacturonase; PME, pectin methylesterase; PRR, pattern recognition receptor; POX, peroxidase; ROS, reactive oxygen species (Bellincamp et al., 2014*).



Although the specific biochemical composition of papillae can vary between different plant species, certain classes of compounds are commonly found, including phenolics, reactive oxygen species, cell wall proteins and cell wall polymers. Among these polymers, (1,3)-β-glucan callose is one of the most abundant and ubiquitous components.

2.1. Callose

Callose is a β-(1,3)-D-glucan polysaccharide with some β-1,6 branches that exists in all multicellular green algae and higher plants.

Callose is deposited between the plasma membrane and the cell wall at the site of pathogen attack, at plasmodesmata and on other plant tissues to slow pathogen invasion and spread.

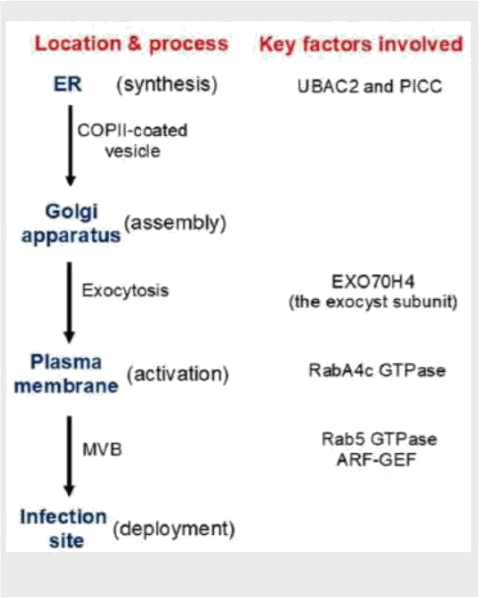


Figure 8.7. Synthesis, transport, activation and recruitment of the Arabidopsis callose synthase PMR4 during plant defense responses. Some of the important factors identified as being involved in these processes are indicated (Wang et al., 2021*).

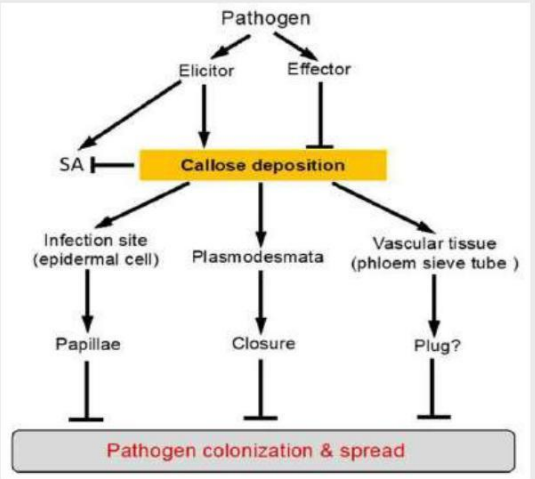


Figure 8.8. Induction and roles of defense-related callose deposition in plants. Pathogen elicitor-activated signaling of innate plant immune responses leads to increased callose deposition at pathogen attack sites, on plasmodesmata and in vascular tissues. The formation of callose-rich papillae at infection sites helps limit penetration and colonization by invading pathogens. Increased callose deposition at plasmodesmata leads to plasmodesmata closure, helping to limit the spread of pathogens. Increased callose deposition in vascular tissues such as phloem sieve tubes could also function as a defense mechanism to reduce colonization and transport of vascular pathogens. In Arabidopsis, pathogen-induced SA signaling is negatively regulated by PMR4-dependent callose deposition. Pathogens contain effector proteins that inhibit or block defense-related callose deposition by both as mechanisms of counter-defense mechanisms (Wang et al., 2021*).

2.2. The Papillae Formation

Figure 8.9. Papilla formation is an induced physical defense mechanism. The papilla (arrow) is formed around the fungal hypha (penetration tip) at the site of penetration, trying to prevent the pathogen from penetrating (Schumann & D'Arcy, 2013*).

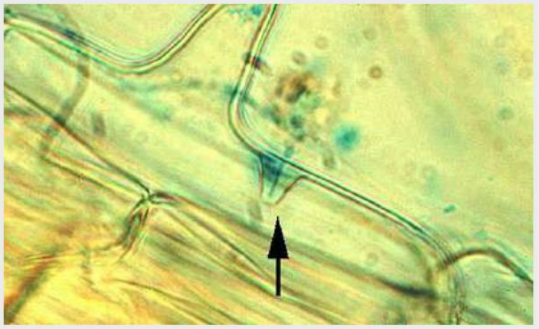


Figure 8.10. Reinforcement of cell wall structure at the site of pathogen penetration.Cell wall-associated structures commonly observed at sites o f interaction with powdery mildew and other fungal pathogens. (A) A fungal penetration attempt stopped by the deposition of a cell wall apposition (blue). The inset image shows a top-down view of the penetration site as usually visualized by light microscopy. (B) A successful penetration event in which the fungus has formed a haustorial feeding structure. The cell wall apposition materials form a neckband or collar around the haustorium neck. (C) A haustorium partially surrounded b y a haustorial envelope.
The envelopes contain materials similar to those found in cell wall appositions. (D) A fully encased haustorium.CW, cell wall; PM, plasma membrane; C, conidiospore; PGT, primary germ tube (note that not all powdery mildew species develop PGTs); AGT, appressorial germ tube; PP, penetration peg; H, haustorium; EHM, extra- haustorial membrane; NB, haustorial neck-band; P, papilla (e.g., cell wall apposition); E, haustorial encasement ((Underwwood, 2012
*).

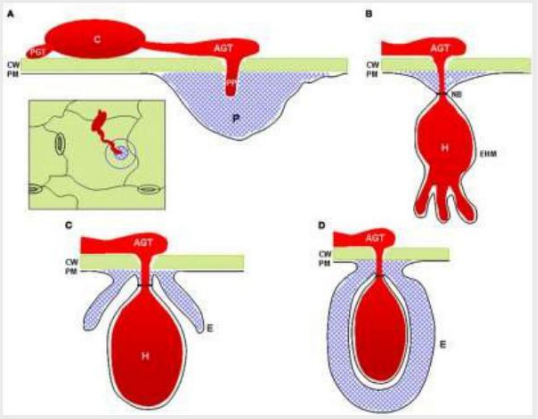
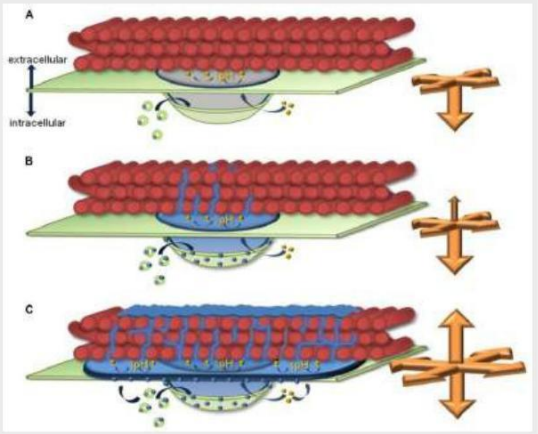


Figure 8.11. Model of callose papilla expansion at infection sites. The model shown highlights similarities and differences in callose papilla expansion and callose/cellulose polymer network formation in Arabidopsis leaf epidermal cells at sites of attempted powdery mildew infection in (A) the pmr4 disruption mutant without pathogen-induced callose deposition in the papilla, (B) wild-type and (C) the penetration-resistant PMR4 overexpression line. Green circles represent possible multivesicular bodies (MVBs) involved in the supply of callose to the papilla.



papilla matrix and/or papilla-forming enzymes (grey dots) and callose synthase PMR4 (blue dots) to the developing papilla. Yellow dots within the papilla matrix indicate a putative involvement of vesicles/vesicle-like bodies in regulating pH at the interphase of papilla matrix and cellulose cell wall to induce callose gel formation (↑pH). Orange arrows indicate the direction and strength of papilla expansion. Green: plasma membrane, red: cellulose fibrils of the cell wall, blue: callose papillae matrix and callose fibrils, grey: non-callose papillae matrix (Voigt, 2014*).

Figure 8.12. Papillae as effective or ineffective means of resistance to infection. The diagram shows a hypothetical model illustrating the deposition of polysaccharides and phenols in the case of effective and ineffective papillae during infection of barley by *Blumeria gramis* f. sp. *hordei* . An efficacious papilla associates large quantities of callose with arabinoxylan, as long as the polysaccharides are bound to ferulic acid, and the penetration point is surrounded by the papilla. In a second stage, the amount of callose deposits falls as arabinoglucan flows in and is surrounded by large amounts of cellulose. Legends: AX, arabinoxylan; FA, ferulic acid (Chowdhury et al., 2014*).

