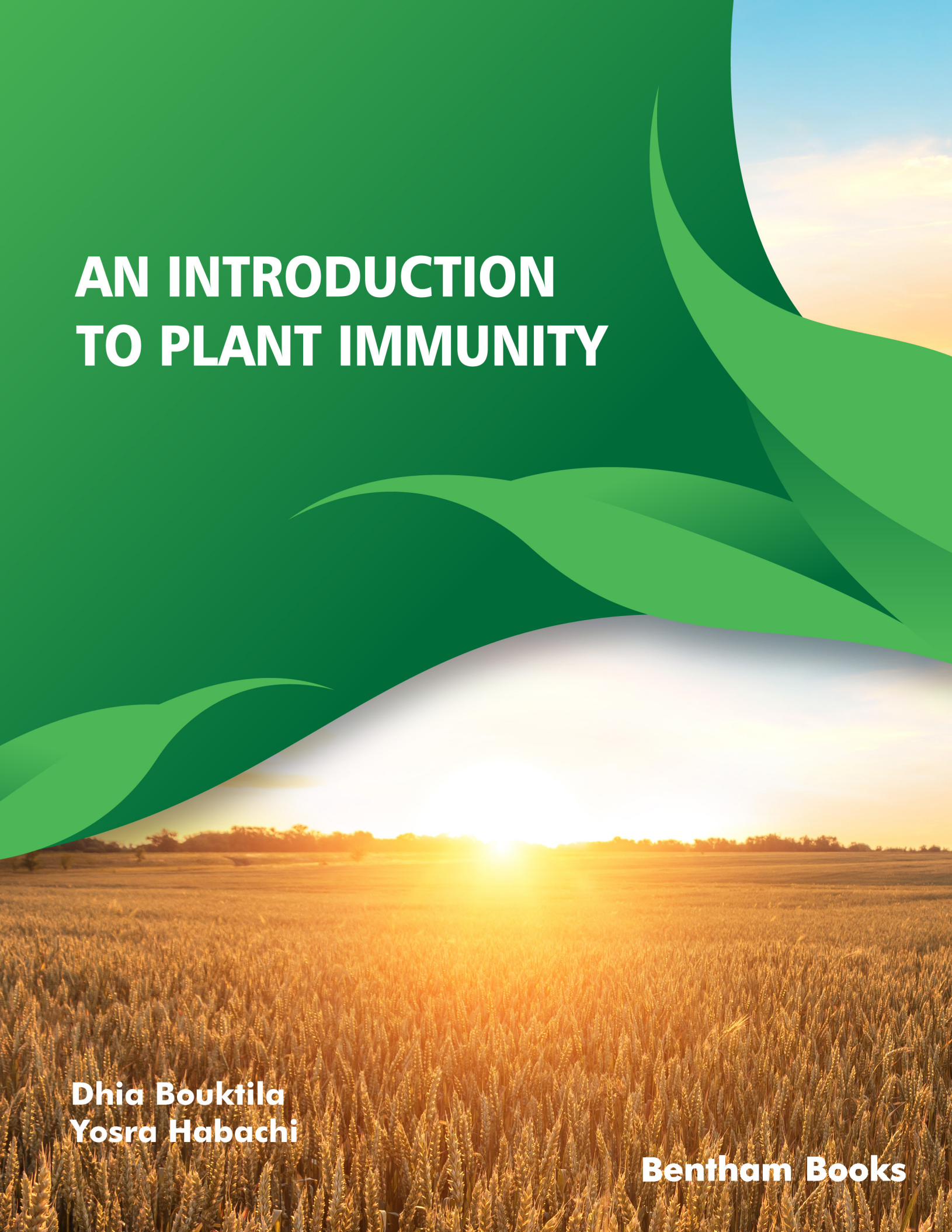
AN INTRODUCTION TO PLANT IMMUNITY



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An Introduction to Plant Immunity

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ISBN (Online): 978-1-68108-802-0

ISBN (Print): 978-1-68108-803-7

ISBN (Paperback): 978-1-68108-804-4

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FOREWORD

It has often been said that plants have neither nervous nor immune systems. However, they are able to react to certain stimuli in the environment and to different stresses whether they are biotic or abiotic. They can fight or resist the germs that surround them. In fact, if they do not have an immune system *per se*, neither dedicated organs nor immune cells, plants have defense mechanisms that can be compared to those of so-called innate immunity in the animal kingdom.

First, they have natural physical (cuticles or spines) or chemical (wax or other compounds) barriers that allow them to prevent or limit infections and control pests. From this point of view, they use the same passive defense strategies as animals (skin, mucous membranes, sweat, sebum, acid secretions).

When pathogens cross these barriers, they encounter active defense mechanisms at the cellular level, with the same molecular systems for the perception of microbial aggressions. These systems involve surface and intracellular receptors PRRs (Pathogen Recognition Receptors) that recognize PAMPs (pathogen-associated molecular patterns) or DAMPs (damage-associated molecular pattern molecules) and trigger signaling pathways aimed at carrying out resistance to infection. Interestingly, these recognition molecules are also described for innate immunity cells in animals. While PRRs identified in plants are primarily, in cell membrane, mammalian receptors can be membrane, cytoplasmic or localized in the endosome membrane. This recognition displays certain specificity and there is a diversity of PRRs recognizing PAMPs, which are conserved patterns, common to different germs and pathogenic microorganisms. Some bacterial PAMPs, such as flagellin (Flg), lipopolysaccharides (LPS) and peptidoglycans (PGN) are recognized in both plants and animals. However, if PRR orthologs in animals do not appear to exist in plants, protein domains such as LRRs (leucine-rich repeats) are conserved between the PRRs of the two kingdoms. For example NOD2 (nucleotide-binding oligomerization domain 2), a cytoplasmic PRR whose mutations are associated with Human Crohn disease, is homologous to resistance *R* proteins in plants.

Similarities between the two kingdoms are also observed in reaction processes including signaling and immune responses. In animals and plants, the perception of PAMPs and DAMPs induces a signaling cascade that leads to the activation of transcription factors and results in the transcription of defense genes. Signal transduction in plants and mammals also involves altering ion flow through membranes, especially Ca^{2+}, as well as producing ROS (reactive oxygen species) or NO (nitrogen monoxide). All of these second messengers also contribute to the expression of a defense transcriptome.

In both cases, pathogen-activated cells have a reprogrammed transcription profile associated with transcription factors (TFs) induced *via* the signaling pathways. These TFs regulate key genes of the protective response. In plants, the target genes, encode in particular, enzymes involved in the synthesis of phytohormones that amplify the immune response and warn neighbouring cells. This is to be compared with what is observed in animal models in which the recognition of PAMPs by TLRs, for example, leads to transduction pathways regulating the expression of genes encoding mediators of inflammation (*e.g.* cytokines and chemokines) that allow the recruitment of specialized defense cells. Moreover, plants and animals synthesize antimicrobial compounds, some of which are common to both kingdoms, such as those of the defensin and thionin family.

In some cases, plant immunity can lead to programmed cell death, called hypersensitive response, which helps to limit the spread of microorganisms. It results in the appearance of localized necrotic lesions at the sites of infection. The mechanisms leading to this cell death can be compared in some ways to the apoptosis or pyroptosis that is observed in animals. Several events of programmed cell death are indeed similar between the two kingdoms.

The adaptation of pathogens to their host is essentially manifested by bypassing the host's immunity. Microbes that infect animals use a variety of escape strategies to reduce the host's defenses and infect them. As in animals, pathogens that infect plants are able to manipulate the cellular functions of their host through effectors (proteins, toxins, *etc*.), thus facilitating their spread. These effectors largely target the cellular signaling pathways that lead to the immune response but also those that induce the opening of the stomata.

Another aspect known for several years in plants is cross-protection. This aspect has been described, in the last decade, in vertebrates and explained by a mechanism known as innate memory or trained immunity. Indeed, it is now accepted that activated innate immunity cells can be maintained in their state of activation for several months with a reprogramming of the transcriptional profile determined, not only by transcription factors, but also by epigenetic changes in DNA and histones methylation profiles, as well as in microRNA expression. Thus, the cells of innate immunity induced into memory cells will persist under an activation state for several months during which they will be more receptive and respond to other infections more effectively. Hence is explained the non-specific cross-protection that can be observed in animals and also in plants where epigenetic change associated to immune response are documented.

Thus, plants use defense strategies against biotic aggressions, which are very similar to those of vertebrates. Knowledge of the mechanisms of innate animal immunity could, therefore, guide research to a better understanding of the defense pathways induced by PAMPs and DAMPs in plants. At a time when agro-ecological concerns are guiding us towards reducing inputs in agriculture, this knowledge will lead to the development of new biocontrol strategies based on stimulating the natural defenses of plants.

This book is a basic document for those who want to understand and deepen their knowledge of immunity in plants. All aspects are dealt with in a gradual and clear way, including the basic concepts, and the subject is treated from its multiple facets: microbiological, phytopathological, cellular, biochemical, genetic, evolutionary, biotechnological and agronomic.

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PREFACE

The world population is increasing day by day, imposing that agricultural production increases proportionally. Unfortunately, the increase in agricultural production is limited due to abiotic and biotic stresses, which have a negative impact on the growth and development of crops and their yields, resulting in considerable economic loss worldwide. In addition, monoculture, which we are witnessing today in agricultural landscapes, and which represents the workhorse of intensive agriculture, weakens plants enormously, while biodiversity protects them. It should be noted that the economic loss attributable to plant diseases and pests, which is estimated at 10-40% of the production potential worldwide, is already being held back by the massive use of phytosanitary products. Although this use of chemicals helps plants to fight against pathogenic microorganisms, it is not without consequences for the environment.

At the sunrise of this new millennium, mankind has an extraordinary chance to enhance development through the scientific improvement of crops and sustainable management of biodiversity. Indeed, advances in Genetics, Molecular Biology and Genomics have evolved to allow what is now called a ***high-tech plant breeding***. Improvements in plant performance can be manifested, among other things, by ***tolerance or resistance to abiotic and biotic stresses***. It follows all the importance that the acquisition of a deep understanding of the genetic mechanisms governing the response of a plant to its invasion by pathogens and pests currently takes. Plants have their own mechanisms for overcoming stress, and activating complex - and sometimes crossed - signal transduction pathways. In recent years, knowledge of the immunogenetic defense mechanisms of plants has improved considerably.

In this perspective, we wanted this book to be an extensive and up-to-date scientific review of the different aspects of knowledge and technologies related to the field of plant immunity. Our goal is to offer a real modern tool for learning and documenting plant immunity for specialist academicians. We hope that students from all over the world will be able to benefit from this informative resource, while allowing teacher-researchers to use it for their teaching and research activities.

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ACKNOWLEDGEMENTS

This work was facilitated by a one academic year teaching leave awarded to Dhia Bouktila, Associate Professor at the Higher Institute of Biotechnology of Beja, in order to focus on completing the writing of this book and achieving this publication. For this, the authors thank the Scientific Council of the Higher Institute of Biotechnology of Beja, the University of Jendouba Council, as well as the Tunisian Ministry of Higher Education and Scientific Research.

The authors want to thank everyone who helped them create this book, especially the students Jihen Hamdi for her valuable help in the preparation of chapters 8 and 14, and Narjess Kmeli and Inchirah Bettaieb for their help in formatting bibliographical entries. Special thanks goes to our editor, **Bentham Science Publishers** who welcomed us into this publishing experience and assisted us with great professionalism.

The authors disclose receipt of the following financial support for the authorship and publication of this book. This work was supported by the Tunisian Association of Psycho-Neuro-Endocrino-Immunology (Association Tunisienne de Psycho-Neuro-Endocrino-Imm unologie) [grant number 2-2020].

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

DEDICATION

To my wife, Rafika, and my kids, May and Marwen: Your support made all of this possible,
To my parents for always loving and supporting me,
To my dear friend and coauthor, Yosra Habachi,
To every student I have taught or advised once in my carreer,
A special dedication to dear friends, Dr. Chokri Belaid, Dr. Anwar Mechri, Dr. Khaled Chatti, Dr. Lotfi Cherni and Dr. Salim Lebbal.

Dhia Bouktila

To my husband, Sami, for his irreplaceable support and his unconditional love.
To my little angels: Seif Allah and Chahed, as a testimony to the attachment, love and affection I have for them.
To my dear friend Dhia Bouktila,
To Dr. Abdelfatteh Zeddini, Head of Laboratory of Pathological Anatomy and Cytology, for his kindness and support.
To my dear colleagues, Asma and Yathreb, for their loveliness and the help that each of them has given me.

Yosra Habachi

CHAPTER 1

Introduction

The survival of most organisms under various environmental conditions depends on the presence of general immune mechanisms, governed by an integrated genetic system. Plants, despite their immobility, have developed various sophisticated and effective mechanisms to recognize and combat pathogens during their attacks. Plant immunity is defined as ***the ability of plants to contain the damaging effect of a pathogen or pest***. Plants contain the genetic information necessary to defend themselves from attack by a multitude of plant pathogens and pests such as viruses, bacteria, insects, nematodes, fungi and oomycetes. This defense can operate at different levels, using either preexisting passive defense systems (cuticle, wax, thorns, chemical compounds, *etc*.), or active defense systems appearing after the perception of aggression. In most cases, the first line of defense is sufficient to repel the pathogen, but sometimes the constitutive barriers are not sufficient and the second, active, line of resistance will be required.

Cell wall penetration introduces microbes to the plant plasma membrane where they will be confronted with extracellular surface receptors that detect pathogen-associated molecular patterns (PAMPs). This detection of microbes on the cells surface sets up ***PAMP-triggered immunity (PTI)***, which hopefully prevents the infection well before the pathogen begins to spread in the plant. That being said, pathogens have evolved strategies to disrupt PTI by secreting specific proteins, called effectors, in the cytosol of plant cells, which affect the efficacy of primary resistance (PTI). Once pathogens have gained the potential to eliminate primary defenses, plants, on the other hand, will establish a more advanced framework for the detection of microbes, termed ***effector-triggered immunity (ETI)***. In the scenario of ETI, the products of major ***resistance (R) genes***, normally intracellular receptors, perceive the associated effector molecules released by the pathogen inside the host cell. Interplay between effectors and intracellular receptors activates a dynamic signaling network to gain disease resistance (McDowell and Dangl 2000). In fact, plant disease resistance conferred by *R* genes is usually supported by an ***oxidative burst***, which is a rapid generation of significant amounts of reactive oxygen species (ROS). This ROS output is necessary for

another component of the resistance process, called ***hypersensitive response*** (HR), a form of programmed cell death that is assumed to restrict pathogen access to the plant.

Finally, at the molecular level, the plant coordinates ***the transcription of a variety of genes*** whose sole objective is resistance. The success of the plant depends on the intensity and speed of the perception of the pathogen signals and their transmission in it to produce an effective response against the pathogen. In *Arabidopsis*, the identification of pathogen-responsive genes is the subject of numerous studies. It has been found that no less than 25% of the genes identified in this model plant species have a transcriptional level affected following the attack of a pathogenic agent. In this way, a deeper knowledge of the basic processes involved in defense responses would make it easier to interpret the interactions between plants and pathogens and allow better resistance of plants, especially in species of agronomic interest.

The relationship between a plant and a harmful organism (*i.e*. pathogen or pest) depends on the environmental conditions, the properties of the harmful organism and the plant's ability to defend itself. The concomitant evolution at the genetic level, including the plant and its pathogenic organism, is a ***coevolutionary process***, which means a specific reciprocal interaction, between the plant and the pathogen. It obviously follows that a large part of the diversity of the living world comes from this coevolution between plants and pathogens, which seems to be an interminable arms race: a species induces a behavioral response to selection pressure imposed by another antagonistic species and the latter changes its behavior in response to the change in the first species. In all coevolutionary systems, the two partner species seek to stabilize with a balanced genetic structure. However, the structure of the genomes of any living organism is constantly modified according to the evolutionary race, *via*, both, small (point mutations) or large-scale (whole-genome duplications) events.

When a pathogen colonizes a plant, or a pest chooses it as a food resource, this will exert a ***selection pressure*** on the plant, thus reducing its fitness. The plant will react in two ways, either it definitively eliminates the aggressor; it is, in this case, a resistant plant, or it accepts the invasion by activating a compensation process; it is, in this case, a tolerant plant. Thus, in-depth knowledge of the genetic defense mechanisms involving resistance genes against biotic stress in plants is a prerequisite for the implementation of management programs and effective control, taking into account of the concomitant evolution of the two protagonists involved.

Contrary to popular belief, the first study linking the development of a disease to a microorganism was not carried out by Robert Koch on the tuberculosis Bacillus in 1890. Instead, at the beginning of the 19th century, the cause of wheat decay was identified by the Swiss Isaac-Bénédict Prévost (1755-1819). This researcher analyzed the cycle of the microscopic parasite responsible for this disease, and developed a mixture capable of eradicating it. However, this work was forgotten because of the preference, in official scientific circles, for the theory of ***spontaneous generation***[1]. In 1861, the German Anton de Bary, considered as the father of phytopathology, did the same by proving that the terrible epidemic of potato late blight responsible for the great famine of Ireland of the 19th century was caused by the filamentous pathogen *Phytophtora infestans* (Matta 2010). More recently, the fungus *Helminthosporium oryzae* was the cause of one of the most significant famines of the 20th century. In 1943, the destruction of rice crops by this fungus was responsible for the deaths of three million people in Bengal (Padmanabhan 1973). The practice of intensive farming since the late 1970s encouraged the development of epidemics. Indeed, monoculture on very large plots and the shortening of crop rotations have led to a loss of diversity in cultivated plants, which are no longer able to resist pathogenic agents on a long-term basis (Ricci *et al*. 2011). Control of phytopathogenic agents is, therefore, a major issue to ensure food security for populations.