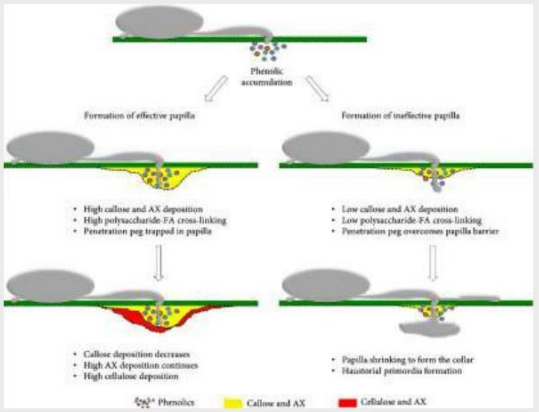
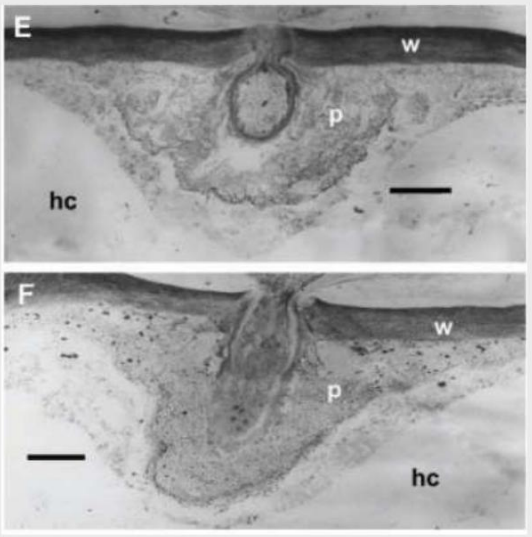


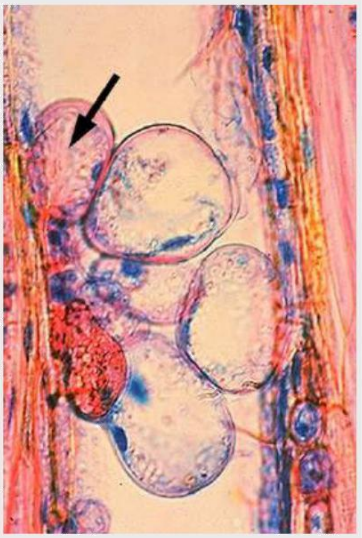
Figure 8.13. Transmission electron micrographs of papillae, 1 day after inoculation with Arabidposis Col-0 powdery mildew.
(E) and *pmr4* (F). Legends: w, host cell wall; p, papilla; hc, host cell.
Scale bar, 0.5 m.



2.3. Tylles

Thylls are excrescences that form in the xylem. This prevents sap circulation and with it the spread of pathogens. Thylls are common in grapevines.

Figure 8.14. Tylles are also defense structures induced after pathogen detection (Schumann & D'Arcy, 2013*).



IX Pathogenesis-Related Proteins

One of the most effective weapons used by plants to respond to infection by a pathogen is the production of pathogenesis-related proteins. These are known as *Pathogenesis-Related Proteins* (*PR proteins*). They are an important component of the plant immune system. PR proteins are mainly associated with the host plant's defensive responses, especially when the interaction is incompatible (resistance), thus hindering the pathogen's progress.

PR proteins can also be synthesized in response to abiotic stresses, pests, the effects of toxins, etc.

Figure 9.1. Effect of PR proteins on plant disease resistance. The wild tobacco genotype is heavily affected by the pathogen *Rhizoctonia solani*, whereas the genotype transformed with the PR proteins AP24 and β-1,3-glucanase is resistant to this pathogen. In addition, these transgenic plants are also protected against *Peronospora hyoscyami f* .sp. *tabacina* and *Phytophthora tabacinae* (Boccardo et al., 2019*).

1. PR Proteins

k *Definition*

PR proteins are a group of different molecules whose synthesis is induced by pathogens, as well as molecules involved in the signalling pathways of the plant immune system.

Caution: PR and R proteins

Not to be confused, R proteins are resistance proteins whose role is to detect and recognize pathogens, and PR proteins whose role is to eliminate pathogens through their antifungal, antibiotic and antiviral activities.

1.1. Characteristics of PR Proteins

PR proteins are a very diverse group of proteins. They are all induced by plant pathogens, as well as by signal molecules (salicylic acid and jasmonic acid). They have a relatively low molecular weight, between 6 and 43 kDa. They are thermostable and resistant to protease activity. They remain soluble even at low pH (<3).

2. Classification of PR Proteins

PR proteins are now classified into 19 biochemical families. This classification is based mainly on :

- Protein sequence similarity
- Enzyme activity,

There are also other traits on which the classification of PR proteins is based. PR

proteins are now classified into 19 families (see table below).

Table 9.1. Classification of PR proteins and their properties (Kaur et al., 2022*).

Family	Specimen	Original plant	Class/Location	Weight (kDa)	Property
PR1	Tobacco PR-1a	*Nicotiana tabacum*		15-17	Antifungal
PR2	Tobacco PR-2	*N. tabacum*	Class III		β-1,3-glucanase
			I, vacuole	33	
			II, III, apoplast	36	
PR3	Tobacco P, Q		Class V	25-30	Chitinase type I,II, IV, V,VI, VII
			I	≈32	
			II	27-28	
			III	28-30	
			IV	28-30	
			v	41-43	

PR4	Tobacco "R"		Class II	15-20	Chitinase type I, II
			I		
			II		
PR5	Tobacco S			22-25	Thaumatin, antifungal, osmotine, zeamatin
PR6	Tomato inhibitor I	*Solanum lycopersicum*		8	Protease inhibitor pr otease inhibitor
PR7	Tomato P69	*S. lycopersicum*		75	Endoprotease
PR8	Cucumber chitinase	*Cucumus sativus*		28	Chitinase type III
PR9	Tobacco lignin- f o r m i n g peroxidase	*S. tuberosum*		35	Peroxidase
PR10	Parsley "PR1	*Petroselinum crispum*	Class III	17	*Ribonuclease-like protein*
			I	11-30	
			II	≈60	
			III	≈60	
PR11	Tobacco "class V" chitinase	*N. tabacum*		40	Chitinase type I
PR12	Radish Rs-AFP3	*Raphanus raphanistrum*	Class IV	3-5	Defensin
PR13	Arabidopsis THI2.1	*Arabidopsis thaliana*		5	Thionin
PR14	Barley LTP4	*Hordeum vulgare*		8.7-9	Lipid transfer protein
PR15	Barley OxOa (germin)	*H. vulgare*		20	Oxalate oxidase
PR16	Barley OxOLP	*H. vulgare*		20	*Oxalate oxidase-like*
PR17	Tobacco PRp27	*N. tabacum*		27	

					Antifungal a nd antiviral
PR18	Carbohydrate oxidases	*Helianthus annuns*		60.9	Charbohydrate oxidases
PR19	antimicrobial protein	*Pinus sylvestris*			Antimicrobial pr o t e i n e

Let's talk about some examples of the PR protein families:

2.1. The PR1 Family

Members of this family are the most common PR proteins. The majority of proteins in this family are secreted into the apoplast (extracellular space).

2.1.1. PR1 protein activities

PR1 proteins have antimicrobial activity. Over-expression of PR1 in transgenic plants increased resistance against fungi, oomycetes and bacteria, but not viruses.

A *Example*

A concentration of 20-200 µg/ml of PR1 protein (depending on the protein (tomato, tobacco)) is sufficient to inhibit germination of 90% of *P. infestans* zoospores.

A *Example*

An exeogenic application of these proteins also inhibits colonization of tomato leaf discs by *P. infestans*.

A *Example*

Tomato P14c and tobacco PR-1a inhibited the growth of Phytophthora brassicae at concentrations of 20 µg/ml, but had no effect on the growth of *Aspergillus niger* or *Botrytis cinerea*.

2.2. The PR2 Family

PR2s show β-1,3-glucanase acitivity. They have hydrolytic activity of the 1,3 β-D-glucosidic bond in β-1,3 glucans. They are abundant proteins in plant tissues and are associated with the formation of calloses and trichomes in leaves and stems.

2.3. The PR3 Family

These are chitinases. These enzymes hydrolyze the β, 1-4 bond between chitin's N-acetylglucosamine residues. Chitinases are endo β, 1-4 glucosaminidases. Plant chitinases are classified into 4 classes based on sequence homology and the presence or absence of the chitin-binding domain. These features are common to PR3, PR4, PR8 and PR11.

PR3s are characterized by a common chitin-binding domain, usually an α-helix, and a catalytic domain containing 2 glutamates.

2.3.1. Class I

These are basic chitinases isolated from tobacco. They have a C-terminus that helps target vacuoles. The N-terminal chitin-binding domain is rich in proline and glycine. 32kDa class I chitinases have also been identified in capsicum.

2.3.2. Class II

They are acidic chitinases, lacking a chitin-binding domain. They also have internal deletions, eliminating one of the four loops required for N-glycosylation. They are closely related to class I.

2.3.3. Class IV

They were originally isolated from beans and are not serologically related to classes II and I. They also show very little sequence homology with classes II and I. They also show very little sequence homology with classes II and I. There are three

deletions in the chitin-binding domain, resulting in hydrolysis of the glycosidic bond closer to the pathogenic surface.
These are acidic chitinases and do not possess a glycosidic binding region.

2.3.4. Class V

This class has only one representative, identified from *Utrica doica*. It is a lectin with precursors having chitinase homology with the chitin-binding domain. The two catalytic residues in the chitin-binding domain are absent, and hence no catalytic activity.

2.3.5. Class VI

The only representative of this class has been identified in sugar beet. This chitinase has 4 deletions of the 8 cysteines in the chitin-binding domain. It has the longest *spacer* region with 135 amino acids, 90 of which are prolines.

2.3.6. Class VII

The only representative of this class is found in rice, and it bears a high resemblance to class IV chitinases. This group of chitinases lacks a chitin-binding domain, but bears a resemblance to the complementary DNA of class IV chitinases.

Note

There are 19 PR protein families. For more details, please read the specialty literature :

Sudisha, J., Sharathchandra, R.G., Amruthesh, K.N., Kumar, A., Shetty, H.S. (2012). Pathogenesis Related Proteins in Plant Defense Response. In: Mérillon, J., Ramawat, K. (eds) Plant Defence: Biological Control. Progress in Biological Control, vol 12. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-1933-0_17

3. Role of PR Proteins

PR proteins have different roles in plant physiology:

- Antifungal activity
- Antibacterial activity
- Antiviral activity
- Resistance to abiotic and other stresses

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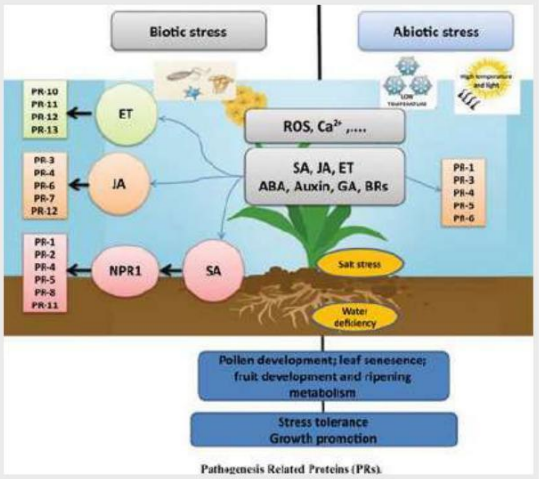
Figure 9.2. Roles of PR proteins. In fact, PR proteins are also involved in resistance to abiotic stress and a host of other physiological processes (Zribi et al., 2021*).



Attention

The synthesis of PR proteins is a late event, from the point of view of the temporal dynamics of infection, and their role in early resistance is limited. They provide long-term protection. They are mainly involved in acquired resistance (local or systemic).

PR proteins are particularly effective in subsequent infections, reducing the severity of symptoms and consequently of the disease.

Fundamental

Certain endophytes and saprophytes (fungi and bacteria) induce the activity of PR proteins, increasing plant resistance (biological control).

4. Mode of action of PR proteins

The different PR proteins have distinct modes of action against pathogens, depending on the type of pathogen and its infection strategy. PR1 proteins generally inhibit pathogen growth by sequestering sterols. This makes sterols unavailable to the pathogen. Other PR proteins have hydrolytic functions: PR-2 (endo-β-1,3-glucanases) and PR-3, -4, - 8 and -11 (endochitinases). They function as antifungal proteins, catalyzing the hydrolysis of the main wall components

of fungi and oomycetes, i.e. β-1,3-glucan (by breaking β-1,3-glucosidic bonds) or chitin (by breaking internal β-1,4-glycoside bonds) respectively, leading to degradation of the fungal cell wall.

Thaumatin-like proteins or osmotin-like proteins such as PR5 inhibit mycelial growth and spore germination by producing transmembrane pores causing high cell permeability in fungal cells and blocking the functions of plasma membrane receptor molecules involved in the cAMP/RAS2 signaling pathways.

Protease inhibitors (trypsin inhibitors and serine inhibitors) belonging to the PR6 family are involved in broad-spectrum (multi-pathogen) immune responses against nematodes, pests, fungi and bacteria. They act by reducing the lytic activity essential for fungal pathogenicity, inhibiting viral replication, and also reducing the activity of nematode and insect digestive enzymes.

Attention

PR proteins show a very high degree of specificity.

5. Types of PR proteins

There are two types of PR proteins:

- Acidic PR proteins
- Basic PR proteins

6. PR protein synthesis

PR proteins are synthesized by all plant organs. Leaves are the organ richest in PR proteins. 5-10% of total leaf proteins are PR proteins.

6.1. Genes encoding PR proteins

Several genes encoding PR proteins have been identified in different plants. Most of these genes belong to multi-gene families. Each family is regulated in a different way from the others.

A *Example: Tobacco*

Sixteen genes encoding the PR protein family, PR-1, are present in tobacco, as are 13-14 genes for the PR-2 family and 2-4 genes encoding acidic and basic chitinases. Five genes for PR-5, 15 for PR-10 and a small multigene family of three genes have been cloned from barley, oats, wheat, arabidopsis, brassica and tobacco.

6.2. Genetic expression

The genes encoding PR proteins are expressed in both constitutive and inductive modes. A small quantity of PR proteins is always synthesized by plants. This is referred to as a basic level of gene expression. These genes are almost silent in healthy plants. However, their expression increases significantly

following detection and recognition of a pathogen. It is also stimulated by signal molecules such as salicylic and jasmonic acid.

Attention

PR proteins show a high level of specificity. The different families can be grouped according to the type of target pathogen:

- Biotrophic: In contrast to biotrophs, plants synthesize PR proteins from the PR1, PR2 and PR5 families. In this case, PR protein synthesis is systemic.
- Necrotrophic: Against this type of pathogen, plants synthesize PR proteins from the PR3, PR4 and PR12 families. Here, the immune response by PR proteins is localized.

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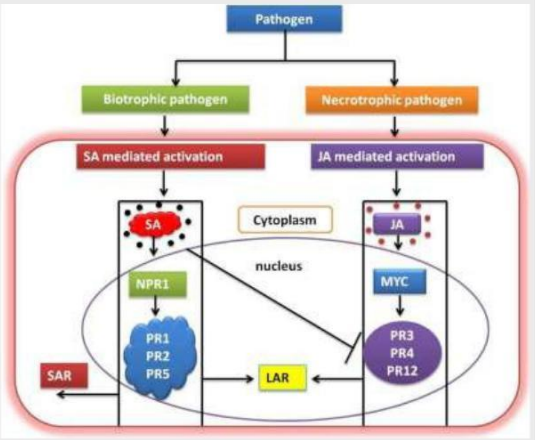
Figure 9.3: Local or systemic expression of PR proteins, depending on the type of pathogen. Against biotrophs, PR proteins are synthesized systemically throughout the plant. Against necrotrophs, on the other hand, they are synthesized locally (Ali et al., 2018). Also against biotrophs, the PR1, PR2, and PR5 families are expressed, while against necrotrophs, the expression o f other PR protein families is stimulated: PR3, PR4, and PR12.



6.3. Secretion

PR proteins have the role of eliminating pathogens regardless of their location. However, some major regions are known to accumulate PR proteins:

6.3.1. Extracellular space

Acidic PR proteins are secreted into the extracellular space.

6.3.2. The vacuole

Basic proteins are transported to the vacuole.

7. Major PR Proteins

There are a multitude of PR proteins. A plant can synthesize several enzymes active against pathogens. Here, we'll look at just a few of them:

7.1. Chitinases

Chitinases degrade chitin, a major component of the fungal wall. They can also degrade chitosans. They play an important role in plant defense and resistance to infection by various pathogens.

A *Example: Morus nobilis*

66 chitinase genes have been detected in this plant. Expression of one of these genes, MnChi18, increases the plant's resistance to *B. cinerea*.

A *Example: Capsicum annuum*

Chitinase-encoding genes positively regulate immune responses, including HR, in *C. annuum.*
against *Colletotrichum acutatum*.

Attention

In addition to their antifungal action, some chitinases have shown antiviral and antibacterial activity.

7.2. Glucanases

Glucanases (especially β-1,3-glucanases) play a major role in plant immune responses to fungi and oomycetes. Cell walls are degraded by β-1,3-glucanases. This degradation generates oligomers of β-1,3/1,6-D-glucans, which in turn act as elicitors (DAMPs), intensifying the plant's immune response.

Table 9.2. Example of some genes encoding PR proteins with glucanase activity that have been used to generate plants resistant to certain pathogens (Kaur et al., 2022*).

Enzyme	Genes	Original plant	Target pathogen
Glucanase	*β-1,3-glucanase*	*Linum usitatissimum*	*Fusarium culmorum*
	HbGLU	*Hevea brasiliensis*	*Rhizoctonia solani*
	β-1,3-glucanase II cDNA	*Hordeum vulgare*	*Fusarium graminearum*
	chi-2, ltp	*Hordeum vulgare, Triticum aestivum*	*Alternaria radicicola Botrytis cinerea*
	McCHIT1	*Momordica charantia*	*Magnaporthe grisea Rhizoctonia solani*
	OsPR4a-e	*Oryza sativa*	*Magnaporthe grisea*
	RC7	*Oryza sativa*	*Rhizoctonia solani*

	BjCHI1	*Brassica juncea*	*Rhizoctonia solani*
	chit cDNA	*Hordeum vulgare*	*Fusarium graminearum*
	Chitinase-I	*Oryza sativa*	*Verticillium dahliae* *Fusarium oxysporum*
	RC24	*Oryza sativa*	*Puccinia striiformis f* .sp. *tritici*
	rcc2 and *rcg3*	*Oryza sativa*	*Puccinia striiformis f* .sp. *tritici*
	LcCHI2	*Leymus chinensis*	*Pseudomonas tabaci,* *A. alternata,* *Exserohilum turcicum,* *Curvularia lunata*

Note

Plant chitinases and glucanases can act synergistically, stimulating strong degradation of pathogen cell walls and boosting plant immune responses.