To limit the damage caused by pathogens in agrosystems, humans have developed various control methods. First of all, cultural practices make it possible to limit the quantities of inoculum, by crop rotation and the burial of residues. Chemical control has also been widely used since the start of the 20th century and has significantly increased yields (Hirooka and Ishii 2013). Chemical treatments of crops effectively fight against phytophagous insects and fungal diseases. However, their possible impact on the environment is a real source of concern. In addition, as it is the case in animals and humans, chemical treatments are powerless against viral plant diseases, except in the rare cases where they attack the organisms that vector them, insects, nematodes or fungi. It is therefore necessary to develop alternative strategies to chemical control, against viruses. Finally, genetic selection is based on the use of ***cultivar resistance*** to fight against pathogens.

Two types of cultivar resistance are differentiated. ***Quantitative resistance*** is controlled by a large number of genes (polygenic resistance) associated with genome portions called Quantitative Trait Loci (QTLs) that contribute to the expression of resistance. It most often gives the plant partial resistance to a pathogen because the defenses of the plant do not completely prevent the invasion of the disease. It is therefore not blocked but only slowed down in its progression, which causes some visible damage to the plants. On the other hand, ***qualitative***

CHAPTER 2

Plant Pathogens and Plant Pests

Abstract: The diversity of plant aggressors is impressive since it includes cellular or sub-cellular pathogens (fungi, bacteria, viruses, mycoplasmas), weeds, animals (rodents, snails), and insects. Before starting a fight against plant disease, it is necessary to identify the pathogen responsible and to know its ecology, its life cycle and its mode of dissemination in the environment. The constant identification of new taxa is continuously accompanied by a revision of the classifications thanks to the new tools brought by molecular biology. In this chapter, the most recent knowledge on classes of plant pathogens and plant pests in relation with their plant hosts is presented.

Keywords: Arthropods, Bacteria, Diversity, Fungi, Host range, Mycoplasmas, Nematodes, Oomycetes, Pathogens, Parasitic Plants, Pests, Viruses, Viroids.

INTRODUCTION

Plants are the hosts of many living things that can be harmful to them. A pathogen is a biological agent responsible for an infectious disease. This definition includes fungi, oomycetes, bacteria, viruses, viroids, mycoplasmas, protozoa, nematodes and parasitic plants. From this definition are excluded ectoparasites which affect plant health by eating plant tissue. These organisms are considered as ***pests***. Pests include arthropods (such as insects and mites), as well as slugs and snails.

In the context of pathogens, the term ***parasites*** is generally reserved for microorganisms - fungi, oomycetes, bacteria, mollicutes (bacteria-like organisms lacking a rigid cell wall) - and for viruses and viroids, even if ***phytophagous agents*** (nematodes, insects and other pests) and parasitic plants can also cause significant damage to crops. An approximate assessment reports at least 8,000 species of filamentous microorganisms (fungi and oomycetes), about 200 species of bacteria and mollicutes, and more than 500 viruses and viroids.

On the other hand, ***crop pests***, also called pests, are animal organisms that attack cultivated plants, or stored crops, causing economic damage to the detriment of farmers. Pests can cause direct damage to cultivated plants by their diet (phytophagous, xylophagous, *etc*.) or their parasitic lifestyle, or indirect when they are vectors of diseases (*e.g*. viruses).

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Phytopathogenic organisms or pests belong to several taxonomic classes, of which we can cite:

1. SUBCELLULAR PATHOGENS

1.1. Viruses

Diseases caused by viruses pose a serious threat to different cultures every year in many parts of the world. Currently, around 1,000 viruses infect different plants. The visible symptoms caused by viruses in plants can vary depending on the virus, the variety or species affected, and the physiological state of the plant. Many viruses cause symptoms of mosaic on the foliage which can be associated with deformations (thread-like or embossed appearance, reduction in size, *etc*.). Other viruses cause yellowing of the leaves. Finally, certain viruses induce more or less generalized necrosis on the leaves, flowers, fruits or stems.

Generally speaking, viral diseases reduce the growth and therefore the overall production potential of a plant.

1.2. Viroids

Viroids are the smallest infectious pathogens known, consisting merely of a short circular RNA without protein coats. Currently, there are 30 recognized viroid species ranging from 170 to 450 kb, which all infect plants, with some causing diseases while others are harmless (Foster and Fermin 2018). They replicate autonomously when introduced into host cells and their replication depends on the host's cellular machinery. Their mode of action is still unknown and it is assumed that they cause interference in the metabolism of cellular RNAs. Viroids generate dwarfism and deformation. Their transmission is mainly mechanical. Viroids are classed in only two families: ***Pospiviroidae*** and ***Avsunviroidae*** (Wilson 2014) Among the viroids identified in plants, we can indicate *Cadang-Cadang coconut viroid* (*CCCVd*), *Potato spindle tuberviroid* (*PSTVd*), *Tomato Chloric Dwarf* and *Apple Fruit Crinkle*.

2. CELLULAR PATHOGENS

2.1. Mycoplasmas (also called Mollicutes)

Mycoplasmas are placed in a separate class, ***Mollicutes***, which removes them from bacteria. Their cellular organization does not differ from that of other prokaryotes (Cacciola *et al*. 2017). However, it is recognized that this group of

organisms is phylogenetically related to Gram-positive bacteria (Garnier *et al*. 2001). Their main characteristics are:

- They lack a cell wall.

- Their plasma membrane lacks peptidoglycan but contains sterols (*e.g*. cholesterol). They are resistant to penicillin and other antibiotics that act on peptidoglycans.

- A small size of their genomes.

- The low guanine (G) plus cytosine (C) content of their genomic DNA.

Plant pathogenic mycoplasmas are responsible for several hundred diseases and belong to two groups:

a. Phytoplasmas

The phytoplasmas (previously called ***MLOs: mycoplasma-like organisms***) represent the largest group of mycoplasmas, which was discovered first. In fact, until 1967, some diseases were attributed to viruses, although no viral particles could be detected in diseased plants. In 1967, thanks to the advent of electron microscopy, specialists (Doi *et al*. 1967) identified, in the phloem of diseased plants [1], polymorphic structures that were absent in the phloem of healthy plants. With the difference that they have no cell walls, the structures observed showed similarity to prokaryotic microorganisms, the mycoplasmas, which have long been known in animals as infectious agents or as saprophytes on mucous membranes. Therefore, they were called mycoplasma-like organisms (MLOs). Following this discovery, many diseases of inexplicable origin have been attributed to mycoplasma-like organisms (MLOs).

b. Spiroplasmas

Only three plant pathogenic spiroplasmas are known today; (a) *Spiroplasma citri*, the agent of citrus stubborn, was discovered and cultured in 1970 and shown to be helical and motile, (b) *S. kunkelii* is the causal agent of corn stunt, and (c) *S. phoeniceum*, responsible for periwinkle yellows, was discovered in Syria (Garnier *et al*. 2001; Cacciola *et al*. 2017).

Due to their organization, plant pathogenic mycoplasmas can only evolve in certain specific cellular niches of the host, mainly in the phloem cells. Phytopathogenic mycoplasmas are transported from a sick plant to a healthy plant

CHAPTER 3

Plant Diseases

Abstract: Broadly defined, disease is any physiological abnormality or significant disruption in the normal health of a plant that changes its appearance or function. Due to viruses, bacteria or fungi and favored by certain environmental conditions (nutrient deficiency, soil degradation, water problems, climate change, *etc*.) and certain pests (sometimes playing a role of vector), plant diseases are sources of considerable economic losses for agriculture and forestry, and as such studied by plant pathology or phytopathology.

Keywords: Controlling methods, Diagnosis, Economic impact, Non parasitic plant disease, Parasitic plant disease, Symptoms.

INTRODUCTION

Studies of the very different accidents or diseases affecting plants during their growth or post-harvest, in addition to the analysis of the alterations of their products, constitute Plant Pathology. If ***biological agents*** are, actually, responsible for many abnormalities, further study of ***plant physiology*** shows that a large number of pathological symptoms are initially caused by a disturbance of different vegetative or reproductive functions, independently of any parasitic cause. Under these conditions, the disease diagnosis becomes very delicate, and the understanding of pathological phenomena will only be total if it takes into account a range of factors relating either to the plant itself (anatomy, physiology, biology), or to the environment where it lives (climate, soil, parasites).

Typically, research leading to a successful (*i.e*. specific and sensitive) diagnosis of a plant pathogen, will be followed by researches aiming to characterize genetically (or genotype) different pathogen races/strains. These studies are crucial for understanding the mechanistic basis of ***why a certain pathogen causes disease in one host plant and not in another*** and also ***why a certain pathogen race causes disease in one host plant cultivar and not in another***. Answering these intriguing questions is a valuable key toward the improvement of methods for controlling plant diseases, especially breeding for cultivar resistance.

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1. DEFINITION OF A PLANT DISEASE

A plant disease can be defined as a succession of invisible and visible responses of the cells and tissues of a plant, following the attack of a microorganism or the modification of an environmental factor, which causes disruption of the form, function or integrity of the plant. These responses can induce a partial alteration or even the death of the plant or of some of its parts.

In the case of parasitic diseases, it is noteworthy that the term disease will exclude plant injuries caused by insects: A plant is diseased when it is ***continuously*** affected by a biotic factor, which causes a disorder in its normal structure or activities, showing some outward signs and/or symptoms of disease. The word ***continuously*** excludes such things as insect injury. Disease is a term usually reserved for those problems caused by parasites: fungi, bacteria, viruses, mycoplasma, **nematodes** and parasitic higher (seed) plants.

2. CLASSIFICATION OF PLANT DISEASES

Plant diseases are caused by agents that are both infectious (fungi, bacteria, viruses and nematodes) and non-infectious (mineral deficiency, sunburn, *etc*.). Infectious diseases of plants are caused by living organisms that attack and feed on the plant they infect. The parasitic organism that causes the disease is called a ***pathogen***, and the plant invaded by the pathogen and used as a nutrient source is designated as a ***host***. A supportive environment is of crucial importance for the development of the disease. Even if susceptible plants are exposed to huge amounts of a pathogen inoculum, they may not develop the disease unless the environmental conditions are favorable.

Thousands of diseases affect plants. On average, each plant can be affected by a hundred diseases[1]. A given pathogen or pest can have a host spectrum of tens or even hundreds of plant species[2]. Plant diseases are sometimes grouped by type of symptoms (root rot, wilt, leaf spots, rust, *etc*.). However, the most useful criterion remains the classification based on the pathogen responsible for the disease, since this approach makes it possible to define the cause of the disease and the control measures to be taken. Basically, plant diseases can be classified as follows:

2.1. Parasitic (Biotic) Diseases

There are over 80,000 different parasitic diseases of plants. These diseases are caused by fungi, prokaryotes (bacteria and mollicutes), parasitic higher plants, viruses and viroids, nematodes and protozoa. Some crops have only one or two

common diseases; others have many more. For example, wheat and tomato have 123 and 76 different parasitic diseases, respectively (The American Phytopathological Society website, Common Names of Plant Disease; https://www.apsnet.org/edcenter/resources/commonnames/Pages/default.aspx). Only a few of these commonly occur and cause economic problems. Quite frequently, the same plant individual may be affected by two or more diseases at one time.

2.2. Noninfectious (Abiotic) Diseases

They may be caused by:

- Temperature (too low or too high).
- Lack or excess of humidity.
- Lack or excess of light.
- Lack of oxygen.
- Atmospheric pollution.
- Nutritional deficiencies.
- Mineral toxicity.
- Soil acidity or alkalinity.
- Pesticide toxicity.
- Poor cultural practices.

3. ECONOMIC IMPACT OF PLANT DISEASES

Plant diseases reduce agricultural yield as well as the selective value of plants.

3.1. Quantitative Effect on Production

On a global scale, pathogens and pests are reducing crop yields by 10 percent to 40 percent[3]. Added to this are losses due to competing plants (weeds) and post-harvest losses. In some countries of the world, the consequences of such losses can go as far as famines. Thus, the effects of plant diseases on production can cause serious social and economic problems. The history of phytopathology is marked by many devastating epidemics, including that of 1942 when the Indian rice crop was destroyed up to 90% by *Heminthosporium orizae*, which caused a famine responsible for the death of thousands of people (the great Bengal famine) (Padmanabhan 1973).

CHAPTER 4

Plant Immunity: An Overview

Abstract: Plants are called upon to defend themselves against a variety of organisms (bacteria, protists, fungi, insects and vertebrates) that use them as a source of nutrients. Plant immunity is the innate or induced ability of plants to detect and counteract invasive species before they can inflict serious harm. Molecules emitted by pathogens are perceived by receptors at the surface of or inside plant cells. This perception induces complex signaling cascades leading to a defensive response to pathogen attack. This chapter gives an overview of the most important defense strategies in higher plants.

Keywords: Acquired immunity, Avoidance, Coevolution, Innate immunity, Immune memory, Non-specific immunity, Programmed cell death, Resistance, Specific immunity, Tolerance, Zigzag model.

INTRODUCTION

Plants are exposed to several pathogens. In comparison to animals, plants are devoid of mobile immune cells serving to eliminate pathogens. They have, therefore, evolved alternative recognition and defense mechanisms. Actually, each plant cell has an innate immune system and the emanation of systemic signals transmitted from the place of infection leads to the generalization of defenses, on the whole-plant scale (Jones and Dangl 2006). The recognition of pathogens in plants calls for two types of perception. The first type of non-specific recognition involves molecules called general elicitors; the second, which is specific, was first described by Flor in 1955, when the concept of gene-for-gene was stated. These two recognition mechanisms constitute the two primary components of the plant immune function (Jones and Dangl 2006).

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1. COEVOLUTION OF PLANT DEFENSE AND PATHOGEN ATTACK MECHANISMS: THE ZIGZAG MODEL

According to the zigzag model presented by Jones and Dangl (2006) (Fig. **1**), plants have two lines of defense: a first called ***basal or non-specific defense*** and a second known as ***specific defense***, often called ***resistance***.

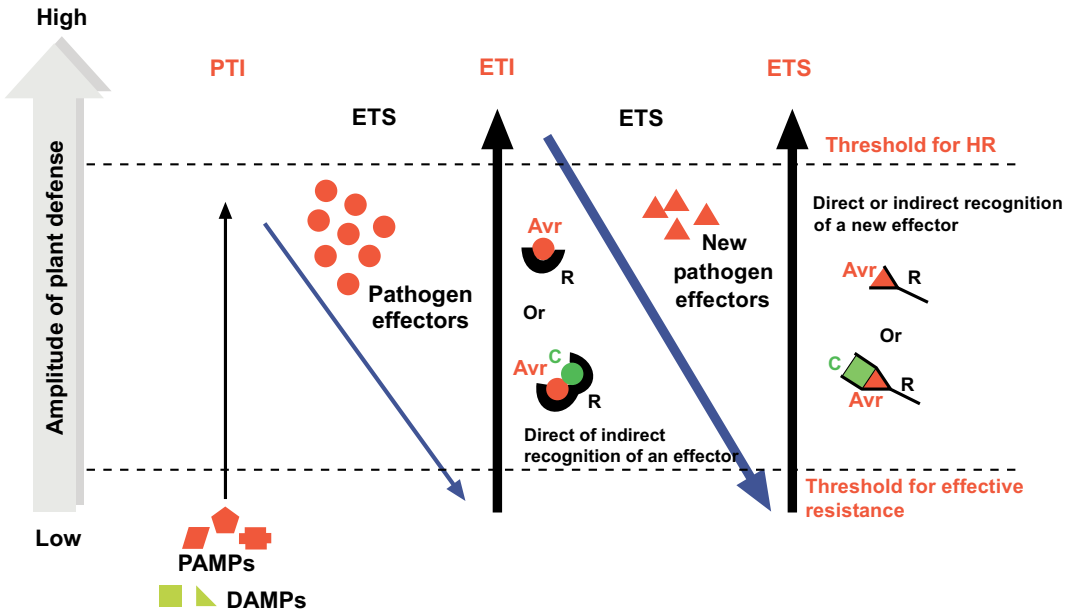

Fig. (1). Zig-zag model illustrating the innate immune system of plants and the coevolution of plant defense and pathogen attack mechanisms (modified from Jones and Dangl 2006).