Attention

Together, chitinases, glucanases and peroxidases act at the start of plant infection.

7.3. Thaumatin-Like Proteins

These proteins belong to the PR5 family. Isolated for the first time from the plant *Thaumatococcus danielli*. They are resistant to extreme pH, temperature and degradation by proteases. They play an important role in plant resistance to biotic and abiotic stresses. Stimulation of their synthesis increases plant resistance to various fungal pathogens. They are thought to act by permeabilizing pathogen cell membranes and degrading cell walls.

Table 9.3: Example of some genes encoding thaumatin-type PR proteins that have been used to generate plants resistant to certain pathogens (Kaur et al., 2022*).

Enzyme	Genes	Original plant	Target pathogen
Thaumatin	*ThaumatinlikeTaLr19TLP1*	*Triticum aestivum*	*Puccinia triticina*
	Tlp	*T. aestivum*	*Fusarium graminearum*
	Tlp	*Oryza sativa*	*A. solani*
	Tlp	*O. sativa*	*R. solani*

	tlp-1	*Hordeum vulgare*	*F. graminearum*
	CsTLP	*Camellia sinensis*	*P. infestans* *Macrophomina phaseolina*
	AdTLP	*Arachis diogoi*	*R. solani*

7.4. Defensins

defensins are peptides of 41-54 amino acids. They have antimicrobial activity. They are

constitutively present in leaves, tubers and flowers,

pods and seeds at very low concentrations... They are also found in the peripheral cell layers and xylem of over 20 different plant species.

These proteins are barely detected in healthy (uninfected) tissue, but accumulate systemically to high levels after localized fungal or bacterial infection.

Table 9.4. Example of some genes coding for defensin-like PR proteins that have been used to generate plants resistant to certain pathogens (Kaur et al., 2022*).

Enzyme	Genes	Original plant	Target pathogen
Defenssines	*Wasabi*	*Wasabia japonica L.*	*M. grisea*
	Wasabi	*W. japonica L.*	*B. cinerea*
	MsDef1	*Medicago sativa*	*F. oxysporum* f. sp. *lycopersici*
	MtDef4.2	*M. truncatula*	*Puccinia triticina*
	RsAFP2	*Raphanus sativus*	*R. solani* *M. grisea*
	RsAFP2	*R. sativus*	*Rhizoctonia cerealis,* *F. graminearum*
	Wasabi	*W. japonica L.*	*A. solani* *F. oxysporum*
	BoDFN	*Brassica oleracea*	*Downy Mildew*
	VrPDF1	*Vigna radiata*	*Weevils*
	TAD1	*Triticum aestivum*	*Typhula ishikariensis,* *F. graminearum*

7.5. Thionins

These are small proteins rich in cysteine. They are constitutively produced in small quantities. Infection stimulates their synthesis. They are found in walls, vacuoles and protein bodies.

Table 9.5. Examples of some of the genes encoding PR thionin proteins that have been used to generate plants resistant to certain pathogens (Kaur et al., 2022*).

Enzyme	Genes	Original plant	Target pathogen
Thionines	*AT1G12660* *AT1G12663*	*A. thaliana*	*R. solani* *F. oxysporum*
	Thionin	*Brassica oleracea* var. acephala, *Nasturtium officinale* *Barbarea vulgaris*	*B. cinerea*
	α-hordothionin (αHT)	*H. vulgare*	*Ceratocystis fimbriata*
	Thi2.1	*A. thaliana*	*F. oxysporum*

X Secondary Metabolites

In addition to wall reinforcement and PR proteins, plants use other strategies to defend themselves against various pathogens. Plants synthesize small molecules that are toxic against phytopathogens and pests too. These molecules are commonly referred to as secondary metabolites.

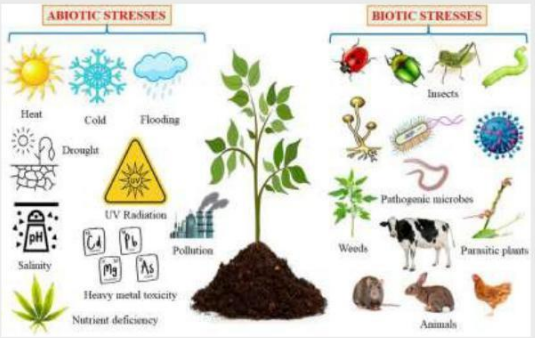


Figure 10.1. Various biotic and abiotic stress factors stimulate the production of secondary metabolites (Anjali et al., 2023*).

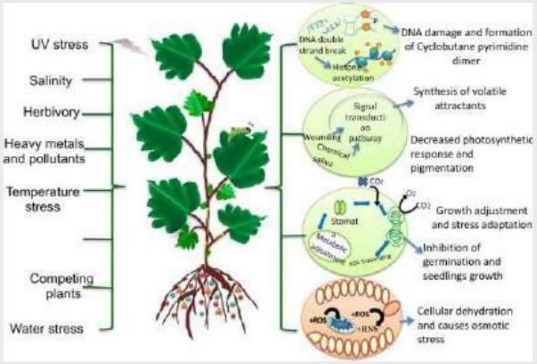


Figure 10.2. Physiological changes that plants undergo under t h e influence of different stresses (Khare et al., 2020*).

1. Secondary metabolites

k *Definition: Secondary metabolites*

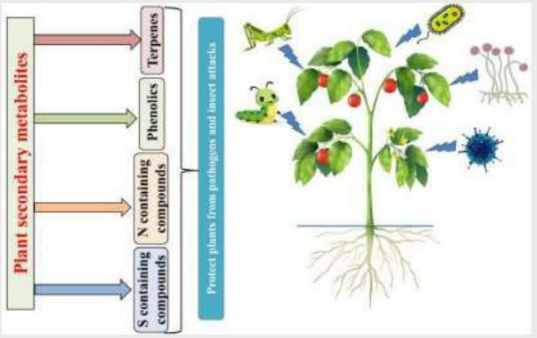
Plant secondary metabolites (SM) are natural by-products of primary metabolic processes. They are organic molecules with a low molecular weight. They r e p r e s e n t a very broad group of

compounds with a wide range of structures that are produced *in-planta* from various primary metabolites or from intermediate molecules of primary metabolites. They are synthesized either constitutively or in response to various environmental stimuli.

2. The role of secondary metabolites

Plants synthesize a multitude of molecules considered secondary metabolites.

Figure 10.3: Roles of secondary metabolites(Anjali et al., 2023*).



Fundamental

The main function of secondary metabolites is to improve plant growth and survival under unfavorable conditions.

They have no direct role in plant growth, metabolism or development, but play an important role in plant defense mechanisms, and are therefore labelled as "secondary compounds".

However, their effects on the growth of either the pathogen or the resistance of the plant that synthesizes them have been proven (see table below).

Table 10.1. Roles of certain secondary plant metabolites in the control of certain pathogens (Anjali et al., 2023*).

Plant Source	Secondary metabolite	Pathogen Target
Citrus reticulate	Reticin A	*Tobacco mosaic virus*
Ageratum conyzoides	Chromenes, terpenoids, flavonoids, coumarins	*Alternaria, Candida, Fusarium, Pythium, Phytophthora*
Solanum nigrum	Glycoalkalids, solamargine, solasonine	*B. cereus, B. thuringiensis, Pseudomonas orientalis, Strenotrophomonas maltophilia*
Pistacia atlantica	Alkaloids	*F. oxysporum* f.sp. *lycopersici*

Allium sativum	Terpenoids, flavonoids, alkaloids, saponins, tannins, glycosides	*Alternaria solani*

Lawsonia inermisAlkaloids	, flavonoids, saponins, steroids, tannins	*F. oxysporum* *Bipolaris oryzae*
Teucrium spp.	Monoterpenes, sesquiterpenes	*Bacillus sp., Candida sp.*
Carica papaya	Alkaloids, flavonoids, terpenes	*Rhizopus stolonifer*, *Fusarium* spp.., *Colletotrichum gloeosporioides*
Ziziphus jujuba	Protocatechuic, catechin, p-coumaric acid, coumarin	*Botrytis fabae*
Pachyrhizus erosus	Rotenone, erosone, paquirrizine, dalinone, dehydroneotenone	*R. stolonifer,* *F.oxysporum,* *C. gloeosporioides*
Cassia alata	Methyl 2 ,4,6-trihydroxybenzoate, aloe-emodin, kaempferol, kaempferol-3-O-glycoside	*Magnaporthe oryzae, Phytopthora infestans,* *Puccinia recondita*
Allium	nigrumCysteine sulfoxides, total polyphenols, saponinsF	*F. oxysporum* f. sp. *cepae*, *. oxysporum* f. sp. *fragariae*, *C. gloeosporioides*
Carthamus tinctoriusTerpenoids	, flavonoids, *alkaloidsAspergillus spp. Myristica fragransErythro-*	
austrobailignan-6	, meso-dihydroguaiaretic acid, nectandrin-B	*A. alternata,* *M. grisea,* *C. gloeosporioides*
Azadirachta indica	Flavonoids, terpenoids, saponins, steroids, coumarins, cardiac glycosides	*A. solani*
Aloe succotrina	Flavonoids, saponins, and tannins	*A. alternata,* *Cladosporium cladosporioides,* *Cochliobolus specife*
Camellia sinensis	α-phenylcinnamic acid	*Colletotrichum gloeosporioides*
Zingiber officinale	Geranial, 1,8-cineole, neral, camphene, α-zingiberene, α-farnesene	*Burkholderia glumae*

Datura metel	Eugenol, pentadecanoic acid, heptacosane, dodecanoic acid, tetradecanoic acid	*Rhizoctonia solani*

3. Types of Secondary Metabolites

In general, there are two types of secondary metabolites, depending on when they are synthesized:

- Anticipins
- Phytoalexins

3.1. Anticipins

k *Definition*

These are molecules with antimicrobial activity, present at the moment of infection (preformed) or released from their storage organs following attempted infection by a pathogen. They are constitutive defense molecules.

Generally speaking, all plant species can constitutively synthesize phytochemical molecules with a potential defensive function.

3.1.1. Saponins

The glycoside units of isoprenoid aglycones are commonly known as saponins, which belong to the steroids or triterpenoids and are found in abundance in flowering crops.

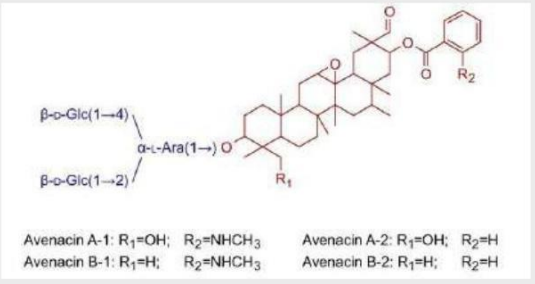
A *Example: Avenacin*

One of the most extensively studied molecules of this type. It is produced b y oats (*Avena* spp.). There are 4 types of avenacin:

- Avenacin A1,
- Avenacin A2,
- Avenacin B1,
- Avenacin B2.

These molecules accumulate in oat roots.

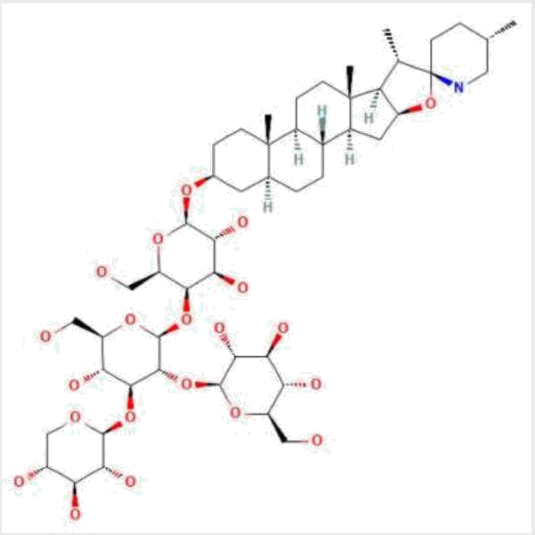
Figure 10.4. Avenacin (Piasecka et al., 2015*).



A *Example: Tomatine*

Tomatoes produce α-tomatine.

Figure 10.5. α-tomatine



Saponins play an important role in the plant immune system. Both α-tomatine and avenacins have been shown to inhibit the development of a variety of pathogenic and non-pathogenic fungi and oomycetes.

Saponin-deficient *A. strigosa* plants are highly susceptible to infection by *Gaeumannomyces graminis* var. *avenae*, *Fusarium culmorum*, and also t o infection by *F. avenaceum*. These plants are also sensitive to non-adapted pathogens: *G. graminis* var. *tritici*, which in normal mode does not infect them.

3.1.2. Glucosinolates

These are β-d-thioglucoside-N-hydroxysulfates derived from amino acids and known as glucosinolates. They are produced mainly by plants belonging to the Brassicales order. They can be derivatives of several amino acids, including alanine, valine/leucine, isoleucine, methionine, phenylalanine/tyrosine, tryptophan and glutamic acid. The result is a highly diverse group (structurally speaking).

However, only a subset of the possible structures is present in particular plant species. For example, the model plant Arabidopsis thaliana (Brassicaceae) accumulates two major groups of these compounds - aliphatic glucosinolates derived from methionine (AG) and indolic glucosinolates derived from tryptophan (IG) - as well as small amounts of benzyl glucosinolates derived from phenylalanine.

Fundamental

Similar to other glycosylated secondary metabolites, glucosinolates are chemically stable and biologically inactive. However, loss of cellular integrity or other environmental stimuli can trigger rapid hydrolysis of glucosinolates by β-thioglucoside glucohydrolases (TGGs), also known as myrosinases. This process leads to the release of aglycones, which are chemically unstable and can break down into various types of molecules, including isothiocyanates (ITCs). The latter are characterized by their high chemical reactivity and biological activity. They are toxic metabolites, especially against pests.

ITCs play an important role in resistance. *Pseudomonas syringae* strains pathogenic to *A. thaliana* have an operon (*Survival in Arabidopsis extracts* (*Sax*)) which codes for proteins involved in the detoxification of these molecules. Strains lacking *Sax* are not pathogenic.

In addition, these *P. syringae* strains lacking *Sax genes* were less virulent on young wild-type Arabidopsis leaves than the GA-deficient *myb28 myb29 double knockout* line. This line is depleted in two myeloblastosis (MYB) transcription factors controlling GA biosynthesis, and consequently does not accumulate any representatives of this class of glucosinolates.

The *myb28 myb29* plants were also found to be more susceptible to the pathogen *Sclerotonia sclerotiorum*, suggesting that the AG function in plant immunity is not limited to bacteria.

3.1.3. Cyanogenic glycosides

They are β-d-glucosides of α-hydroxynitriles that can be derived from tyrosine, phenylalanine, valine, isoleucine and leucine. Similar to glucosinolates, cyanogenic glycosides are constitutively stored and are not intrinsically biologically active. For them to be biologically active, they must be hydrolyzed by their respective cyanogenic β-glucosidases.

These molecules are best known for their pest control properties.

3.1.4. Benzoxazinone glycosides

This group of anticipins is mainly found in poaceae: wheat, barley, corn and others. In wheat and maize, the main compound in this group is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside (DIMBOA-Glc). Wild barley and rye, on the other hand, mainly synthesize 2,4-dihydroxy-1,4-benzoxazin-3-one glucoside (DIBOA- Glc).

In addition to members of the Poaceae, benzoxazinone glucosides are present in Ranunulales and Lamiales.

Glucoside benzoxazinone aglycones have antifungal activity, at least in-vitro, against a number of pathogens: *G. graminis*, *Fusarium moniliforme*, *F. culmorum* and *Setosphaeria turcica*. DIMBOA accumulates in the apoplast of maize leaves following inoculation with *S. turcica*. Maize plants unable to synthesize DIMBOA (*bx1* mutants) are extremely sensitive to this pathogen.

It should be emphasized that infection is not intended solely to trigger hydrolysis of the benzoxazinone glucosides into their respective aglycones. In maize, challenge with several adapted and non-adapted parasitic fungi, including *S. turcica*, *Fusarium graminearum*, *Bipolaris maydis*, *Curvularia lunata* and *Alternaria alterna*, induces the accumulation of a 2-hydroxy-4,7-dimethoxy-1,4-benzoxazine -3-one glucoside (HDMBOA-Glc).

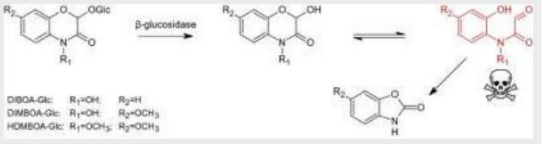


Figure 10.6. benzoxazinone structures and their metabolism by β-glucosidase. The ring-opened tautomeric form of benzoxazinone aglycone, highly reactive and potentially toxic to pathogens, is highlighted in red. DIBOA, 2,4-dihydroxy-1,4-benzoxazine-3-one; DIMBOA, 2,4- dihydroxy-7-methoxy-1,4-benzoxazine-3-one; HDMBOA, 2-hydroxy- 4,7-dimethoxy-1,4-benzoxazine-3-one (Piasecka et al., 2015*).