The first level of defense is formed by all of the physical barriers of the cell, in particular the cuticle and the cell wall, and by non-specific defense reactions. This first basal defense (or PTI, for PAMP-Triggered Immunity) is activated when plants perceive *via* their membrane receptors (Pattern Recognition Receptor, PRRs) the pathogen-associated molecular patterns (PAMPS). Although this defese line is not specific, it makes it possible to limit the spread of pathogens. For example, the lignified cell walls represent an important barrier as they are impermeable to pathogens. In addition, some plants secrete antimicrobial molecules (such as alkaloids, phytoanticipins, phytoalexins and phenylpropanoic compounds) and generate reactive oxygen species (ROS) to limit the spread of the pathogen (Jiang and Tyler 2012).

However, a number of pathogens manage to get rid of these physical barriers by infecting plants *via* natural openings (stomata, wounds). Pathogens also produce

effectors that favor their development while suppressing the basal defense (PTI). Therefore, the plant becomes susceptible due to these effectors. This stage of plant-pathogen interaction is called ***Effector-Triggered Susceptibility (ETS).***

Parallel to this evolution of pathogens' virulence, the plant has developed specific cytosolic receptors capable of perceiving these effectors and allowing the triggering of the second line of defense (specific defense). This specific defense system involves complex cellular events which lead to resistance to certain pathogens. Therefore, concomitant host-pathogen ***coevolution*** has enabled certain plants to develop specific resistance (*R*) genes that directly or indirectly recognize the *Avr* genes of pathogens and confer ***Effector-Triggered Immunity (ETI)*** (Jones and Dangl 2006). At this stage, the ***incompatible*** interaction of *R* and *Avr* genes activate complex signaling cascades, which can locally lead to a ***hypersensitivity reaction (HR)***, as well as to the establishment of systemic resistance at the level of whole plant.

The diagram can be described based on four co-evolutionary phases. Phase 1: Plants recognize the microbe/pathogen-associated molecular patterns (MAMP / PAMP, red patterns) using PRRs to induce PAMP-triggered immunity (PTI). Phase 2: Successful pathogens produce effectors able to overcome PTI, leading to effector-triggered susceptibility (ETS). Phase 3: An effector (full red circles: Avr) is perceived by a resistance (*R*) protein, directly or indirectly by means of a cooperating protein (C), thus activating effector-triggered immunity (ETI), an enhanced variant of PTI, frequently surpassing the induction threshold for hypersensitive reaction (HR). Phase 4: Some isolates of the pathogen gain new effectors (full red triangles: Avr), which can help them overcome the ETI. The selection will later favor plant new alleles that encode the modified resistance proteins, which are able to neutralize the newly recruited effectors. As a consequence, ETI will be once again established.

2. COMPONENTS OF PLANT IMMUNITY

Plant defense against biotic stresses has been classified as ***innate*** (either broad-spectrum or race-specific) and ***acquired*** (either local or systemic) immunity.

2.1. Innate Immunity

The plant has two forms of inherent (*i.e*. innate) defense; ***non-specific*** (*i.e*. basal or general resistance) and ***specific*** (cultivar/pathogen race specific). Therefore, the plant employs a two-level perception system: one used in non-specific defense

CHAPTER 5

Passive Defenses

Abstract: Plants shield against microbes using structural defenses, antimicrobial compounds and secondary metabolites. Most plants have impermeable obstacles, such as waxy cuticles, or morphological adaptive transformations like thorns, which retard the pathogen's progression into plant tissues and prevent the release of deleterious substances such as enzymes for the degradation of walls or toxins. If a pest crosses the barriers of a plant, it is usually confronted with biochemical weapons including, but not limited to, secondary metabolites with antimicrobial ability.

Keywords: Biochemical defenses, Cuticle, Mechanical defenses, Phenolic compounds, Preformed defenses, Phytoanticipins, Wax.

INTRODUCTION

When plants are attacked by pathogens and pests, they react by active and/or passive defenses. In fact, passive immunity relies on the defenses that are constitutively exhibited by the plant body, while active immunity depends on defenses that are inducible by disease or infestation. In particular, passive defenses, which are present before infection, are used to prevent entry and spread of pathogens, and include both physical barriers and chemicals.

1. PRE-EXISTING MECHANICAL DEFENSES

The very first line of protection in the plant is a robust and impermeable major obstacle: the cuticle. First, this cuticle, consisting of ***cutin*** (a lipid substance) and ***wax*** [1], coats the surface of the aerial parts of the plant, increasing their thickness. The thickness of a cuticle varies greatly between different parts of the plant and between different plant species (Wójcicka 2015). Secondly, the ***pectocellulosic wall***[2] is a kind of exoskeleton that envelops the plant cell plasma membrane. This wall can incorporate ***lignin***, a diverse and complex polymer of phenolic compounds that gives the cells rigidity. Lignin is a major component of wood, but the concentration of lignin may be even higher in bark than in wood (Dou *et al*. 2018). Lignified cell walls (for example of the bark) are particularly resistant to pathogens and unpleasant for insects to ingest. These two mechanical elements

(cuticle and bark) safeguard plants against herbivores (including insects) and pathogens. Additional adaptative strategies against grazing vertebrates encompass leaves or branches modified into ***thorns*** (Fig. **1**). These organs discourage herbivores through inducing physical harm such as skin irritation, stomach pain or allergic reactions.

Fig. (1). The thorn-modified leaves in cactus plants act as a mechanical defense against predators (Image source: publicdomainpictures).

We can also cite the ***mutualistic relationships*** established between certain species of acacias (*Acacia* sp.) and the ants of acacia tree, *Pseudomyrmex ferruginea*; the acacias provide shelter and food to aggressive biting ants, which in turn defend the trees by attacking herbivores who try to feed on the tree leaves (Palmer *et al*. 2008) (Fig. **2**).

In addition to the structural barriers cited above, we can add to it the following (reviewed in Doughari 2015):

- **The Ctoskeleton:** Actin cytoskeleton is an important structural feature of eukaryotic cells. Particularly in plants, actin cytoskeleton stands as a major barrier experienced by pathogens in the site of infection, and its disruption was followed by cellular infiltration of many non-host fungi into the crops, as reported in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), tobacco (*Nicotiana tabacum* L.) and cucumber (*Cucumis sativus* L.) (reviewed in Doughari 2015).

- **Nectarthodes:** Hydathodes are pores present naturally in leaf ends, whose role is to excrete sugary nectar, which limits the access of sugar-intolerant microbes into the plant.

- **Leaf Hairs and Trichomes:** Leaves are covered by hairs that act as barriers to pathogen penetration. For example, in chickpea, the rich hair of the leaves and the pods represents a defensive line against *Ascachyta rabei*[3] intrusion (Doughari 2015). Trichomes are thin hairs or appendages that can grow on the surface of different parts of a plant (on roots, stems or leaves); they provide combined physical and chemical immunity especially against pest species. For example, soybean (*Glycine max*) trichomes stop the access of insect eggs into the epidermis, causing the larvae to starve after hatching (Freeman and Beattie 2008).

Fig. (2). Opened *Acacia cornigera* thorn, bearing adult and immature *P. ferruginea*.
(By Meixiaotian, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php? curid=76585564).

2. PRE-EXISTING BIOCHIMICAL DEFENSES

The external protection of a plant can be compromised by mechanical damage (wounds) that can constitute an entry point for pathogens. It is also possible that some micro-organisms manage to overcome the physical barriers of the plant by infecting it through natural openings such as stomata, or even through the action of hydrolytic enzymes. As soon as the primary level of plant defenses has been violated, the plant will have to use a new range of protective mechanisms based on chemical substances, such as toxins and enzymes.

Plants are rich in ***secondary metabolites***, which are not necessary for the plant. Secondary metabolites are molecules that are not derived directly from photosynthesis and are not required for respiration or for the growth and

CHAPTER 6

Basal or Nonspecific Plant Defense

Abstract: Non-specific defense against plant pathogens can be passive (constitutive) or active (induced by microbes). The activation of general resistance follows the perception of the pathogenic threat. The first class of plant receptors recognizes molecular patterns associated with pathogens / microbes (PAMPs / MAMPs) in a non-specific way. These are resident membrane receptors, also called pattern recognition receptors, PRRs. Plant PRRs are the source of extremely complex molecular signaling immune machinery. A transmembrane receptor that binds to a ligand then triggers the signalling would be the most simplistic scenario. Yet, in many cases, the recognition scheme would also include co-receptors, as well as regulatory proteins, which activate PRRs leading to the signal trasduction intiation. It is, therefore, reasonable that our current knowledge is only touching the surface of a remarkably intricate immune strategy.

Keywords: EF-TU Receptor (EFR), Elongation factor Tu (EF-Tu), Elicitor, Flg22, FLS2, Pathogen Associated Molecular Pattern (PAMP), Pattern recognition receptor (PRR), PAMP-triggered immunity (PTI).

INTRODUCTION

The plant defensive arsenal includes a broad variety of constitutive defenses. Besides these, a sophisticated system of responses is induced upon infection that is based on the capability of plants to recognize and identify the invader.

Acting according to the wisdom prevention is better than cure, plants, first, develop protective barriers against infection. These ***constitutive, passive defenses*** are either mechanical or chemical in nature[1]. These barriers and molecules practice regardless of the presence of a well-defined pathogen, and are, therefore, considered non-specific. Their role is to protect the plant from invasion by any eventual biotic agent, by providing it strength, rigidity and vigilance.

The ***inducible, active defenses***, such as PAMP-triggered immunity (PTI), are adopted by the plant as a second resort, due to the high energy costs and metabolism mobilization, essential to their set up, activation and maintenance. However, if the pathogen has the necessary weapons to invade a plant passive

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defense arsenal, or the plant does not detect it, then the outcome of the interaction can be fatal for the host plant, which, therefore, will be forced to make the necessary energy expenditure. Ultimately, the plant will manage to confine the aggressor to the site of attack thanks to the establishment of active defenses triggered by its presence.

1. PASSIVE (CONSTITUTIVE) DEFENSES

The first barriers that pathogenic organisms must overcome are the physical and chemical barriers of the plant[2]. This immunity is qualified as passive because it does not imply recognition of the pathogen by the plant. The cuticle, made up of cutin (a lipid substance) and waxes, protects the surface of the aerial parts of the plant (Fig. **1**). The pectocellulosic wall envelopes each plant cell. This wall may contain lignin, a heterogeneous polymer of phenolic compounds which gives cells their rigidity. The lignified cell walls are notably impermeable to pathogens and difficult to consume by herbivores, including insects.

Some plants add antimicrobial chemical molecules (these are called secondary metabolites) to these physical barriers, such as:

a. Alkaloids, for example ***taxine alkaloids*** produced by ***yew plants***, *Taxus* spp. [3] (Wilson *et al*. 2001),

b. Terpenoids, such as digitoxin from digitalis, *Digitalis purpurea* and *Digitalis lanata* (Jackson Seukep *et al*. 2014),

c. Phenolic compounds are derived from the phenylpropanoid pathway (Kundu and Vadassery 2019), and are documented to exert a role against both insect and mammalian herbivores (Boeckler *et al*. 2011). Some examples include:

   - ***Chlorogenic Acid*** (3-caffeoyl quinic acid) is a derivative of caffeic acid and quinic acid (Kundu and Vadassery 2019), which provides defense against diverse insect herbivores, including the tomato fruitworm, *Heliothis zea* Boddie (Elliger *et al*. 1981) and beet armyworm, *Spodoptera exigua* Hübner (Shapiro *et al*. 2009). Consequent to wounding due to feeding by the insect, polyphenol oxidase (PPO), which is present in the chloroplast, interacts with a vacuolar phenolic substrate[4]. This interaction results in the oxidation of the substrates, which catalyzes the conversion of chlorogenic acid to an insect-toxic substance, chlorogenoquinone (CGQ).

- ***Glycoside Phloridzin*** and its aglycone, phloretin, inhibit scab fungus *Venturia inaequalis*, in apple (Jha *et al*. 2009).

All of these substances are effective against a large number of pathogens. In most cases, these preexisting passive defenses constitute a sufficient obstacle against most pathogens[5].

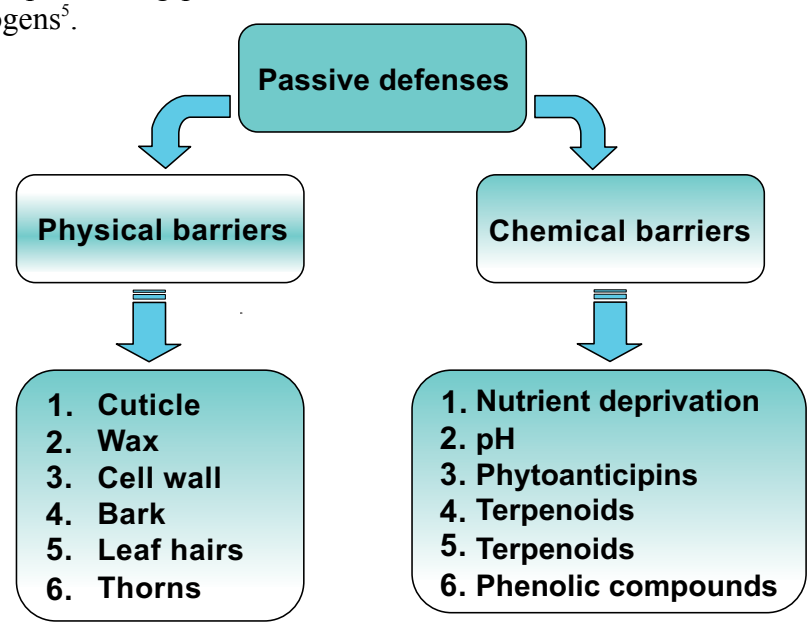

Fig. (1). The constitutive, physical and chemical, barriers of the plant.

2. ACTIVE (INDUCIBLE) DEFENSES

Some microorganisms manage to overcome passive defenses, by infecting the plant through natural openings such as stomata, through injuries, or even directly, through mechanical force and enzymatic softening of the cell wall substances[6] (Fig. **2**). These molecules can either degrade the cuticle and the cell wall, or neutralize toxic molecules by metabolizing them.

Plasma membrane-localized receptors play a major role in detecting pathogen-associated molecular patterns (PAMP) or endogenous signals released after attack, so called danger-associated molecular patterns (DAMP). Recognition of PAMPs by the appropriate pattern recognition receptors (PRR) in plants gives rise to PAMP-triggered immunity (PTI), a basal immune function active against a wide range of microbes.

CHAPTER 7

Pathogen Race-Specific Resistance

Abstract: As part of a host-pathogen coevolution, plants have developed very specific resistance proteins (*R* proteins), which directly or indirectly recognize *Avr* proteins and thus activate host resistance, or effector-triggered immunity (ETI). ETI can be thought of as an enhanced version of PTI and is often described as leading to localized programmed cell death in infected tissue: the hypersensitive response (HR). This chapter aims to clarify what we know and to identify areas that require further investigation.

Keywords: Effector-triggered Immunity (ETI), Effector recognition, Gene-for Gene resistance, Host cultivar, Hypersensible reaction (HR), Nucleotide Binding Site domain (NBS), Pathogen race, Pathogen effector.

INTRODUCTION

In specific resistance, the ***strains of the pathogen*** are capable of infecting all or part of the genotypes of the host plant species. Therefore, this is a second level of plant recognition, when the pathogen is able to cross the barrier of nonspecific recognition.

The initial model, labeled ***gene-for-gene***, assumed the existence of a physical interaction between an *Avr* elicitor and an *R* protein. This model was developed in the 1940s by the American biologist Harold Flor who studied the interactions of flax and the flaxseed rust pathogen, *Melampsora lini*. This hypothesis states that for each resistance (*R*) gene in a plant, there is a corresponding avirulence (*Avr*) gene in the pathogen. The interaction between the two corresponding genes, leads to incompatibility (resistance). This model hypothesizes that there could be a direct or indirect physical interaction between a ligand produced by the pathogen and a corresponding plant receptor, which ultimately triggers the activation of defense genes downstream.

This hypothetical model was later confirmed by molecular evidence of direct contact between the two types of proteins. Indeed, Martin *et al*. (1993) provided first evidence of direct interaction of tomato *Pto* gene with *avrPto* from *Pseudomonas syringae* pv. tomato. Later, additional evidence on this model was

provided, such as ***Pita*/*AvrPita*** in rice (Jia *et al*. 2000), ***RRS-1/PopP2*** in *A. thaliana* (Deslandes *et al*. 2002) [1], and ***L/AprL567*** in flax (Dodds *et al*. 2006; Wang *et al*. 2007) [2]. However, cases of direct R-Avr interaction remain rare, suggesting a more complex relationship (Ellis *et al*. 2000).

It has been demonstrated that the establishment of resistance in the plant requires the presence of a gene in each of the two partners: in the plant, a gene called resistance (*R*) gene, and in the pathogenic agent, an avirulence (*Avr*) gene. In the absence of one of the players, the disease develops. On the basis of this genetic model called gene-for-gene resistance, an interpretation of the model has been proposed according to which the *R* proteins act as receptors which specifically and directly or indirectly [3] bind to a corresponding Avr ligand protein, in order to activate the plant's defense mechanisms (Gabriel and Rolfe 1990).