3.2. Phytoalexins

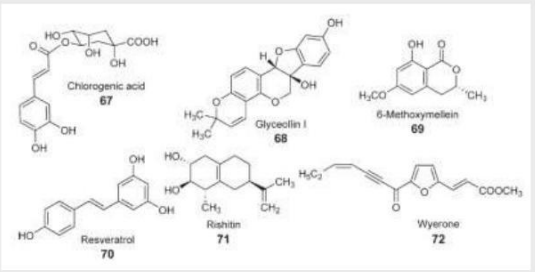
The term "phytoalexin" was originally coined to describe a hypothetical defensive substance that accumulated in the tissues of potato tubers when inoculated with an incompatible strain of the oomycete pathogen responsible for *Phytophthora infestans* late blight.

k *Definition*

phytoalexins are defined as low-weight antimicrobial metabolites that are synthesized and accumulate in plants after infection by a pathogen.

Hundreds of phytoalexins have been isolated and characterized from different plant species.

Figure. 10. 7 Examples of phytoalexins (Spiteller. 2008*).

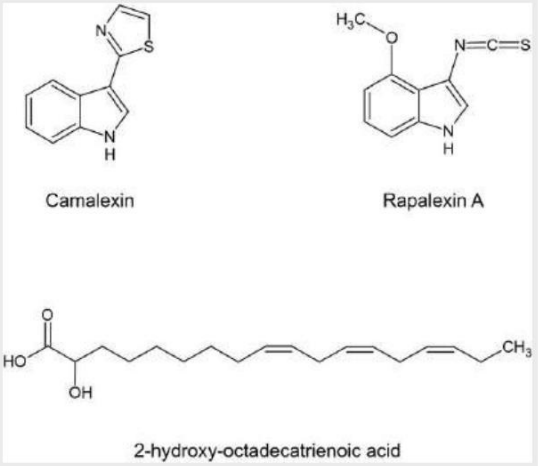


Phytoalexins

3.2.1. The Camalexines

Camalexins are the model phytoalexins of the *Brassicaceae*. They are sulfide-containing alkaloid derivatives of tryptophan that accumulate in response to pathogen infection.

Figure 10.8. Chemical structures of some camalexins identified in Arabidopsis: camalexin, rapalexin and 2- hydroxy-octadecatrienoic acid (Piasecka et al., 2015*).



camalexin contributes to post-invasive resistance by limiting the further development of pathogens and their spread to neighboring cells.

Mutant Arabidopsis plants that do not accumulate camalexin are susceptible to necrotrophic pathogens, including *Alternaria brassicola* and certain isolates of *B. cinerea*.

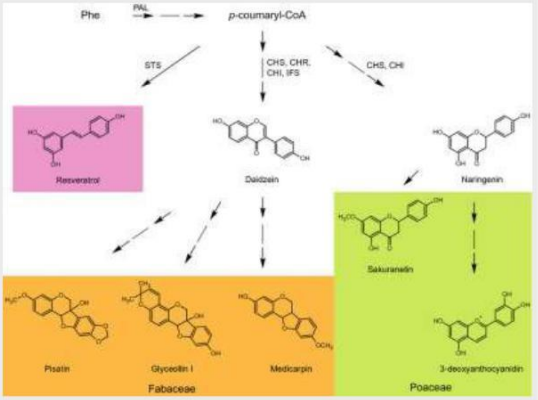
3.2.2. Phenylaalanine Derivatives

These are phenylpropanoids derived from phenylalanine. A representative compound in this phytoalexin category is resveratrol. Resveratrol is synthesized following infection in several plant species, including grapevines and peanuts.

Figure 10.9. Biosynthesis of phenylalanine-derived phytoalexins in different plant species. The presence of resveratrol a

been reported at several phylogenetically unrelated species.

Legend: PAL, phenylalanine ammonia-lyase; STS, stilbene synthase; CHS, chalcone synthase; CHR, chalcone reductase; CHI, chalcone isomerase; IFS, isoflavone synthetase (Piasecka et al., 2015*).



Other molecules belonging to a different group of phenylalanine derivatives are found in the fabaceae, including :

- Glycerolins: soy
- Pisatin: chickpea
- Medicarpine: alfalfa

These molecules have antimicrobial effects that have been tested *in-vitro* and also proven *in-vivo*.

Poaceae also synthesize phytoalexins of this type:

- Sakuranetine: rice
- Apigeninidin: sorghum
- Luteolinidin: sorghum

....

3.2.3. Terpenoids

Rice also synthesizes momilactone A and momilactone B following infection, which are diterpenoids. In fact, rice synthesizes 2 types of diterpenoid phytoalexins:

- Oryzalexins
- Phytocassanes

Maize also produces a large number of phytolexins in this group, including: Kauralexins: which are

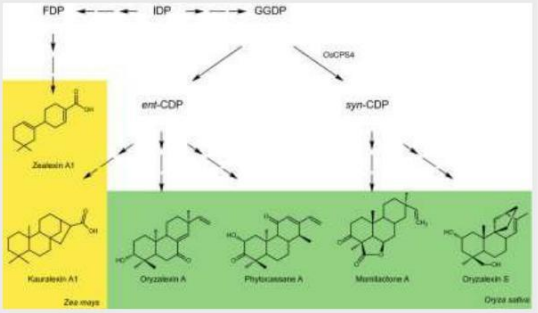
diterpenoids

Zealexins: are sesquitermpenoids

Figure 10.10. Simplified diagram of biosynthesis and representative structures of isoprenoid phytoalexins in rice and maize. Captions:

IDP, isopentenyl d i p h o s p h a t e ; FDP, farnesyl diphosphate GGDP, geranylgeranyl diphosphate; CDP, copalyl diphosphate;

CPS4, copalyl diphosphate synthetase 4 (Piasecka et al., 2015*).



4. Modes of action of secondary metabolites

Although they share the common goal of limiting disease development, the molecules known as secondary metabolites form a highly heterogeneous group. As a result, their modes of action differ.

Figure 10.11. The different hypothetical functions of isothiocyanates (ITCs) and indole glucosinolates (IGs) in Arabidopsis immunity. (a) ITCs act directly as antibiotics and reduce reduced glutathione (GSH) levels. GSH depletion affects the generation of reactive oxygen species (ROS) which, in turn, can have an impact on programmed cell death and stomatal closure. GSSG, oxidized glutathione; GS-ITC, glutathione-ITC conjugate. (b) The products of GI metabolism control the entry of fungal and oomycete pathogens into epidermal cells. In addition, they can affect callose deposition and programmed cell death. (Piasecka et al., 2015*).

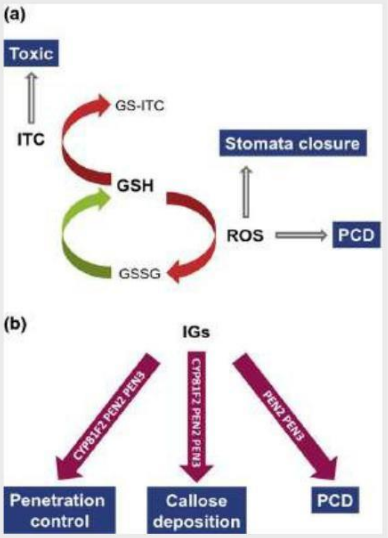
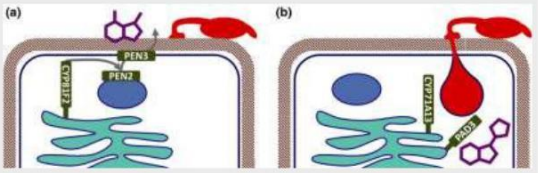


Figure 10.12. Mode of action of secondary metabolites. Sequential function of phytoantycipins and phytoalexins in pre- and post-invasive resistance illustrated by indole glucosinolates (IG) and camalexin. (a) CYP81F2, Penetration2 (PEN2) and Penetration3 (PEN3) function in a coordinated manner in pre-invasive resistance to generate and deliver to host-pathogen interface products of IG metabolism. (b) During successful pathogen invasion, CYP71A13 and Phytoalexin-deficient3 (PAD3) P450 monooxygenases produce camalexin, which can limit the growth of post-invasive pathogens. P450 monooxygenases are anchored in the ER membrane, PEN2 is associated with peroxisomes, PEN3 localizes to the plasma membrane (Piasecka et al., 2015*).



A *Example: Le Zest des Citrus*

Reticin A isolated from *Citrus reticulata* fruit peel extract (zest) induced a local hypersensitivity reaction (HR), systemic accumulation of H_2O_2 and systemic induction of salicylic acid (SA) and PR protein synthesis, culminating in induction of SAR, in tobacco against tobacco mosaic virus (TMV).

A *Example*

Several phenylpropanoid phytoalexins, such as phaseolin, glycinol and 3-deoxyanthocyanidins, have been shown to be involved in disrupting (pathogen) membrane function either directly or indirectly, by disrupting processes crucial to membrane function.

5. Secondary Metabolite Synthesis

Plants synthesize a very large number of molecules known as secondary metabolites (which are not primary metabolism molecules). A certain category of plants is widely recognized for this quality, and is commonly referred to as aromatic plants.

As these molecules are not peptides or proteins, the genes involved in their synthesis code for enzymes involved in their synthesis. This would imply that a very large number of genes are involved in this process.