1. THE FLOR MODEL

Harold Henry Flor's hypothesis corresponds to an explanatory model proposed in the middle of the 20th century on the flax/flaxseed rust pathosystem. *For each gene conditioning resistance in the host plant, there is a specific complementary gene conditioning pathogenicity in the pathogen*. In other words, a plant carrying a given *R* gene will only be resistant to a strain of pathogen carrying the corresponding (specific for this *R* gene) *Avr* gene. This model has been shown to be extremely fertile and remains the basis of phytopathology today. Today, ***avirulence proteins are considered to be protein effectors***. These are proteins from the pathogen that are potentially involved in suppressing the host plant's defense reactions. When these are recognized by resistance proteins from the plant cell, the reactions triggered from this recognition lead to an incompatibility reaction.

2. PATHOGEN EFFECTORS

The notion of effector refers to any secreted molecule associated with an organism, which alters the physiology, structure or function of another organism. In particular, effectors are pathogenic molecules which can modify or even suppress the defense mechanisms induced by PTI, to thereby facilitate access to nutrients, proliferation and growth of the pathogen (Göhre and Robatzek 2008).

The effectors mainly refer to small secreted proteins, which are rich in cysteine and do not have a clear homology with other known proteins (Göhre and Robatzek 2008). Secreted effectors attain their cellular target either at the intercellular interface of the host and the pathogen cells (***apo effectors***) or inside

the host cells (***cytoplasmic effectors***) (Kamoun 2006; Schornack *et al*. 2009; Djamei *et al*. 2011) (Fig. **1**).

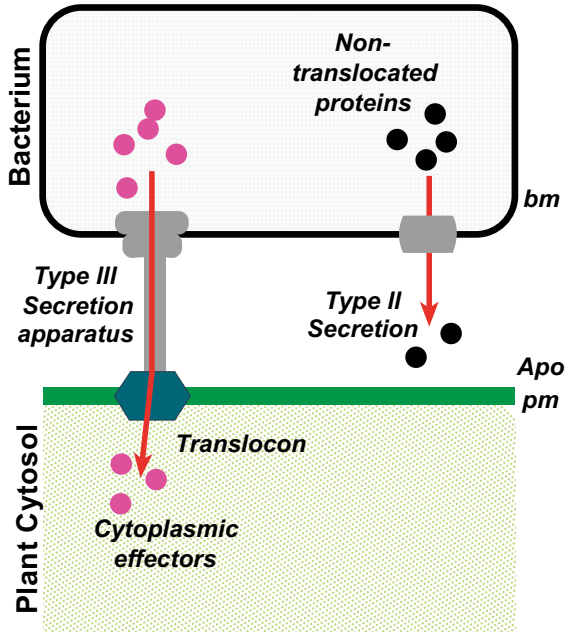

Fig. (1). Schematic view of the two classes of effectors, apoplastic and cytoplasmic, secreted by phytopathogenic bacteria (modified from Schornack *et al*. 2009). Apoplastic effectors (black circles) are secreted in the intercellular space using type II secretion system. Once in the apoplast, they interfere with the apo defenses of plants. Cytoplasmic effectors (magenta circles), on the other hand, are translocated inside the cytoplasm of the host cell. They must cross both the membranes of the pathogen and the plant. Host translocation is achieved by the type III secretion apparatus. Plant plasma membrane (pm), bacterial plasma membrane (bm), apoplast (apo).

Although the presence of effectors has been inferred for many years and their activity has been hypothetically linked to the gene-for-gene hypothesis, the application of molecular techniques, in last decades, has allowed the characterisation of a few gene-specific elicitors (*i.e*. effectors). Since the 1980s', a series of race-specific peptide products of the avirulence genes of *Fulvia fulva*, a biotrophic pathogen of tomato, has been identified. These peptides were first isolated from intercellular fluids of infected leaves and have since been found around the infection site (De Wit *et al*. 1986; Schottens-Toma and DeWit 1988). Recent research is, now, beginning to reveal the function of increasing numbers of fungal effectors bringing forward new technologies that improve our understanding in plant-pathogen interactions (Table **1**).

CHAPTER 8

Acquired Resistance and Elicitors of Natural Plant Defense Mechanisms

Abstract: Unlike innate resistance, acquired resistance is a defense system activated mainly by an earlier infection and allowing plants to resist later attacks by harmful organisms. Its mode of action does not depend on the direct destruction or inhibition of the invading pathogen, but rather on physiological changes which lead to the increase of the physical or chemical barrier of the host plant. The idea of using this ability of plants to defend themselves, to the aim of protecting them from their bio-aggressors is a completely realistic strategy that can be reached by using certain molecules, which have eliciting properties. These molecules, called natural defense stimulators (NDSs), can be of natural or synthetic origin and are capable of putting the plant on a state of alert in order to respond quickly and effectively in subsequent attacks. This innovative strategy greatly contributes to reducing the risks associated with pesticides, and also has great promises for the future, in terms of both socio-economic impact and technology transfer. This chapter provides a summary of the remarkable progress made in recent years in understanding the mechanisms involved in the acquired resistance of plants to various pathogens.

Keywords: Elicitors of natural plant defense, Induced Systemic Resistance (ISR), Jasmonic Acid (JA) et Ethylene (ET) signalling, Local Acquired Resistance (LAR), Pathogenesis-Related (PR) Proteins, Plant growth promoting rhizobacteria (PGPR), Salicilic Acid signalling, stress memory, Systemic Acquired Resistance (SAR).

INTRODUCTION

Plants are sessile organisms that grow within complex, sometimes adverse environments, and are always faced with many stresses of a biotic and abiotic nature. These stresses can lead to significant reductions in crop yield and quality (Atkinson *et al*. 2011; Singh *et al*. 2002). In order to overcome these constraints, plants have developed ***innate defensive strategies*** that allow them to resist different types of threats. In general, there are two types of defense in plants: ***passive defense***, [1] involving preformed or constitutive barriers which the plant has acquired following environmental adaptations (*e.g*. the cuticle), and ***active resistance***, involving instantly formed barriers in response to stress (Llorens *et al*. 2017). When a pathogen succeeds in bypassing the first line of passive defense,

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the active resistance system will be set up, leading to considerable modifications in the metabolic activity of plant cells, resulting in a cascade of events intended to restrict the progression of the infectious agent (Benhamou and Rey 2012). First, broad spectrum defenses are induced, which are often linked to the detection by plants of pathogen-associated molecular patterns (PAMPs) (Jones and Dangl 2006). This is called ***PAMP-triggered immunity (PTI)***[2]. Several membrane proteins involved in the recognition of PAMPs have been identified, in particular the FLS2 protein in *Arabidopsis*, a receptor associated with the perception of flagellin-type bacterial proteins (Chinchilla *et al*. 2006). Plants are also capable of responding to the presence of pathogens *via* the detection of pathogen effectors; in this case, they are using ***effector-triggered immunity (ETI)*** (Jones and Dangl 2006) [3].

Apart from innate immunity (basal and race-specific), ***acquired resistance*** occurs when the inoculation of a plant with an incompatible pathogen strain (one that does not succeed to invade the host plant because of either race-specific or basal resistance) induces a plant defense response that prevents infection by a subsequent inoculation with a normally virulent pathogen.

1. ACQUIRED RESISTANCE

The defensive strategies adopted by plants, in the event of a pathogenic attack, generally result in a hypersensitivity reaction (HR), which is, in fact, a programmed death of plant cells in the area of infection (Jones and Dangl 2006). Following this reaction (HR), signals are sent to uninfected cells and this contributes to the establishment of a ***permanent standby state*** that allows the plant to be alert in the event of a potential attack and to respond quickly to aggression (Jourdan *et al*. 2008); it is, therefore, an ***acquired resistance***. This resistance takes place, first of all, over a slightly wider area than that infected, this is the case of ***local acquired resistance (LAR)***, which is characterized by an increase in the resistance of tissues adjacent to the infection site (Kombrink and Schmelzer 2001). Furthermore, the resistances implemented by plants are not limited to local responses only; plants are also capable of deploying systemic resistance, that is to say generalized to all of their tissues (Durrant and Dong 2004; Jourdan *et al*. 2008). This is the case with S***ystemic Acquired Resistance (SAR)*** and ***Induced Systemic Resistance (ISR)***.

1.1. Systemic Acquired Resistance (SAR)

Acquired Systemic Resistance (SAR), also known as ***Acquired Physiological Immunity***, concerns the plant as a whole (Durrant and Dong 2004). Since the

1930s, Chester has already mentioned the possibility that acquired physiological immunity may exist in plants (Chester 1933). However, it was only after the 1960s that Ross took up this concept. He found that following a first infection of tobacco plants (*Nicotiana tabacum* L.) by the tobacco mosaic virus (TMV), a second infection caused less damage to the whole plant (Ross 1961a; 1961b). Generally, SAR appears throughout the plant from 30 minutes to several hours after initial infection and results in a much more effective defense in subsequent attacks. Even more remarkable, this exacerbation of defenses is effective against any pathogen (viruses, bacteria or fungi), and not just that involved in the first attack (Klarzynski and Fritig 2001). This resistance can last for several weeks. In cucumbers, for example, it has been shown that after the first inoculation with a pathogen, the plants were protected until flowering (Madamanchi and Kuc 1991). Generally, this type of resistance is accompanied by the accumulation of ***salicylic acid (SA)*** and ***Pathogenesis-Related Proteins (PR proteins)*** (Fig. **1**). Indeed, the accumulation of salicylic acid after infection is necessary to activate pathogenesis-related (PR) defense genes (Vallad and Goodman 2004). For example, assays of salicylic acid have shown that its concentration increases considerably (x 100) in several plants following a pathogenic infection (Yalpani *et al*. 1993). In addition, it has been reported that transgenic tobacco and *Arabidopsis thaliana* L. plants unable to synthesize salicylic acid no longer express SAR against various pathogens (viral, fungal and bacterial) (Delaney *et al*. 1994 ; Dong 1998). However, recent studies have highlighted several metabolites that may be involved in ***long-distance SAR signaling***, such as dehydroabietinal (DA), azelaic acid (AzA) and pipecolic acid (Pip) (Dempsey and Klessig 2012; Shah and Zeier 2013).

Disease Acquired Resistance is a paradigm for the existence of a form of ***plant memory***. This state of resistance can be compared to vaccination practiced in humans, with the difference that it protects against many pathogens of different natures.

1.2. Induced Systemic Resistance (ISR)

The protective effect conferred by SAR is phenotypically similar to another phenomenon triggered by interaction with a ***non-pathogenic microorganism***. This immunization of the plant is called induced systemic resistance (ISR) (Bakker *et al*. 2013); in fact, it is a form of induced resistance specifically stimulated by rhizobacteria better known as ***Plant growth-promoting rhizobacteria*** (PGPR). These include $Gram^+$ bacteria such as *Bacillus pumilus*, *B. subtilis*, and *B. thuringiensis* (Kloepper *et al*. 2004), or $Gram^-$ bacteria, most belonging to the genus *Pseudomonas*, which are most studied in the context of

CHAPTER 9

Quantitative Resistance

Abstract: If breeders use a limited number of genes in their new resistant varieties, the adaptive capacity of pathogenic populations will ultimately lead to more or less rapid overcoming of these resistances, thus limiting the sustainability of their effectiveness. Qualitative resistance is considered less durable than quantitative resistance since the latter oppose less selection pressure on the pathogen and they are often governed by several resistance genes. Quantitative disease resistance has been observed within many crop plants but is not as well understood as qualitative (monogenic) disease resistance and has not been used as extensively in breeding. Mapping quantitative trait loci (QTLs) is a powerful tool for genetic dissection of quantitative disease resistance.

Keywords: Additive effect, Marker-Assisted Selection, Molecular markers associated to QTLs, Quantitative trait loci (QTL), Resistance durability.

INTRODUCTION

Plant pathogens are major limiting factors in crop production and this has led to the extensive use of chemicals to control them. Plant genetic resistance is a promising key alternative to control crop diseases and pests. However, pathogens frequently adapt to and overcome genetic resistance especially when it is determined by major genes. The mechanism for bypassing qualitative resistance is explained by the existence of gene-for-gene interactions between the plant's resistance gene and the pathogen's avirulence gene, since a simple mutation in the gene for avirulence can allow it to escape recognition by the resistance gene and overcome this resistance. Thus, quantitative resistance has gained interest in recent years to address the major challenge of genetic resistance durability. Several genes usually control quantitative resistance and are associated with genomic regions or QTL (quantitative trait loci) which contribute, each with variable effect, to the phenotype of resistance to a pathogen. However, a combination of resistance QTLs can lead to total resistance in some cases, especially when QTLs have strong effects (Niks *et al*. 2015). For example, three QTLs, *rx1, rx2*, and *rx3* were found to confer a high level of resistance of tomato to *Xanthomonas campestris* (Stall *et al*. 2009). Over the past 20 years, since the development of molecular markers, many resistance QTL detection experiments

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have been conducted in all major crop species (Stall *et al*. 2009; Huang and Han 2014; Desgroux *et al*. 2016; Corwin and Kliebenstein 2017).

1. MOLECULAR MECHANISMS ASSOCIATED WITH QUANTITATIVE IMMUNITY

While the molecular mechanisms underlying the main *R* genes have been widely described (Michelmore *et al*. 2013), those associated with quantitative immunity are much less known (Fig. **1**).

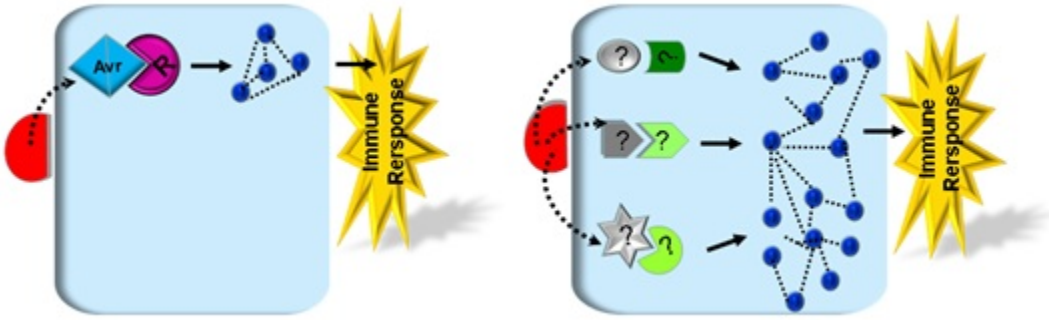

Fig. (1). Simplified model of the molecular bases of plant-pathogen interaction in the case of qualitative resistance (left) and the complexity of molecular bases assumed in quantitative resistance (right) (Red = pathogen cell, blue = plant cell).

The genes that underlie resistance QTLs are far less known and described than the *R* genes. Few resistance QTLs have been cloned to date and have shown a variety of underlying functions (Niks *et al*. 2015). The main QTLs cloned by positional cloning are *Lr34*, *Yr36*, *Pi21*, *Rhg1*, *Rhg4* and *Pi35* (Lavaud 2015). Their recognized functions are disparate, which include ABC transporter proteins, tRNA protein (HIS) guanylyltransferase, Proline-rich protein, Serine hydroxymethyltransferase or NBS-LRR domain-containing proteins (Kou and Wang 2010 ; Michelmore *et al*. 2013; Niks *et al*. 2015). These QTL cloning results fuel the multiple ***hypotheses*** synthesized by (Poland *et al*. 2009) on the ***function of resistance QTLs***. Among these:

a. Resistance QTLs would be correlated to the plant morphology and development.

   Many correlations have been described between QTLs of resistance, on one hand, and architecture and development of the plant, on the other hand. In potato, colocalizations have been observed between QTLs of resistance to

Phytophthora infestans and plant maturity and vigor (Collins *et al*. 1999; Gebhardt *et al*. 2004). In pea, QTLs of partial resistance to *Didymella pinodes*[1] have been located in the same regions as genes controlling the elongation of internodes (plant height), flowering date and photoperiod sensitivity during the initiation of flowering (Prioul *et al*. 2004; Giorgetti 2013).

b. Resistance QTLs would be involved in the production of antitoxic compounds.

Biochemical studies of the *Arabidopsis-Botrytis* pathosystem have shown that camalexin[2] levels are correlated with the level of quantitative resistance. In fact, the decrease in the level of camalexin in the plant caused greater sensitivity towards the pathogen (Denby *et al*. 2004).

c. Resistance QTLs would be involved in the transduction of the plant defense signal.

In *A. thaliana*, mutants of the transcription factor WRKY (involved in the signaling pathways regulated by salicylic acid and jasmonic acid) showed an increased sensitivity to the necrotrophic pathogen *Botrytis cinerea* (Zheng *et al*. 2006). Conversely, increased resistance to *Pseudomonas syringe* pv. tomato, and *Peronospora parasitica* has been observed in mutants of *A. thaliana* in MAP kinase 4 protein (MPK4) (Petersen *et al*. 2000). In *A. thaliana*, QTL RKS1 confers resistance to *Xanthomonas campestris* and corresponds to a protein kinase potentially involved in signal transduction (Huard-Chauveau *et al*. 2013).

d. QTLs would be *R* genes with weak effects or *R* genes overcome by pathogen strains yet maintaining a residual effect.

Several studies have shown colocalization of QTLs and overcome *R* genes in several plants (Poland *et al*. 2009). In corn, QTL RCG1 has been identified as an NB-LRR gene involved in partial resistance to anthracnose (Broglie *et al*. 2006). In rice, substitutions in the LRR domain of the *Xa21* gene have conferred partial resistance to *Xanthomonas oryzae* pv. *oryzae* (Wang *et al*. 1998). In wheat, the *Yr36* gene conferring quantitative resistance to yellow rust is involved in the transfer of lipids and includes a lipid-binding domain (Fu *et al*. 2009). The partial effect of a QTL could therefore come from the modification of recognition sites and / or transducing sites, leading to a weak interaction between the pathogen and the plant and consequently, would trigger less important and/or slower defense responses.

CHAPTER 10

Molecular Models of Specific Host-Pathogen Recognition

Abstract: Although some of the resistance strategies rely on simple physical or chemical barriers, modern concepts of plant immunity emphasize the role and evolution of protein receptors in the plant cell. These immune receptors, made up of multidomain proteins, are the key elements in the recognition of pathogen elicitors / effectors, leading to the susceptibility or resistance of plants. Numerous pairs of plant R proteins and corresponding pathogenic Avr proteins have been identified as well as cellular proteins which mediate R/Avr interactions, and the molecular analysis of these interactions has led to the formulation of models on how *R* gene products recognize pathogens. Data from several R/Avr systems indicate that specific domains within R proteins determine recognition specificity. However, recent evidence suggests that R proteins have recruited cell recognition cofactors that mediate interactions between Avr proteins and R proteins. Overall, to explain this direct or indirect interaction, at least four models are currently widely approved. This chapter highlights the current trends in understanding host–pathogen interactions through a variety of models.

Keywords: Avirulence (Avr) protein, Compatible/incompatible reaction, Decoy model, Gene-for-gene model, Guard model, Helper NLRs, Integrated decoy model, NLR-IDs, R-Avr recognition, Recognition cofactors, Resistance (R) protein, Resistance gene, Receptor-ligand model.

INTRODUCTION

In their struggle against attacks by viruses, bacteria, fungi, protozoa, nematodes and insects, plants have developed an integrated defense mechanism against the invasion of pathogenic organisms and pests. This integrated mechanism limiting the proliferation of harmful agents to the plant, is called ***resistance***. Genes that confer a resistant phenotype are called ***resistance genes***. Even though ***Flor's model*** has been experimentally confirmed in several models, such as *Pita* / *AvrPita* (Jia *et al*. 2000), *PopP2* / *RRS-1* (Deslandes *et al*. 2002), and *L* / *AvrL567* (Dodds *et al*. 2006), cases of direct *R-Avr* recognition remain rare, suggesting a more complex relationship (Ellis *et al*. 2000).

Since 1998 (Van Der Biezen and Jones 1998b), there has been a new hypothesis called ***guard hypothesis***, which involves a third component, a ***guardee*** protein,

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mediating the interaction during the recognition process. According to this hypothesis, the attachment of the elicitor (Avr) would induce the formation of the guardee-*R* protein complex, causing signal transduction and the defensive response. This hypothesis then explains the fact that certain resistance proteins are capable of recognizing multiple unrelated Avr proteins.

In 2008, Van der Hoorn and Kamoun proposed the ***Decoy model*** in addition to the guard model. They postulated that in most cases, the guardee would not be the target of the effector. A ***decoy protein***, by mimicry with the real target, would trap the effector by competing with its virulence target.

1. « RECEPTOR – LIGAND » MODEL

The first model, called the ***gene-for-gene or receptor - ligand model*** (Fig. **1**), involves the direct effect of a plant receptor that recognizes an effector specific to the pathogen. This model was formalized by Flor (1971), based on studies carried out on rust resistance in flax (*Linum usitatisimum* L.). The initial hypothesis put forward by Flor postulates that for each resistance gene in the host, there is an avirulence gene in the pathogen. This hypothesis revolutionized the genetics of plant breeding for disease resistance, forcing plant breeders and geneticists to study the evolution and migration of pathogens. Fifty years after its discovery, the gene-for-gene theory still remains valid for understanding the genetics of host-pathogen systems (or pathosystems) (Table **1**).

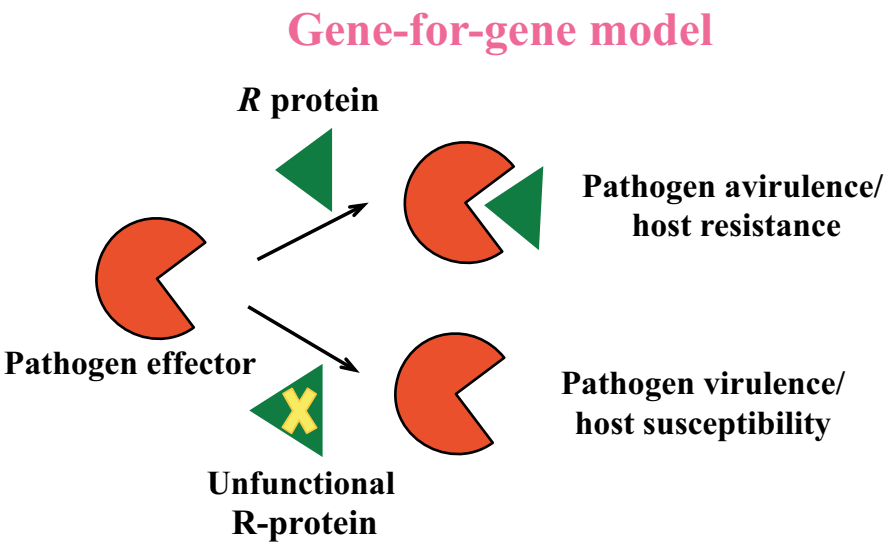
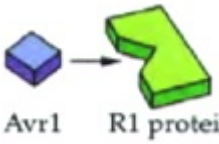
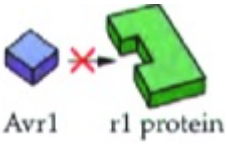
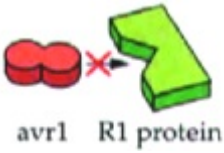
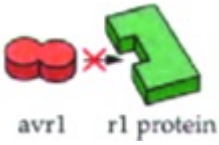


Fig. (1). Mechanism of plant-pathogen recognition according to the gene-for-gene model (modified from Glowacki *et al.* 2011).

Table 1. Host plant and pathogen genotypes leading to compatible or incompatible reactions between plant and pathogen, in gene-for-gene interaction.

		HOST GENOTYPE	
		RR / Rr	***rr***
PATHOGEN GENOTYPE	***Avr Avr / Avr avr***	 **Plant and pathogen are incompatible** **RESISTANCE**	 **Plant and pathogen are compatible** **DISEASE**
	avr avr	 **Plant and pathogen are compatible** **DISEASE**	 **Plant and pathogen are compatible** **DISEASE**

The advances made since 1970 in Molecular Biology, have made it possible to better understand the gene-for-gene theory, initially emitted by Flor. In fact, it has been shown that the proteins encoded by resistance genes act as receptors localized at the level of the cell membrane, acting as sensors recognizing in a specific way products (effectors) encoded by the avirulence genes of the pathogen. Whenever these receptors are in contact with their specific ligand, the signal is transferred inside the cell. This signal transduction leads to a hypersensitivity reaction.

2. THE « GUARD » MODEL

This second model (Van Der Biezen and Jones 1998b; Innes 2004) represents an extension of the gene-for-gene model. According to the Guard model, the resistance reaction does not occur simply as a consequence of the direct recognition of the pathogen effector by the resistance protein, but additional cooperation is also necessary between the resistance protein and certain intracellular receptors present in certain hosts, and playing the role of ***guarded protein (guardee)*** in this tripartite, indirect recognition (Fig. **2**).

CHAPTER 11

PRRs and WAKs: PAMPs and DAMPs Detectors

Abstract: The perception of environmental signals and the ability to react accordingly are essential for the survival of organisms. In plants, extracellular recognition of microbe- and host damage-associated molecular patterns leads to the first layer of inducible defenses, termed pattern-triggered immunity (PTI). Pattern recognition receptors (PRRs) can perceive pathogen/microbe-associated molecular patterns (P/MAMP) from different microbes such as bacteria, fungi, oomycetes or viruses. Danger-associated molecular patterns (DAMPs) correspond to cell wall fragments that can be released by the plant after wounding or pathogen attack. An important group of PRRs is the family of wall associated kinases (WAK) that perceives pathogens indirectly, *via* DAMPs, and activates oligogalacturonide-dependent defense responses. The present chapter will address the most important perception systems used by plants to perceive pathogen attack and initiate efficient defense responses.

Keywords: Bacterial flagellins, Damage Associated Molecular Patterns (DAMP), Pathogen-associated molecular pattern (PAMP), Pattern-Recognition Receptors (PRRs), PAMP-triggered immunity (PTI), Plant innate immunity, Plant lectin receptors, Wall-associated kinases (WAKs).

INTRODUCTION

The immune responses of plants are triggered by the perception of nonself, which can be of a general type or more specific depending on the nature of the recognized molecules. The general-type response (*i.e*. nonspecific) follows the recognition of molecular patterns, common to many microorganisms, called elicitors and more recently PAMPs or MAMPs (pathogen / microbe-associated molecular patterns). When tissues undergo an alteration in their integrity associated with the death of cells, the latter release molecules that are called ***danger signals*** or damage-associated molecular patterns (DAMPs). Unlike mammals, plants do not have mobile defense cells or an adaptive immune system. Plants contain an innate immune system ***embedded in each cell*** and emit systemic signals from infected sites (Marina-García *et al*. 2008; Cassel *et al*. 2009; Duewell *et al*. 2010). In all cases, the perception of PAMPs or DAMPs is carried out by receptors called ***pattern recognition receptors (PRRs)***, which constitute the first level of recognition of the plant immune system (Jones and Dangl 2006). These

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receptors confer broad-spectrum resistance because they are able to recognize these conserved molecules that are widely present in microorganisms. These can be receptors with a kinase domain (Receptor-like Kinase: RLK) or protein receptors (receptor like protein: RLP).

In recent years, considerable progress has been made on the functional analysis of PRRs. The first pattern recognition receptor was identified in plants [1] and this led to a ***paradigm change*** in the plant defense field and instigated the recognition of PTI as a critically important component of the plant defense machinery. Since then, several new PRR candidate genes have been identified.

1. PATTERN-RECOGNITION RECEPTORS (PRRS)

1.1. An Overview: Nature of PAMPs and Biochemical Structure of PRRs

Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) to microbial infection constitutes an evolutionarily ancient type of immunity that is characteristic of all multicellular eukaryotic living things. Microbial patterns (PAMPs) activating plant PTI have been conserved through evolution ; in other words, these are molecules present in microorganisms, and which are slowly evolving due to the critical functions they exert for survival. These molecules, which are detected by PRRs, are diverse: bacterial (flagellin, elongation factor EF-Tu, and peptidoglycan) (Gust *et al*. 2007), fungal (chitin, xylanase) (Kaku *et al*. 2006), oomycete (β-glucan and elicitins) (Du *et al*. 2015), viral (double stranded RNA) (Niehl *et al*. 2016), and insect (aphid-derived elicitors) (Prince *et al*. 2014).

Plant Pattern-Recognition Receptors (PRRs) mediate microbial pattern sensing and subsequent immune activation. PRRs include a range of non-specific receptors. These receptors often possess leucine-rich repeats (LRRs) that bind to extracellular ligands, transmembrane domains necessary for their localization in the plasma membrane, and cytoplasmic kinase domains for signal transduction through phosphorylation (Zipfel 2014). LRRs are highly divergent, associated with their ability to bind to diverse elicitors. Numerous PRRs rely on the regulatory protein brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1) and other somatic embryogenesis receptor-like kinases (SERKs) (Prince *et al*. 2014)[2].

1.2. Best Known Examples of Bacterial and Fungal PAMPs and their Cognate Pattern Recognition Receptors

The LRR-RLKs FLS2, EFR and Xa21 are capable of detecting bacterial peptides such as flg22 from flagellin, elf18 from EF-Tu and AxYs22 from Ax21, respectively (Kaku *et al*. 2006; Gust *et al*. 2007).

Another example is the plasma membrane LysM receptor-like kinase1 (CERK1/LysM-RLK1), which has been shown to be essential for chitin signaling in *A. thaliana*. Actually, in mutants knocked-out for this gene, chitin-induced resistance against pathogens is suppressed (Miya *et al*. 2007).

Another example is the tomato LeEix1 and LeEix2, which are capable of perceiving the fungal ethylene-inducing xylanase protein EIX (Ron and Avni 2004).

1.3. Focus on FLS2-flg22 Interaction

A very good example is that involving bacterial flagellin, which induces immune responses in many plants (Zhou *et al*. 2010). Mobility due to flagellin is very important for the pathogenicity of bacteria in plants (Schroder and Tschopp 2010). A synthetic peptide of 22 amino acids, flg22, constitutes a conserved domain of flagellin. The plant PRR binding flg22 is a receptor with kinase activity containing an extracellular LRR called FLS2 (Flagellin Sensentive) (Yegutkin 2008). FLS2 engages a cascade of MAPK, WRKY transcription factors and defense effector proteins (Schenk *et al*. 2008) [3]. It is to note that the human counterpart of FLS2, TLR5 (Toll-Like Receptor-5), recognizes different domains of flagellin (Schroder and Tschopp 2010), which suggests a mechanism of ***convergent evolution***.

2. A PARTICULAR PRR CLASS: WALL-ASSOCIATED KINASES (WAKS), DAMPS RECEPTORS

Unlike other PRRs that detect pathogenic molecules during infection, other receptors perceive the damage by recognizing the cellular components (DAMPs) that have been disrupted by pathogenic enzymes. Specialized receptors are used by the plant to carry out ***indirect recognition of pathogens via DAMPs***. This has been shown in *Arabidopsis* with the perception by WAK1 of oligogalacturonides (Brutus *et al*. 2010) and the perception by DORN1/LecRK-I.9 of extracellular ATPs (Choi *et al*. 2014). WAK1 and WAK2 from *Arabidopsis* perceive oligogalacturonic acid, resulting from the degradation of pectin in the plant cell wall by fungal enzymes (Brutus *et al*. 2010).

CHAPTER 12

NLRs: Detectors of Pathogen Effectors

Abstract: Defense response by NBS-LRR proteins (NLRs) is a sophisticated strategy that induces effector-triggered immunity (ETI). The NBS-LRR proteins are encoded by one of the largest and most important gene families involved in disease resistance in plants. These NBS-LRR proteins are mainly intracellular, and they can specifically recognize effectors secreted by pathogens either directly or indirectly. This will trigger downstream signaling pathways leading to implementation of plant defense response against various classes of pathogens including bacterial, fungal, viral, nematode and insect. In the present chapter we discuss about the present knowledge pertaining to NBS-LRR class of proteins; their structural organization, genomic ditribution and evolution.

Keywords: CNL, Coiled-Coil domain (CC), Gene duplication, LRR (Leucine-Rich Repeats), Nucleotide-Binding Site (NBS), TNL, Toll Interleukin Receptor (TIR).

INTRODUCTION

Cell surface-localized plant innate immune receptors, addressed in the previous chapter, are capable of recognizing diverse evolutionary conserved pathogen components. Conversely, intracellular nucleotide-binding leucine-rich repeat (NBS-LRR or NLR) receptors recognize race-specific pathogen effectors delivered inside host cells. These NBS-LRR receptors are functionally responsible for the detection of a variety of pathogens, including bacteria, viruses, fungi, nematodes, insects and oomycetes. With the advent of high-throughput molecular tools, genomic analyzes have revealed a variable number of putative genes containing an NBS domain, in different cultivated species and models, even if the actual number of functional or expressed *R* genes, in many plant species, remains unknown. Even though NLRs are a stereotyped family of immune receptors, many mechanistic facts remain poorly understood.

1. THE MAIN STRUCTURAL DOMAINS OF NBS-LRR PROTEINS

An NLR tpyically has a modular structure with three domains (N-terminal, central and C-terminal), with distinct roles for each of these three domains. Most *R*

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products would combine a C-terminal receptor domain and a central effector domain (Hammond-Kosack and Jones 1997), which perform two major functions: firstly, the recognition of elicitor molecules by protein-protein interaction mechanisms and, secondly, the direct or indirect activation of transduction signals (Blumwald *et al*. 1998). These signals activate the local hypersensitivity reaction (Fritig *et al*. 1998).

1.1. The C-terminal Region

1.1.1. Leucine-Rich Repeats (LRR) Domain

The C-terminal Leucine-Rich Repeat (LRR) domain is very variable and composed of repeated leucine-rich motifs (and/or other hydrophobic residues). The repeated motif size is 23 amino acids with the consensus (LxxLxxLxxLxLxx(N/C/T)x(x)LxxIPxx) (Jones and Jones 1997). The LRR domains are involved in protein-protein or protein-polysaccharide interactions, as well as in other peptide-ligand associations (Kobe and Kajava 2001). They would therefore carry the specificity of recognition of the avirulence protein (DeYoung and Innes 2006).