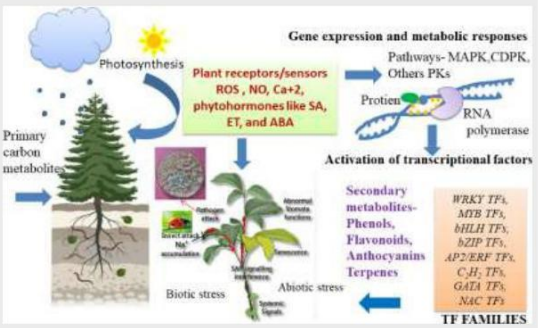


Figure 10.13. Inducers of secondary metabolite synthesis in plants

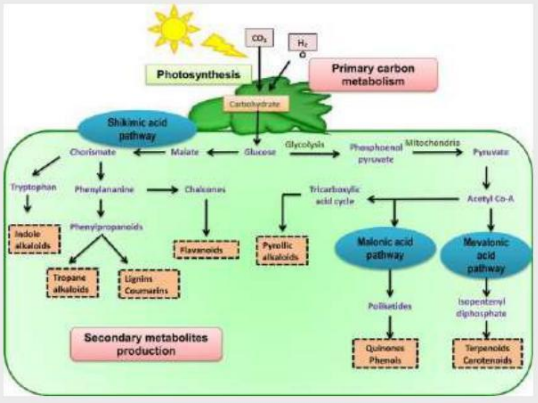


Figure 10.14. Secondary metabolite synthesis pathways

5.1. Genetic Control and Regulation of Secondary Metabolite Synthesis

The synthesis of secondary metabolites involves a multitude of genes. These are the genes coding for the enzymes involved in this process. In reality, several genes are involved in the synthesis of a single molecule.

In addition to these genes, there are also transcription factors, which control the rate of synthesis of a molecule either positively or negatively, by stimulating/inhibiting the expression of the genes involved (Table 10.2).

Table 10. 2. Transcription factors involved in immune responses through regulation of secondary metabolite synthesis

Family of F. transcription	F. Transcription	Secondary metabolite	Culture	Disease	Pathogen
WRKY	StWRKY1	Hydroxycinnamic acid amide	Potatoes	Downy mildew	*P. infestans*
	StWRKY8	Benzylisoquinoline alkaloids	Potato	Downy mildew	
	ZmWRKY79	Terpenoid phytoalexins	Corn		*Rhizoctonia solani*
	VviWRKY24/03 VvWRKY8	Resveratrol	Vine		*Botrytis cinerea*
	HvWRKY23	Hydroxycinnamic acid amide	Barley	Fusarium head blight	*Fusarium graminearum*
	G h M K K 2 GhNTF6	Flavonoid Terpenoid	Corn		*F. graminearum*
	TaWRKY70	Hydroxycinnamic acid amide	Wheat	Fusarium head blight	*F. graminearum*
MYB	AtMYB34/51 /112	I n d o l i c glucosinolate	Arabidopsis		*Plectospharella cucumerina*
	Vv MYB 14 VviMYB14	Resveratrol	Vine		*B. cinerea*
	CsMYB1	Flavonoids Hydroxycinnamic acid amide	Citrus		*Elsinoe fawcettii*
	CsMYB2/26	Flavonoids	Tea		*Exobasidium vexans*
	CsMYB96	Lignin, Coumarins, caffeic acid, salicin	Citrus	Blue rot	*Penicillium italicum*

	GhODO1	Lignin	Cotton	Verticillium wilt	*Verticillium dahliae*
bZIP	MdHY5	Anthocyanin	Apple		*Venturia inaequalis*
	CAbZIP1	Flavonoids	Peppers		*X. campestris pv. vesicatoria*
AP2/ERF	GbERF1	Lignin	Cotton	Verticillium wilt	*V. dahliae*
	VqERF114	Resveratrol	Vine		*B. cinerea*
	PnERF1	Saponins	Chinese ginseng	Root rot	*F. monilliforme var. intermedium*
	TaAP2-15	Terpenoids	Wheat	Yellow Rust	*Puccinia striiformis f. sp. tritici*
	ORA59	Hydroxycinnamic acid amide	Arabidopsis	Rot	*B. cinerea*
NAC	ANAC042	Camalexin	Arabidopsis	Black spots	*Alternaria brassicicola*
	TaNAC032	Lignin	Wheat	Fusarium head blight	*F. graminearum*
	MdNAC52	Anthocyanin proanthocyanidin	Apple	Leaf spots	*X. campestris pv. vesicatoria*
	SlNAP1	Terpenoids	Tomato	Wilt	*Ralstonia solanacearum*

Transcription factors are proteins that bind to DNA in the region of the target gene promoter, modulating the probability of transcription. To regulate the expression of genes involved in defense, transcription factors can be internally or externally signaled, thus regulating the rate of synthesis of secondary metabolites.

5.2. Secondary metabolite biosynthesis

Secondary metabolites are synthesized according to the type of molecule:

5.2.1. Anticipin biosynthesis

Anticipins are synthesized before the arrival of the pathogen. The genes controlling their synthesis are constitutively expressed. They are released when the pathogen is detected.

5.2.2. Phytoalexin biosynthesis

Phytoalexin synthesis begins at the moment of pathogen detection and recognition (PAMPs, DAMPS, or effectors). Their synthesis can also be induced by signalling molecules.

Table 10.3: Example of signal molecules affecting the synthesis of some of the secondary metabolites in cereals (Meyer et al., 2015*).

Plant	Secondary metabolite	Molecule Signal
Avena sativa	Avenanthramides Avenacines	SA+ BTH+
Zea mays	Kauralexin Zealexin DIMBOA	(JA+Et)+ (JA+Et)+ (JA+Et) ABA+ ; (JA+Et)+
Triticum aestivum	DIMBOA	JA+
Oryza sativa	Sakuranetine Phytocassanes Oryzalexins Momilactones	JA+
Sorghum bicolor	3-deoxyanthocyanidins	SA+ ; (SA+JA)+-

Legend: JA: jasmonic acid, SA: salicylic acid, ET: etylene, BTH: benzothiadiazole derivatives, +: positively regulated, -: negatively regulated.

5.3. Secondary metabolite storage

Secondary metabolites can also be toxic to the plant. These molecules are isolated and stored separately to avoid autotoxicity. The plant must isolate them from compartments and organelles sensitive to their actions (membranes, proteins, enzymes, etc.).

For anticipins, the plant stores these molecules as precursors, separately from the enzyme that activates them. Benzoxazinone glucosides are stored in the vacuole and β-glucosidases in the plastids. In Brassicaceae, glucosinolates and myrosinases are stored in different specialized cells.

Table 10.4: Examples of phytoanticipin locations and their activating enzymes (Chappell, 2023*).

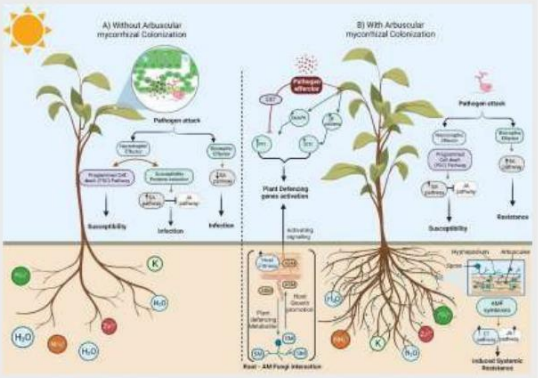
Phytoanticipin class	Phytoanticipin localization	Localization of β-Glucosidase

Cyanogenic glucosides (linamarin)	Vacuole	apoplast
Cyanogenic glucoside (dhurrin)	Cytoplasm, epidermal cells	Plastids, mesophyll cells
Glucosinolates (glucoraphanin)	Vacuole, S Cells	Vacuole, Myrosin cells
Benzoxazinoids (DIMBOA)	Vacuole	Plastides
Saponin glycosides (avenacosides)	Cytoplasm, Root tip cells	Unknown

6. Secondary Metabolites of Symbiotic Fungi

In addition to stimulating the plant immune system by detecting symbiotic fungi, these organisms synthesize other molecules that stimulate plant defense and/or are toxic to pathogens.

Figure 10.15. Diagram showing the different responses of plants to pathogenic fungi in the absence and with colonization by arbuscular mycorrhizal fungi (AMF). (A) The absence of AMF root colonization causes more damage than mycorrhized plants due to the development of symptoms following infection by necrotrophic and biotrophic pathogens. In addition, host plants with undeveloped root systems have a low capacity t o absorb nutrients from the soil, leading to plant death in the end. (B) A symbiotic relationship between plant roots and arbuscular mycorrhizal fungi (AMF) significantly alters ecosystems and impacts plant production via the promotion of plant growth due to enhanced mineral nutrient acquisition through the extensive AM fungal hyphal network (mycorrhizosphere) with a massive mycorrhizal network around the root system. In addition, host plants can thrive under a variety of abiotic / biotic stresses (including drought, salt, herbivory, temperature, metals and pathogens) due to the symbiotic localization of arbuscular mycorrhizal fungi (AMF) via complex signaling communications that increase the photosynthetic rate of plants. Consequently, the release of strigolactones (SL) as part of root exudates induces branching of AMF hyphae to promote mycorrhization. Changes in root exudate patterns induce changes in the soil microbial community, possibly by attracting



pathogen antagonists. In addition, there are different ways in which AMF-induced biotic stress tolerance in plants via competition with soil pathogens and nutrient uptake, modified root exudates that support beneficial microbes and suppress phytopathogens in the rhizosphere, AMF-colonized roots have little or no space f o r pathogen entry... . Interestingly, an overall reduction in damage and disease incidence caused by soil-borne pathogens has been noted as a result of the plant's priming defense power. The role of phytohormones (e.g. JA and ET) in the relationship between the host plant and its symbiotic fungi is well known. Phytohormones participate as signaling molecules and enhance the ISR (induced systemic resistance) of the host plant. In contrast, the development of necrotrophic pathogens in plant-fungal pathogen interaction signals is limited by jasmonate-regulated plant defense mechanisms.