A number of proteins with LRR have been described in mammals and *Drosophila*. In these animal proteins, it has been shown that the LRR domain interacts with pathogen-specific proteins (Dangl and Jones 2001). In addition, a great diversity is observed among the different alleles of known resistance genes. In particular the diversity of LRR domains would be at the origin of the diversity of the resistance protein specificities. This assumption is supported by studies carried out on the various alleles of the *L* and *P* genes of flax (Ellis *et al*. 1999; Dodds *et al*. 2000). However, it has been shown that domains of resistance proteins, other than the LRR, also play a role in this specificity (Ellis *et al*. 2000; Luck *et al*. 2000), and that the LRR domain can sometimes have a role in the transduction of the resistance signal (Warren *et al*. 1998; Hwang *et al*. 2000).

1.1.2. Other Domains of the C-Terminal Region

The C-terminal domain in plant NLRs generally contains a Leucine-Rich repeats (LRR) domain; this domain often has detection[1] and self-regulatory functions (Yuen *et al*. 2014). However, the C-terminal region may instead consist of other repeats forming a superstructure (Kobe and Kajava 2000), for example Armadillo (ARM), Ankyrin (ANK), HEAT, Kelch-like repeats, Tetratricopeptide (TPR), WD40, and Pentatricopeptide repeats (PPR) (Sharma and Pandey 2015). Majority of these motifs correspond to repeated units of conserved stretches of 20–40

amino acids, that contain distinctive structures (α-helices or β-sheets) providing them flexibility to bind diverse ligands and proteins.

1.2. The Central NOD Region

This region contains a Nucleotide-Binding Site (NBS) domain with regulatory and oligomerization functions (Inohara and Nunez 2001; Dyrka *et al*. 2014). The central NBS domain corresponds to a site for attachment and hydrolysis of the nucleotide triphosphates ATP and GTP. The analogy of this domain with animal proteins potentially involved in apoptotic cell death phenomena has led to the notion of the NB-ARC domain[2] (Van der Biezen and Jones 1998a). Numerous studies have highlighted the existence of eight major motifs in the NBS domain, some of which are characteristic of one of the CNL or TNL classes and others common to both (Table **1**). Indeed, the four motifs P-loop, kinase-2, RNBS-B (or kinase-3a) and GLPL are common to both classes. However, the two motifs RNBS-A and RNBS-D show no similarity, and the RNBS-C motif has a low similarity between CNL and TNL (Meyers *et al*. 1999). The eighth motif is MHDV, which is highly conserved in the CNL class. The peptide segment containing all of these eight motifs (from P-loop to MHDV) corresponds to the NBS domain, which has a size of around 300 amino acids.

Table 1. Major motifs present in the NBS domain, classified according to their position (Cordero and Skinner 2002; Meyers *et al.* 2003).

S no	Motif	Consensus Aminoacid Sequence	Position Inside NBS Domain
1	P-loop	GVGKTT	1
2	RNBS-A (CNL)	FDLxAWVCVSQxF	20
	RNBS-A (TNL)	FLENIRExSKKHGLEHLQKKLLSKLL	23
3	Kinase-2	LLVLDDVW	74
4	Kinase-3a (synonyme RNBS-B)	GSRIIITTRD	102
5	RNBS-C	YEVxxLSEDEAWELFCKxAF	122
6	GLPL	CGGLPLA	162
7	RNBS-D (CNL)	CFLYCALFPED	223
	RNBS-D (TNL)	FLHIACFF	219
8	MHDV (CNL)	VKMHDVVREMALWIA	~ 300

CHAPTER 13

Molecular Classification of Plant Resistance Genes

Abstract: Plant resistance (*R*) genes exhibit conserved domains, each of which performs discrete functions in the resistance to pathogens. The most abundant *R* genes belong to the classes of nucleotide binding site leucine rich repeats (NBS-LRR), receptor-like kinases (RLK), and receptor-like proteins (RLP). The list also includes genes encoding proteins with a unique transmembrane domain, genes encoding toxin reductases, genes encoding CC and transmembrane proteins and genes encoding an intracytoplasmic protein kinase. This chapter sheds light on recent advances in the classification of *R* genes, based on their conserved structural characteristics. Knowledge about the R proteins cellular localization and advances in the molecular cloning of *R* genes are also treated.

Keywords: Classes of plant disease resistance (*R*) genes, Cellular localization, Domain architecture, Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR), *R*-gene cloning, Receptor-Like Kinase (RLK), Receptor-Like Protein (RLP).

INTRODUCTION

Since 1990, the application of molecular approaches such as positional cloning strategy (case of *Pto* in tomato or *RSP2* in *Arabidopsis thaliana*), transposon tagging (case of *N* in tobacco, *L6* in flax and *Cf-9* in tomato) have made it possible, until today, to clone a considerable number of resistance (*R*) genes. A total of 153 reference [1] disease resistance genes have been stored in the latest release of PRGdb database (PRGdb 3.0; www.prgdb.org; Osuna-Cruz *et al*. 2018), in addition to 177 072 putative candidate Pathogen Recognition Genes (PRGs).

Despite the large diversity of parasites to which they confer resistance (fungi, bacteria, viruses, nematodes, *etc*.), sequence comparison reveals the conservation of many structural motifs involved in the recognition and signal transduction. Based on structural protein domains of these *R* genes products and their cellular localization, five major classes, which will be treated in the current chapter, are recognized according to recent literature (Sanseverino *et al*. 2010; Sekhwal *et al*. 2015).

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1. WHY STUDY *R* GENES?

Plant resistance genes are the subject of several research studies not only because of their fundamental relevance to genetic research, but also because of the serious implications that findings in this area of research can have on humans. Plant improvement for resistance to biotic stresses is a major concern, due to the economic losses that pathogens and pests induce in world agricultural production every year. Pathogens and pests limit the yield and performance of crop plants, and threaten food safety at household, national and global scales. Recently, Savary *et al*. (2019) estimated losses in yields due to 137 pathogens and pests associated with wheat, rice, maize, potato and soybean worldwide. Worlwide estimates of yield losses were 21.5% for wheat, 30% for rice, 22.5% for maize, 17.2% for potato and 21.4% for soybean.

Chemical treatment, as a disease and pest controlling method, continues to be widely applied to date, despite its complete inefficiency against certain microbes (viruses, viroids, and mycoplasma) and, above all, its tremendous economic and environmental harmful effects. In this context, plant breeding focusing on the development of cultivars carrying resistance factors to diseases is emerging as an alternative approach that can cancel or minimize costs due to the application of chemicals during agricultural production and, at the same time, reduce their ecological consequences. It is in this context that there is an urgent need to develop our understanding of the immune function of plants. Considering the significance of this problem and the commitment of many scientists, studies in this field are progressing steadily, given the complex and dynamic relationship involving plants and pathogens.

From the point of view of breeders and basic research, the major resistance genes have a number of advantages, such as ***efficacy*** (at least in the short term), ***clear phenotypes*** and ***simple inheritance***. These advantages have enabled the positional cloning of many *R* genes and almost 20 years after the first successes, we now have many sequences. Knowledge about major *R* genes (including the availability of their sequences) allows to introgress a trait of resistance from one genotype to another by conventional selection or, possibly, by genetic engineering tools.

2. CLASSES OF PLANT DISEASE RESISTANCE GENES BASED ON STRUCTURAL FEATURES

2.1. The Two Classes of Coiled Coil-Nucleotide Binding Site-Leucine Rich Repeat (CNL) and Toll-Interleukin Receptor-Nucleotide Binding Site-Leucine Rich Repeat (TNL)

Proteins of these two classes have a conserved central NBS domain (Nucleotide-

Binding Site), which is the central component of a larger conserved segment called NB-ARC, which is shared by *R* genes in plants, the APAF-1 (Apoptotic protease-activating factor1) in humans and the CED4 protein in the model nematode *Caenorbabditus elegans*[2]. In the C-terminal region, the NB-ARC domain is linked to a variable Leucine-Rich Repeat (LRR) domain, composed of leucine-rich repeated units. A third domain is present in the N-terminal position, which can be either a Coiled-Coil (CC) or a Toll-Interleukin Receptor (TIR) (Pan *et al*. 2000), hence the subdivision into two classes: CC-NBS-LRR (or CNL) and TIR-NBS-LRR (or TNL). For example, the flax *L6* gene that confers resistance to the fungus *Melampsora lini*, encodes a TIR-NBS-LRR (TNL)-type protein (Lawrence *et al*. 1995), whereas the tomato gene *I2*, which provides resistance to race 2 of the ascomycete *Fusarium oxysporum* f. sp. *lycopersici*, the tomato wilt disease agent, encodes a CC-NBS-LRR (CNL)-type protein (Giannakopoulou *et al*. 2015). The NBS-LRR genes are one of the largest families of genes in plants (McHale *et al*. 2006), encoding for cytoplasmic receptors capable of detecting the presence of pathogen avirulence proteins secreted into the cytoplasm[3].

2.2. The two classes of Receptor-Like Protein (RLP) and Receptor-Like Kinase (RLK)[4]

The proteins of the Receptor-Like Proteins (RLP) and Receptor-Like Kinase (RLK) classes have an extracellular LRR domain in the N-terminal region, a transmembrane domain allowing their anchoring to the cell membrane, and a C-terminal domain, which may contain a kinase domain. The RLP class has an intracellular domain, while the RLK class has a C-terminal cytoplasmic Serine/Threonine kinase domain. It is known that the LRR domains, located in the extracellular region, are highly versatile in number of repeated motifs, allowing a very wide range of protein-protein interactions. These include homo- or hetero-dimerization of receptors, in addition to ligand binding.

RLPs are represented, for example, by the family of *Cf* genes conferring race-specific resistance to the biotrophic fungus *Cladosporium fulvum* in tomato (Hammond-Kosack and Jones 1997). The *Xa21* gene is a representative of the RLK class controlling the resistance of rice to *Xanthomonass oryzae* pv. *oryzae* (Song *et al*. 1995).

The **RLK and RLP** R protein classes possess a ***transmembrane domain*** and function primarily as cell-surface sensors for the recognition of pathogenic patterns. It is not the case for the **TNL and CNL** classes, seen above, which usually lack anchoring domains on the membrane, and therefore function mainly as ***cytoplasmic receptors***, directly or indirectly[5] identifying pathogenic molecules, which settle in the host cell after the basal resistance has been defeated.

CHAPTER 14

Strategies and Mechanisms for Plant Resistance Protein Function

Abstract: Given the current constraints to sustainable agricultural production, with increasing crop losses due to plant pests and diseases and climate change, considerable advances are required in crop improvement approaches for enabling durable disease resistance. Interestingly, advances in fundamental understanding of the plant immune system will have far reaching implications for genetic resistance development, appropriate for effective and durable disease control and global sustainable agriculture. In particular, a deeper understanding of the molecular and functional mechanisms of resistance (*R*) genes would make it possible to engineer new resistances for future agriculture. In general, there are currently two main strategies, which include nine recognized molecular mechanisms for *R* genes, most of them (all but one, mechanism 6: executor genes) have been used against various types of biotic stress and tend to be widely applicable among plants.

Keywords: Active/passive loss of susceptibility, Direct/Indirect perception, Executor genes, Extracellular/Intracellular perception, Integrated domain, Resistance mechanism.

INTRODUCTION

Resistance (*R*) genes occupy a frontal position in plant immune responses. In innate immunity (with its two branches: specific and nonspecific), the detection of generic or specific molecules emitted by pathogenic attackers is the first stage of the immune reaction. Overall, we can count ***nine distinct molecular mechanisms*** through which *R* proteins can provide resistance. They are grouped into two general strategies: (I) perception and (II) loss of susceptibility (analyzed in: Kourelis and van der Hoorn 2018).

The ***Perception-based strategy*** comprises three distinct modes: Extracellular perception (Ia); Intracellular perception (Ib); and Executor genes (Ic). Until date, more than 300 *R* genes have been cloned and characterized, with resistance function confirmed in different plant species (Araújo *et al*. 2019). Most *R* genes encode cell surface or intracellular receptors[1].

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The ***Loss-of-susceptibility strategy*** offers long-lasting resistance, but has a high cost to the host plant and hence can result in reduced yields (Kourelis and van der Hoorn 2018). These authors defined three mechanisms for loss-of-susceptibility: active loss of susceptibility *by* ***actively disrupting pathogen processes***; passive loss of susceptibility *through* ***inactivation of pathogen targets*** *inside the host*; and loss of susceptibility *through* ***metabolic reprogramming of the host***.

1. STRATEGY (1): PERCEPTION

1.1. Mode (1.1): Extracellular Perception

In this mode, plant cells recognize different pathogen-associated molecular patterns (PAMPs) by plasma membrane-localized RLKs or RLPs. This recognition may be performed ***directly (mechanism 1)*** or ***indirectly (mechanism 2)***.

a. Mechanism 1: Direct Extracellular Perception

Several PAMPs are immediately sensed by RLKs and RLPs on the cell surface. Bacterial flagellin (Felix *et al*. 1999)[2] is the best studied PAMP in plants. In *Arabidopsis*, flagellin epitope flg22 is recognized directly by the receptor kinase (RLK) FLAGELLIN-SENSITIVE2 (FLS2) (Chinchilla *et al*. 2006). The FLS2 gene is ubiquitously expressed among plants (Gómez-Gómez and Boller 2000). However, plants with mutated FLS2 gene exhibit inadequate interaction with flg22, and are, therefore, vulnerable to infection by pathogenic bacteria (Zipfel *et al*. 2004). A number of additional PAMPs are perceived directly, in a way comparable to flagellin. These comprise EF-Tu[3], peptidoglycan, chitin, lipopolysaccharide, and other elements of the bacterial cell wall (Zipfel 2014).

b. Mechanism 2: Indirect Extracellular Perception

The sensing of pathogen molecules, on the cell surface, may also take place indirectly through the detection of altered host factors. A typical case of this mechanism is the perception of the tomato leaf mold, *Cladosporium fulvum* (syn. *Passalora fulva*) by the tomato *Cf-2* protein. The *Cf-2* tomato gene (Dixon *et al*. 1996) encodes an RLP receptor protein with an extracytoplasmic LRR domain that interacts with an extracellular ligand, but lacks a kinase domain[4]. The *Cf-2* gene product activates resistance to strains of the biotrophic fungus *C. fulvum*, which carry avirulence gene *Avr2* (Dixon *et al*. 1996). After being originally thought to have an exclusive receptor affinity to the fungal pathogen, *C. fulvum*,

Cf-2 was also found to mediate resistance to the root parasitic nematode, *Globodera rostochiensis,* because it is capable of recognizing the nematode effector GrVap1 (Lozano-Torres *et al*. 2012). Both Avr2 and GrVAP1 serve as protease inhibitors that cannot be processed by the cysteine protease Rcr3 and directly interact with it (Rooney *et al*. 2005; Lozano-Torres *et al*. 2012). Therefore, Rcr3 is an additional, intermediate, protein that is explicitly needed for *Cf-2*-dependent disease resistance (Luderer *et al*. 2002). This case is one of the rare (if not the unique) examples of an RLP cell surface receptor (*Cf-2*) acting indirectly for Avr2/GrVap1 perception in the apoplast, *via* a guardee/decoy protein, Rcr3 (van der Hoorn and Kamoun 2008).

1.2. Mode (1.2): Intracellular Perception

Plants are not only able to detect pathogen patterns (PAMPs) outside the cell, but also to detect pathogen effectors inside the cell[5]. The majority of cloned *R* genes encode NLRs that are cytoplasmic receptors. In recent years, the central role of the NLR protein family has increasingly been studied in innate immune responses. The NLR protein family, one of the largest multigene families known in plants (Steuernagel *et al*. 2018), may have originated in green algae (Andolfo *et al*. 2019; Gao *et al*. 2018) and existed in early land plant lineages (Yue *et al*. 2012). These NLRs can recognize effectors ***directly (mechanism 3)***, ***indirectly (mechanism 4)***, or ***through integrated domains (IDs) (mechanism 5)***.

c. Mechanism 3: Direct Intracellular Recognition

The direct sensing of effectors is not limited to the cell surface (as explained above), as several effectors are recognized by intracellular NLRs to initiate defense responses. A number of well-known examples illustrating this mechanism are available in literature. Among these, we can cite:

- The direct recognition and physical interaction between RRS1-R and PopP2, an effector of the bacterial wilt, *Ralstonia solanacearum* type III, in the resistant Nd-1 *Arabidopsis thaliana* ecotype (Deslandes *et al*. 2003).
- The direct recognition and physical interaction between the resistance protein Pi-ta and the effector AVR-Pita, during the interaction between rice, *Oryza sativa*, and the rice blast fungus, *Magnaporthe grisea* (jia *et al*. 2000).
- The direct protein interaction involving R proteins encoded by the polymorphic L locus in flax (*Linum usitatissimum*), on one hand, and Avr proteins in various strains of the flax rust fungus (*Melampsora lini*), on the other hand (Dodds *et al*. 2006).

CHAPTER 15

Signal Transduction Pathways Activated During Plant Resistance to Pathogens

Abstract: The signaling pathways play an indispensable role and act as a connecting link between recognizing the stress molecules and generating an appropriate physiological and biochemical response. Recent studies using genomics and proteomics approach enabled decoding and understanding these signaling networks, which increased our knowledge regarding signaling pathways. This chapter offers a review of the most important bases of plant signaling pathways during various stresses.

Keywords: Abiotic/biotic stress responses, Calcium Signaling, Ethylene, Jasmonic acid (JA), MAPK cascades, Plant signaling pathways, Phytohormone signalling, ROS (Reactive Oxygen Species), Salicylic acid (SA), Signaling cross talk.

INTRODUCTION

Pathogen recognition leads to the execution of intracellular signaling events that contribute to the activation of of the plant's adaptive response (Jones and Dangl 2006). These events may involve a large number of actors. Among the first signaling events detecetd following the perception of a pathogen are ***ion flows*** through the plasma membrane, notably ***influx of*** Ca^{2+} and ***effluxes of*** NO^{3-} ***or*** Cl^- ***then*** K^+. These ion movements trigger a membrane depolarization; and the amplitude and duration of this depolarization depends on the elicitor (Garcia-Brugger *et al*. 2006).

These studies have been, more recently, complemented by the discovery of the participation of certain phytohormones (initially known for their involvement in development) in the implementation of adaptive responses of plants to biotic and abiotic stresses (Pieterse *et al*. 2009). This is the case for ***brassinosteroids***, ***auxins (AUX)***, ***gibberellins (GAs)***, ***cytokinins (CK)*** and ***abscissic acid (ABA)***. These various hormones play a pivotal role in regulating the signaling network of the plant defense system. However, the different pathways specific to each of these

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hormones can be interdependent and, thus, cross-communicated in an antagonistic or synergistic manner, giving the plant with a powerful ability to finely regulate its immune response (Pieterse *et al*. 2009).

1. PHYTOHORMONE SIGNALING

In addition to their involvement in many physiological processes, phytohormones are signaling molecules that regulate plant immunity (Pieterse *et al*. 2012). All phytohormones, including salicylic acid (SA), jasmonic acid (JA), ethylene, abscissic acid (ABA), giberillin (GA), auxin (Aux), cytokinin (CK) and brassinosteroid (BR) participate in plant immunity and form complex signaling networks to coordinate responses to various stresses. Among them, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are the main immune phytohormones, whose major role in resistance to pathogenic microorganisms has been demonstrated since many years (Glazebrook 2005; Lorenzo and Solano 2005; Broekaert *et al*. 2006; Loake and Grant 2007; Balbi and Devoto 2008).

1.1. Salicylic Acid (SA)

Studies carried out on the physiological role of salycilic acid (SA) have demonstrated that this compound has a key role in the establishment of basal resistance (innate immunity), in the execution of RH, and in the establishment of SAR. A large number of mutants or transgenic plants relating to this defense pathway have been characterized. The biosynthesis of SA can be initiated in plants at the level of chloroplasts where two enzymatic pathways using ***chorismate*** as a precursor have been identified (Vlot *et al*. 2009). Studies on tobacco suggested that SA is derived from the phenylpropanoid pathway initiated by phenylalanine ammonia lyase (PAL); it would be synthesized from trans-cinnamic acid and benzoic acid (Shah 2003) in the cytoplasm. Other work has shown that the isochorismate pathway is a major source of SA during SAR in *A. thaliana* (Wildermuth *et al*. 2001).

1.2. Jasmonic Acid (JA) and Ethylene

Two other hormones play an important role in hormone signaling pathways: jasmonic acid (JA) and ethylene (ET). These two hormones often act synergistically when building resistance. The three main components of the JA pathway are coronatin insensitive 1 (COI1), jasmonate resistant 1 (JAR1) and Jasmonate ZIM Domain 1 (JAZ1) (Fonseca *et al*. 2009). COI1 is a protein involved in the degradation of proteins *via* the proteasome 26S, and is necessary

for almost all the responses implemented by the JA. JAR1 encodes a JA amino acid synthetase involved in the synthesis of isoleucine-JA (Ile-JA), a bioactive molecule that can diffuse from cell to cell and induce the expression of genes associated with resistance to necrotrophic pathogens and insects (Staswick and Tiryaki 2004). Ile-JA promotes interaction between COI1 and JAZ1. Finally, JAZ1 is a repressor of the transcription of genes responding to JA (Thines *et al*. 2007), and the interaction between COI1 and JAZ1 leads to the degradation of JAZ1 and to the removal of the inhibition of defense genes.

Ethylene is an important factor in responses to various stresses in plants, such as mechanical injury and infection by pathogens (Guo and Ecker 2004).

2. CALCIUM SIGNALING

Calcium plays a key role in the growth and development of plants. Ca^{2+} signaling has been implicated in various pathways and responds to many extracellular stimuli, such as light, abiotic and biotic stress factors, all causing cellular calcium levels to change.

Two opposing reactions may occur within the plant cell: Ca^{2+} influx through channels or Ca^{2+} efflux through pumps. Actually, the removal of Ca^{2+} from the cytosol against its electrochemical gradient to either the apoplast or the intracellular organelles requires energized 'active' transport. The high level of Ca^{2+} can be recognized by calcium sensors or Ca^{2+} binding proteins, which together can activate protein kinases (Mahajan *et al*. 2006). These activated protein kinases can phosphorylate many regulatory proteins, including transcription factors, which regulate the level of gene expression, resulting in a change in metabolism, followed by a physiological response to stress resistance/tolerance. The physiological response may consist of an inhibition of plant growth or cell death, which will depend on the number and type of genes that are up- or down-regulated in response to high calcium (Tuteja and Mahajan 2007).

Several proteins exist in plants that bind to Ca^{2+} to modulate several processes. For example, we can cite the ***Calcineurin B-like proteins (CBL)*** in *Arabidopsis*, which form a complex with the CIPKs (Albrecht *et al*. 2001; D'Angelo *et al*. 2006). It has been proposed that this property of the CBL/CIPK complex contributes to efficient signal transduction.

Another calcium sensor in plants is ***Calmodulin (CaM)***. It is a highly conserved Ca^{2+} sensor, devoid of intrinsic enzymatic activity and functioning by regulating the activities of its targets (CaM-binding proteins: CaMBP). The accumulated data, in *Arabidopsis* and other organisms, indicate that Ca^{2+}/CaM is involved in

CHAPTER 16

Transcriptional Reprogramming in Plant Defense

Abstract: In order to set up an optimal response to the invading pathogen, the host plant uses transcriptional reprogramming. This phenomenon, involving both DNA-binding transcription factors (TFs) and their regulatory molecules, occurs at several levels of resistance, such as the expression of resistance components (*e.g*. intracellular and membrane receptor proteins), and downstream defense signalling. In this chapter, we address the structure and function of main TF families associated with plant defense against biotic stress, as well as the role played by MAPK cascades and Ca^{2+} signaling in regulating transcriptional complexes during plant-pathogen interactions.

Keywords: BZIP, ERF/DREB, MYB, NAC, Pathogen-triggered cellular responses, Signalling network, Transcription factors, WRKY.

INTRODUCTION

In multicellular organisms, the expression of genes coding for proteins is subject to complex regulation in space (tissue) and time (developmental stage). Such specificity of gene expression is partially attributable to the action of proteins capable of activating or repressing transcription: ***transcription factors (TFs)***. They are generally composed of a DNA-binding domain, a nuclear localization signal (NLS) and a transcription activation domain allowing them to modulate the level of transcription of their target genes. The transcription factor (TF) genes represent an important group targeted for crop improvement. These factors play an active role in the initiation of the transcription process as well as the control of development and response to external stress throughout the life cycle of an organism. They are able to induce a cascade of metabolic pathways, by modifying the transcription profile of a plant cell. These metabolic adjustments help plants react to harsh environmental conditions, since plants are sessile and cannot escape environmental stress.

In particular, several transcription factors are critical players in stress resistance and plant adaptation to external constraints, because they govern the transcription of almost all stress-sensitive genes, by binding to their cis elements.

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1. MAJOR TRANSCRIPTION FACTOR FAMILIES ACTIVE IN PLANT IMMUNITY

Numerous TF families, including WRKY, MYB, NAC, and bZIP (Table **1**), are involved in stress response, and the expression of their coding genes is associated with the enhancement of resistance/tolerance in both crop and model plant systems (Wang *et al*. 2016a; Baillo *et al*. 2019).

Table 1. Summary features of the discussed transcription factor (TF) families.

TF Family	DNA-Binding Domain	*Cis*-acting Element	Structural Features
WRKY	WRKYGQK domain	W-box (TTGACT/C)	• **N-terminus**: WRKY domain of ~60 amino acid residues. • **C-terminus**: Zinc-finger structure ($Cx_{4-5}Cx_{22-23}HxH$ or $Cx_7Cx_{23}HxC$).
NAC	NAC domain	NACRS (TCNACACGCATGT)	• **N-terminus**: NAC domain of 150 amino acids residues. • **C-terminus**: Variable transcription regulatory.
MYB	MYB domain	MYBR (TAACNA/G)	Multiple repeats each of about 52 amino acids, forming a helix–turn–helix (HTH) structure.
ERF/DREB	AP2/ERF domain	GCC box (AGCCGCC) and (TACCGACAT)	A conserved domain of 50-70 aminoacids consisting of three parallel β-sheets and an α-helix.
bZIP	bZIP domain	C-box (GACGTC), A-box (TACGTA), G-box (CACGTG), PB-like (TGAAAA), and GLM (GTGAGTCAT)	A sequence of 60–80 amino acids divided into :1) a conserved 18-amino acids basic region N-x7-R-K-x9 responsible of DNA-binding; and 2) a leucine zipper.

1.1. WRKY Transcription Factors

The family of WRKY transcription factors is distinguished by a strongly conserved 60 amino acid sequence denoted the WRKY domain, which contains a standard WRKYGQK motif, supplemented by a zinc finger motif Cys2His2 or Cys2HisCys (Eulgem *et al*. 2000). WRKY proteins bind specifically to the W-Box element [(T)TGCA(C/T)] (Du and Chen 2000). Seventy-four WRKY transcription factors were reported in *Arabidopsis* (Eulgem *et al*. 2000).

WRKY transcription factors are among the major groups of regulators of gene transcription in plants and are an essential part of the signaling pathways that govern multiple physiological functions in plants. New discoveries show that

WRKY TFs have a role in regulating important plant functions. In addition, the regulation of many apparently different processes may be provided by a single WRKY transcription factor (Lee *et al*. 2018). Signaling and transcriptional regulation mechanisms have been dissected, discovering the interaction of the WRKY with multiple protein collaborators, such as *mitogen-activated protein kinase* (MAPK or MAP kinase), MAPKKs, calmodulin, Histone deacetylase, resistance proteins as well as other WRKY transcription factors (Rushton *et al*. 2010; Banerjee and Roychoudhury 2015).

WRKY factors have been studied in the gene response to diseases in several species such as wheat (Gupta *et al*. 2019), corn (Wei *et al*. 2012b), chickpea (Waqas *et al*. 2019) and rice (Ramamoorthy *et al*. 2008). WRKYs are also involved in the response to abiotic stress (Marè *et al*. 2004). In particular, the factor WRKY70 seems to play a role in the interconnection between SA and JA signaling pathways.

Furthermore, WRKY TFs can form a convergence point in cross-talk of ovelapped pathways of abiotic and biotic stress response. For instance, Lee *et al*. (2018) demonstrated that rice OsWRKY11 can stimulate drought tolerance and resistance to the bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae*, through positively regulating both abiotic and biotic stress-responsive genes.

1.2. NAC Transcription Factors

The NAC family is one of the largest families of plant-specific transcription factors, and the expression of its members is modulated during the development of the plant and its response to biotic and abiotic stresses (Olsen *et al*. 2005). The acronym NAC is derived from NAM (No Apical Meristem), ATAF (*Arabidopsis* Transcription Activation Factor) and CUC2 (Cup-shaped Cotyledon), which are genes that are independently characterized and all contain a NAC domain. Souer *et al*. (1996) described the first NAC gene as linked to the development of the shoot apical meristem and to the determination of the position of the meristems and primordia (first leaves after differentiation of the meristems) in petunia. NAC genes were then identified in all groups of terrestrial plants, halophytes but also in streptophyte green algae, which indicates that the emergence of NAC transcription factors even precedes the advent of land plants (Maugarny-Calès *et al*. 2016; Khedia *et al*. 2018).

Because of their role as transcription factors, NAC proteins regulate the expression of target genes through DNA-binding, either alone or by forming regulatory complexes with other proteins. A typical NAC protein contains, in its N-terminal region, a well-characterized, evolutionarily conserved DNA-binding

CHAPTER 17

Insights into the Role of Epigenetics in Controlling Disease Resistance in Plants

Abstract: Plants are masters of epigenetic regulation. All of the major epigenetic mechanisms known to occur in eukaryotes are used by plants, with the responsible pathways elaborated to a degree that is unsurpassed in other taxa. DNA methylation occurs in plant genomes, in patterns that reflect a balance between enzyme activities that install, maintain, or remove methylation. Histone-modifying enzymes influence epigenetic states in plants and these enzymes are encoded by comparatively large gene families, allowing for diversified as well as overlapping functions. RNA-mediated gene silencing is accomplished using multiple distinct pathways to combat viruses, orchestrate development, and help organize the genome. The interplay between DNA methylation, histone modification, and noncoding RNAs provides plants with a multilayered and robust epigenetic circuitry that has a tangible impact on the control of plant genes conferring resistance to different biotic and abiotic stresses, either directly or indirectly. Eventually, plants with the most suitable epigenome may be subject to selection.

Keywords: Epigenetic control, Epigenetic modifications, Epigenome, MicroRNAs (miRNAs), Non coding small (sRNA), Plant DNA methylation changes, Transgenerational epigenetically acquired resistance, Transposable elements (TEs).

INTRODUCTION

The genetic information encoded by DNA is packaged in a structure called chromatin. All of the changes undergone by chromatin (DNA methylation, post-translational changes in histones) induce changes in the expression of genes transmissible by cell division. The study of the regulatory mechanisms related to the expression of genes has shown that the latter in addition to being under the control of regulatory sequences (involving nucleotide sequences upstream: place of fixation of factors modifying the access to transcriptional machinery), are also dependent on another level of regulation. This additional level is called ***epigenetics*** (Silveira *et al*. 2013). However, epigenetics is a recent discipline, which has been in full development since the 2000s, and studies are still essential

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to estimate the extent of the epigenetic phenomena and their role in the evolution of species.

Particularly in plants, epigenetics would play an important role in the response of the organism, leading to the establishment of an advantageous phenotype which could be permanently fixed to result in an adaptation (Schlichting and Wund 2014; Rey *et al*. 2016). A recent study in *Arabidopsis* has shown the strong link between the DNA methylation profiles in more than 1,000 collections and their adaptation to their original climate (Kawakatsu *et al*. 2016). Another study also showed, this time on oak, that the SNPs found in loci that contained DNA methylation variants (SMP) showed a greater differentiation, which is in agreement with a role DNA methylation in the local adaptation of plants (Platt *et al*. 2015).

Current research aims to better understand ***the share of epigenetic mechanisms within the framework of the theory of evolution***, a subject which is currently controversial (Danchin*et al*. 2011; Miska and Ferguson-Smith 2016). Another very important goal of understanding epigenetic mechanisms is to improve cultivated plants (Rodríguez López and Wilkinson 2015 ; Bilichak and Kovalchuk 2016). Indeed, the ability to fix the memory of stress through generations but also the identification of genes affected during the stress response represent relevant tools for breeders (Rodríguez López and Wilkinson 2015). Moreover, an epigenetic component of complex traits (epiQTL) has been reported in *Arabidopsis* by exploiting the famous epiRIL (Cortijo *et al*. 2014). Some authors suggest to couple epigenetic data with emerging biotechnologies (Transcription Activator-Like Effectors (TALEs) - and nuclease-defective Cas9 (dCas9) -based designed transcription factor systems) to modify the transcription profiles on specific loci in order to generate plants more tolerant of environmental constraints (Moradpour and Abdulah 2019).

1. DNA METHYLATION

DNA methylation reveals common characteristics among plants and animals (Zhang *et al*. 2008; Lister *et al*. 2008; Zemach *et al*. 2010). The presence of methylation in the gene bodies [1] in the two kingdoms indicates that this phenomenon is an ancestral feature common to eukaryotic genomes (Zemach *et al*. 2010). In addition, preferential methylation of exons, in comparison to introns[2], also seems to be an ancestral phenomenon common to animals and plants (Feng *et al*. 2010). Interestingly, methylation in gene bodies shows a correlation with gene expression; for example, in *Arabidopsis*, genes methylated in their gene bodies are expressed constitutively, while genes methylated in the promoter are expressed specifically in certain tissues (Zhang *et al*. 2006). Overall, this

correlation between the level of gene-body methylation and the level of gene expression is parabolic, which means that the genes expressed at an intermediate level are the most methylated (Zhang *et al*. 2006). All these observations indicate a certain role of methylation in gene expression (initiation, elongation, and transcriptional termination or even alternative splicing).