XILThe Reaction Hypersensitivity

1. Introduction

The *Hypersensitive* Reaction (HR) is found in all higher plant species. It is an extreme plant defense mechanism against pathogenic aggressors.

k *Definition: Hypersensitivity reaction*

The hypersensitivity reaction is the rapid death of plant cells at the point of penetration of the pathogen, in order to prevent its spread. It is associated with resistance.

Figure 11.1. Hypersensitivity reaction of a leaf after inoculation with a pathogen (Schumann & D'Arcy, 2013*).

Fundamental

HR is the hallmark of ETI.

HR is suicide cell death to limit the spread of the disease. The pathogen is killed along with the dead cells involved in HR.

HR can be considered a form of programmed cell death. Programmed cell death is the death of a cell in any manner, controlled by an internal program within that cell.

2. HR levels

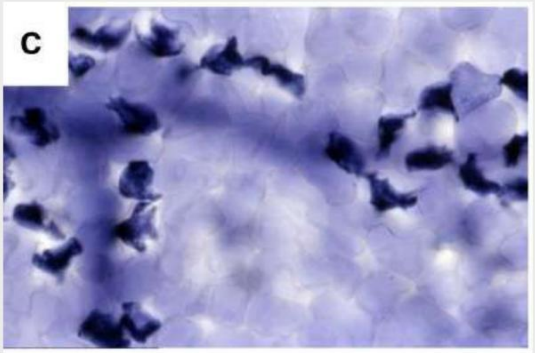
HR can be observed on several levels:

- HR can involve a single cell: only the attacked cell dies. In this situation, HR is no longer visible to the naked eye (figure 11.2).
- HR can be oberserved in a group of infected cells (figure 11.3).
- RH can involve part of a plant tissue: RH can be easily observed on leaves (figure 10.1, and 10.4).

Figure 11. 2. The HR reaction at cellular level. A bean cell has committed suicide following infection by Colletotrichum limdemuthianum, agent of bean anthracnose ((Picture source: G. Johal (Purdue University) *in* Balint-Kurti, 2019*).

HR can be oberserved in a group of infected cells (figure 11.3).

Figure 11.3. The microscopic HR Reaction *in* Balint-Kurti, 2019*).



RH can involve part of a plant tissue: RH can be easily observed on leaves (figure 10.1, and 10.4).

Figure 11. 4. HR observed with the naked eye. *in* Balint-Kurti, 2019*).

3. HR control

Fundamental

Because of the potentially high costs of inappropriate activation, plants use several mechanisms to suppress inappropriate HR activation and constrain it after activation. It must be completely suppressed under non-pathological conditions, as inappropriate activation will lead to a spontaneous cell death phenotype that can be highly detrimental to plant growth. Conversely, it must be activated rapidly when needed. These constraints have led to the evolution of several levels of control.

3.1. R gene expression

HR is induced by TNL-type R proteins (TIR-NBS-LRR) and seems to be particularly based on PTI signal transmission.

Note

HR is not induced by PTI. It is part of ETI.

HR is controlled at the level of protein accumulation and stability. In particular, the molecular chaperone HSP90 and two interacting co-chaperones RAR1 and SGT1 form a complex that interacts with numerous NLRs, stabilizing them and enabling their maturation and proper function. Silencing or mutation of any of RAR1, SGT1 or HSP90 in many interactions is sufficient to abolish HR and cause a reduction in NLR protein levels.

In reality, the R proteins associate (an oligomer of R proteins (homo or heteromeric) with each other to form a so-called Resistosome. It is this resistosome that is responsible for HR induction.

In addition to the formation of resistosomes by R sensor proteins, R helper proteins are responsible for downstream signal transduction.

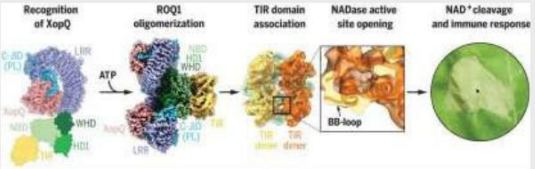


Figure 11.5: Proposed mechanism o f ROQ1 activation. The LRR and C-JID domains of the ROQ1 protein recognize the pathogen's XopQ effector. ROQ1 becomes an oligomer (several ROQ1 molecules associate together) via the NB-ARC domain (NBD, HD1, WHD) in an ATP-bound state. The association of the TIR domain induces a conformational rearrangement of the BB-loop domain, opening up the NADase active site. Catalytic activity of the TIR domain The catalytic activity of TIR domains also signals the immune response, leading to cell death (Martin et al., 2020*).

3.2. Temperature

In most cases, resistance is not expressed at high temperatures, including RH.

In Arabidopsis, HR is slow, and immune responses (controlled by the *RPS2*, *RPM1* and *RPS4* genes) are compromised for plants raised at 28°C, compared with those raised at 22°C, when infected by certain races of *P. syringae* pv *tomato*.

3.3. Light

Light dependence of HR is generally observed. This dependence is thought to be due to the production of ROS by chloroplases. The other major source of ROS is the mitochondria.

3.4. Relative Humidity

High relative humidity cancels or delays RH in certain situations.

4. Consequences of HR

4.1. The resistance

HR has always been considered a resistance phenomenon. HR is a response of resistant plants to infection by a pathogen. Initially, cell death during HR was considered to be due to the pathogen's arrest of growth. Now, with the characterization of several resistance proteins, we know that cell death can occur during HR independently of the presence of the pathogen.

Note

There's always debate about the role of HR in resistance. Is HR a consequence or a by-product of resistance, rather than the cause of resistance?

Attention

In some situations there is no relationship between resistance and HR:

- RPS6 resistance protein (TIR-NBS-LRR type) confers resistance without any visible cell death
- RIN13 gene improves RPM1-induced resistance but eliminates visible HR
- In Arabidopsis, the RPS4 (NLR) protein confers resistance to P. syringae in Col-0 and Ler accessions. HR is observed in Ler but not in Col-0.

Note

In situations where R proteins confer resistance very quickly and at very high levels, HR may not take place.

4.2. Sensitivity

Some necrotrophic pathogens can induce HR. In some cases, such pasthogens induce the expression of certain susceptibility genes encoding resistance proteins or PRR-like proteins. These proteins induce HR, facilitating necrotroph penetration.

Attention

For hemibiotrophs, HR is an effective means of resistance only during the biotrophic phase of the pathogen's life. If HR is delayed and occurs during the necrotrophic phase, it becomes a susceptibility factor.

Note

For hemibiotrophs, the biotrophic phase differs from one pathogen to another:

- *P. infestans*: 3-4 days
- *Septoria tritici*: 7 days,....

4.3. Systemic Resistance

HR generally induces systemic plant resistance. Broad-spectrum resistance is activated following localized infection by a pathogen generating HR. It is salicylic acid-dependent, and associated with the accumulation of PR proteins (PR1, PR2 and PR5).

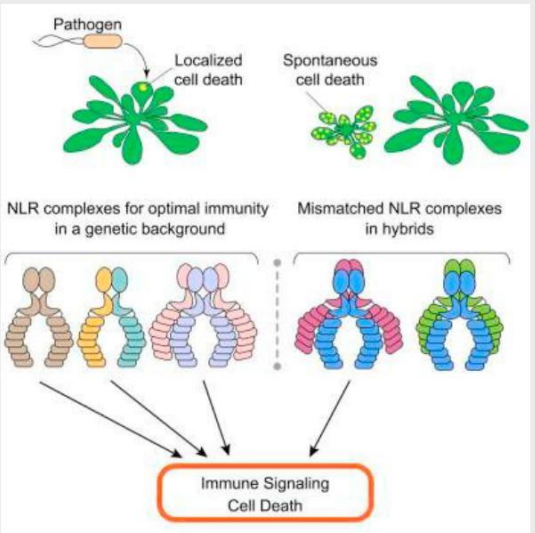
4.4. Autoimmunity phenomena

In certain situations, cell death is observed, which is associated with autoimmunity. This phenomenon may be associated with the formation of the resistosome. This phenomenon is mainly observed in hybrids. These hybrids have limited growth and show necrotic spots on the leaves. This is known as hybrid necrosis.

4.4.1. Control

HR is controlled by the interaction of R proteins (resistosome) either for activation or inhibition. It is thought that when two or more R-proteins interact, with genes that have not evolved together and are in the same gene pool, this phenomenon is activated, giving rise to autoimmunity.

Figure 11.6: Autoimmunity in plants (Trans et al., 2016*).



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Webography