1.1. Reduced DNA Methylation and Defense-Related Genes Priming

In tobacco, the *NtAlix1* gene, which is potentially involved in programmed cell death after attack by the tobacco mosaic virus (TMV), is specifically expressed in hypomethylated *NtMET1* mutants (Wada *et al*. 2004). Likewise, in *Arabidopsis*, DNA methylation has been shown to be an important element in suppressing the development of *Agrobacterium tumefaciens* (Bond and Baulcombe 2015). Also, the *A. thaliana* mutant *nrpe1*, showing a global hypomethylation of DNA, is more resistant to the biotrophic pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) whereas two hyper-methylated mutants were more susceptible to this pathogen. On the other hand, *nrpe1* was more susceptible to the necrotrophic fungus *Plectosphaerella cucumerina*. Conversely, DNA methylation had opposite effects on *ros1* mutant, showing overall hypermethylation of DNA. The latter is more sensitive to *H. arabidopsidis* while it is more resistant to *P. cucumerina* (López Sánchez *et al*. 2016).

There is converging evidence from several recent studies, suggesting that ***reduced DNA methylation increases the responsiveness of the plant immune system*** (Espinas *et al*. 2016). This 'priming' of plant defense allows a faster induction of defense-related genes after pathogen attack, resulting in an increased resistance (Furci *et al*. 2019).

1.2. Plant Methylation Changes During Pathogen Infection

The methylation rate in a plant can be changed by infection with different pathogens. There are multiple examples of ***dynamic changes in DNA methylation*** during pathogen infection. For example, in *Arabidopsis*, infection with *P. syringae* DC3000 induces hypomethylation of the genome (Pavet *et al*. 2006; Hewezi *et al*. 2017). Genome hypomethylation has also been observed in soybean during infection with *Heterodera glycines*[3] (Rambani *et al*. 2015). Similarly, Dowen *et al*. (2012) described numerous stress-induced differentially methylated regions in the ***DNA methylome*** of plants exposed to the biotrophic pathogen *Pseudomonas syringae* pv.*tomato* DC3000 (*Pst*).

CHAPTER 18

Plant Defense Gene Expression and Physiological Response

Abstract: The perception of the pathogen signal by the host specific receptors and its transduction by various signaling pathways culminate in the synthesis and synchronized accumulation of defensive molecules some of which play a structural role while others exercise a direct antimicrobial function. Biochemical mechanisms include, among others, the synthesis of peptides and antimicrobial proteins, hydrolytic enzymes as well as the production of phytoalexins and secondary metabolites with high antimicrobial potential. This chapter provides a synthesis of the remarkable progress made in recent years in terms of understanding the mechanisms applied in the molecular and physiological alterations that occur during the defense response of plants.

Keywords: Defensins, Hypersensible response (HR), Protease inhibitors, Phytoalexins, Pathogenesis-related proteins (PRs), Secondary metabolites.

INTRODUCTION

From their first contact with plants, phytopathogenic microorganisms are confronted with the barriers of constitutive defenses, which prevent the pathogen from penetrating the tissues of the host. When the microorganisms come to the apoplasm[1], the plant triggers more advanced defense mechanisms: perception and recognition of its aggressor, transduction of cellular signals. Finally, the outcome of the interaction between a plant and a pathogen, *i.e*. resistance or disease, will be dependent on the effectiveness of the arsenal of activated defenses. On the other hand, the speed with which the plant's response is expressed is, at this stage, a crucial criterion in the outcome of a plant-pathogen interaction since it will determine the implementation of resistance or the expression of the disease.

1. HYPERSENSIBLE RESPONSE (HR)

HR is one of the most spectacular and effective plant defense mechanisms. It is generally associated with specific host resistance but also with certain types of non-specific resistance. HR is a genetically programmed form of cell death that

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takes place very quickly and is limited to the area infected with the pathogen. Two roles are assigned to this defense response. On the one hand, the death of the plant cells surrounding the pathogen would considerably disturb the development of the latter. In fact, it is found in an environment devoid of nutrients, at an unfavorable pH and humidity, and containing toxic compounds released following the death of cells. On the other hand, the HR would play a role of ***alarm signal***, informing the other parts of the plant of the attack. This signal generated during HR would spread to the whole plant, authorizing the establishment of ***systemic defenses***.

2. ENZYMES AND ENZYME INHIBITORS

Protease inhibitors (PIs) are defensive proteins characterized by their ability to inactivate proteolytic enzymes of endogenous or exogenous origin (Ryan 1990). On the one hand, they protect the plant against uncontrolled endogenous proteolytic activity and, on the other hand, they control the exogenous proteases secreted by herbivorous insects or pathogens. They are subdivided into four categories according to the type of proteases with which they are associated:

a. **Serine Protease Inhibitors**, very widely distributed in plants;
b. **Cysteine Protease Inhibitors**, also abundant in plants;
c. **Metalloprotease Inhibitors**, which are relatively rare in plants;
d. **Acid Protease-specific Inhibitors**, rarely present in plants (Selitrennikoff 2001).

In collaboration with other molecules such as ***phytoalexins***, PIs play an important role in the defensive arsenal of plants (Schimoler-O'Rourke *et al*. 2001). Many studies have focused on the effect of ingestion of PIs on insect survival. All results obtained under experimental conditions converge on the idea that the PIs ingested by insect larvae are ***antimetabolic*** and ***antinutritive*** molecules, which inhibit digestive proteases, thus depriving the pests of the amino acids essential for their growth and development (Zhu-Salzman and Zeng 2015). However, the selection pressure, exerted by the plant, often leads to the establishment in insects and pathogens of various adaptive mechanisms allowing them to overcome the toxic, repulsive or anti-palatable[2] effects of defense molecules (Kessler and Baldwin 2002). The relationships between plants and their aggressors are therefore constantly evolving, which seems to be responsible for biodiversity on earth.

3. DEFENSINS

Plant defensins are small, highly stable, basic, cysteine-rich[3] peptides that are a part of the plant innate immune system. They are termed plant defensins because they are structurally related to defensins found in other types of organism, including humans (Wong *et al*. 2007). The first members of the family of plant defensins were isolated from wheat and barley grains in 1990 (Colilla *et al*. 1990). A query of the UniProt database (www.uniprot.org/) currently reveals publications of 1,929 plant defensins available for review from TrEMBL section and 390 reviewed, manually annotated plant defensins from SwissProt section and that the *Arabidopsis* genome alone contains more than 330 defensin-like (DEFL) proteins [4].

Unlike the insect and mammalian defensins, which are mainly active against bacteria, plant defensins, with some exceptions, do not have antibacterial activity (Stotz *et al* 2009). These exceptions include *Cp-thionin II* from cowpea (Franco *et al*. 2006), *DmAMP1* from *Dahlia merckii*, *CtAMP1* from *Clitoria ternatea*, *ZmESR-6* from maize (Balandín *et al*. 2005), fabatin from broad been (Zhang and Lewis 1997), *SOD2* and *SOD7* from spinach (Segura *et al*. 1998), *MtDef4* and *MtDef5* from *Medicago truncatula* (Sathoff *et al*. 2019), all of them reported to exhibit antibacterial activity against a range of Gram-positive and Gram-negative bacterial pathogens. In contrast, most plant defensins have previously been shown to primarily inhibit the growth of fungal plant pathogens, such as *ZmDEF1* from *Zea mays*, *Psd1* from *Pisum sativum*, *NaD1* from *Nicotiana alata*, *TPP3* from *Solanum lycopersicum*, *BjD* from *Brassica juncea* (Indian mustard), and *MtDef4* and *MtDef5* from *Medicago truncatula*.

Most plant defensins were isolated from plant seeds; for example in radish, defensin proteins represents 0.5% of the total protein in seeds, and the amount of released proteins is sufficient to suppress the fungal growth in the soil (Terras *et al*. 1995). Yet, defensins have also been identified in other tissues from a variety of plants, including leaves, pods, tubers, fruit, roots, bark and floral organs (cited in Stotz *et al* 2009). These peptides have a biotechnological potential as they can be overexpressed in transgenic crops, often resulting in improved resistance to pathogen as was the case in tobacco, tomato, oilseed rape, rice and papaya (cited in Stotz *et al* 2009).

Antifungal plant defensins spectrum of organisms and mode of action: Plant defensins differ considerably in the spectrum of organisms inhibited and modes of action. Plant defensins interactions with fungal-specific components can be active as follows (cited in Sathoff *et al*. 2019):

CHAPTER 19

Contribution of Genomics to the Study of Resistance in Cultivated Plants

Abstract: Nowadays, agricultural genomics, or agrigenomics (the application of genomics in agriculture), continues to drive sustainable productivity and offer solutions to the mounting challenges of feeding the global population. Omic sciences (genomics, transcriptomics, proteomics, metabolomics) open today opportunities to create plants with high yields, independently of biotic and abiotic stresses. Plant genomic research, genome-wide computational tools and association analyses can assist in the identification of Resistance gene analogs (RGAs) from strategic plant species, and the detection of disease resistance QTLs. This chapter summarizes some of the large-scale genomic tools and studies that have clarified the plant – pathogen interactions.

Keywords: Plant Genomic Research, Computational Analyses, Genome-Wide Analyses, Big Biological Data, Agrigenomics, Biotic Stresses, R-gene Analogs (RGAs), NBS-LRR-encoding Genes, TILLING, RNA-seq, GWAS.

INTRODUCTION

The origin of the term genomics is recent since it was proposed by Tom Roderick in 1989 to designate the science having for subject the study of genomes. This new discipline aims to identify all the genes of a living organism. The first sequence of a genome, that of the bacteriophage PhiX174 (5386 bp) was published in 1977 (Sanger *et al*. 1977). Several virus, chloroplast and mitochondrial genomes were then sequenced for the next 20 years.

The advent and improvement of next-generation sequencing (NGS) technology has rapidly expanded the genomic information of numerous organisms and accelerated the generation of ***multiomic*** (genomic, transcriptomic, proteomic and metabolomic) data, leading to a new era of '***big biological data'***.

For plants, in particular, genetic resources are crucial for crop-breeding programs. Rich plant genetic resources have become available. As of March 2020, the genomes of ~ 100 angiosperm species have been completely sequenced and their genome data are hosted in well-constructed customized databases. Most of these species are plants of high economic importance or their wild relatives. Never-

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theless, the number of sequenced plant species (without customized databases) is much higher and is above 230 angiosperms (Chen *et al*. 2018).

A crucial challenge that emerges from genome data availability, resides in structuring (integrating and organizing) these plant omic data and linking them to particular phenotypes, which will contribute significantly to broadening and deepening our understanding of the molecular and genetic mechanisms that underly plant growth and adaptation to surrounding constraints, including abiotic and biotic stresses, therefore efficiently assisting in the breeding programs of major agricultural crops.

1. PLANT GENOMIC RESEARCH

In agriculture, the sequencing of the genomes of the main economic food crops and livestock is a project that has been going on for a long time. In particular, plant genomics was kick-started in 2000, when the common weed, *Arabidopsis thaliana*, was sequenced and promoted to a status of celebrity, as a model species. However, advances in many important plant species were hindered by the complexity of their genomes. It took almost 20 years for most of the genomes of agricultural crops around the world to be sequenced. Almost four years after the launch of an international rice genome sequencing consortium (International Rice Genome Sequencing Project, IRGSP), the rice genome was completely sequenced in 2002 (Goff *et al*. 2002). It is the second plant genome, after that of *A. thaliana* published in 2000. In 2005, an international consortium for the sequencing of the wheat genome (International Wheat Genome Sequencing Consortium, IWGSC) was launched. A draft of this genome was published in November 2012 (Brenchley *et al*. 2012). This research was particularly long to carry out because of the complexity of the wheat genome, comprising between 94,000 and 96,000 genes, five times more than that of humans. Just one year later, Chinese teams published the genomes of two species of spontaneous wheat; *Triticum urartu* (the supposed donor of genome A to cultivated wheat hexaploid) (Ling *et al*. 2013) and *Aegilops tauschii* (the supposed donor of genome D to cultivated wheat hexaploid) (Jia *et al*. 2013). At the time of writing these lines, the latest publication reporting a whole-genome sequence of a plant species is Chen *et al*. (2020) (Epub ahead of print, 20 april 2020) reporting the genome sequences of five cotton (*Gossypium*) allopolyploid species.

Since the genome sequence of the model plant *Arabidopsis thaliana* was published in 2000 (Arabidopsis Genome Initiative 2000), around 100 plant genome sequences have been published (plaBi database; https://www.plabipd.de /portal/web/guest/home1)[1]. To this number, we can add that of plant transcriptome assemblies available. As of March 2020, the One Thousand Plant Transcriptomes

Initiative database (One Thousand Plant Transcriptomes Initiative 2019) (https://sites.google.com/a/ualberta.ca/onekp/) includes >1300 plant transcriptomes[2].

Genomic analysis of an organism makes it possible to understand its cellular physiology, to grasp a very large number of its biological processes and metabolic activities, either by experimental evidence, or by ***analogy with a model system***. The sequencing of complete genomes can also highlight ***gene transfers*** (Quispe-Huamanquispe *et al*. 2017) and allow a better understanding of the coevolutionary mechanisms of virulence and immunity.

Agrigenomics will help us develop new varieties of food crops with better nutritional content and ***better resistance against pathogens***. However, genomics has also a variety of vocations. For example, genomics will help improve the environment by defining how diseases work in forest crops and by using the traits of living organisms to clean up polluted environments. Industrial biotechnology will continue to use genomics (and proteomics) to create many useful products, such as biofuels, antibodies, vaccines, plastics and biodegradable cosmetics.

2. FROM PLANT GENOMES TO PLANT PHENOTYPES: THE ANNOTATION OF PLANT GENOMES AS A FIRST STEP INTO THE IMPROVEMENT OF PLANTS FOR RESISTANCE TO BIOTIC STRESSES

Next-generation sequencing has triggered an explosion of available genomic and transcriptomic resources in plant sciences. However, whole genome sequencing is still only the first step to identifying the function of genes present in the genome of a plant species. Other tools are necessary for the precise identification of the function of these genes, including the functional annotation of genes.

Especially regarding plant disease resistance genes, pinpointing the mechanism and causes of disease requires more effort than sequencing a specific genome. For that, bioinformatics and computing technology is helping to unravel the coding DNA and predict gene sites in the genome as well as gene function based on similarity to other deposited sequences, which should provide many insights. However, similarity does not always translate into equivalent function especially when comparing across distant taxa. Furthermore, large portions of sequenced genomes in plants have not yet been assigned a putative function based on homology to known proteins.

CHAPTER 20

State of the Art and Perspectives of Genetic Engineering of Plant Resistance to Diseases

Abstract: The improvement of plants, in order to give them better resistance to diseases, relies on the existence of diverse natural populations. Traditionally, the breeder's role has been to crossbreed populations to obtain varieties possessing the desired traits. Modern advances in genetic engineering, in association with omic sciences, allow breeders to increase the genetic diversity of the populations on which selection operates, and to introgress new traits using various molecular methods, such as chemical or irradiation-based mutagenesis, genetic transformation or genome editing. It is most often a question of modifying existing varieties in order to obtain new ones which have the properties desired by researchers, according to the needs expressed by the various actors concerned. Here, we review the increasing usefulness and applicability of biotechnology and genetic engineering approaches for accelerating variety development and crop improvement.

Keywords: Bacterial Harpins (*hrp*) genes, *Bacillus thuringiensis* (*Bt*) delta-endotoxin genes, Biotechnology, Crop improvement, CRISPR-Cas9, Genetic Engineering, Genetically engineered crops, Plant Protease Inhibitors (PI), Plant genome editing, RNA-based antiviral resistance.

INTRODUCTION

Conventional breeding (based on intervarietal crosses) plays an essential role in crop improvement but generally involves the examination of large populations of crops over several generations, which is a long and laborious process. Genetic engineering, which refers to the direct modification of an organism's genetic material, using biotechnology, offers several advantages over conventional breeding. First, crops with the desired agronomic characteristics can be obtained in fewer generations than conventional breeding. Second, the genetic material that can be exploited for the introgression, deletion, modification or regulation of genes of specific interest is not limited to the genes available within the target species, because genetic engineering allows the transfer of genetic material between distinct species, even between distant or belonging to different kingdoms. Finally, genetic engineering makes it possible to improve vegetatively

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propagated plants, such as the banana tree (*Musa* sp.) and potato (*Solanum tuberosum*). These characteristics make genetic engineering a powerful tool for improving resistance to plant pathogens.

In addition, the induction of mutations in plants is made possible by two main methods, namely irradiation and treatment with chemical mutagens (Leitao 2011). The most commonly used chemical mutagens cause, almost strictly, single base substitutions. The main benefit of the chemical mutagens is that they can be used to prepare mutant populations with high mutation levels, which facilitates the detection of specific mutations in a population (Szarejko *et al*. 2017). A major change in the use of mutants occurred following development of effective TILLING [1] (Targeting Induced Local Lesions in Genomes) techniques (McCallum *et al*. 2000). Actually, TILLING made reverse genetics approaches pertinent, because this technique is intended to detect mutations in specific, known genes. This now makes it easier to find mutations in any pre-defined gene from across the genome of a crop plant, if the DNA sequence of the gene is identified and if an appropriate mutant population is accessible (Jankowicz-Cieslak *et al*. 2017).

All these techniques set a new scene for assessing the precision of new breeding techniques.

1. GENETICALLY ENGINEERED CROPS

The first transgenic plant was produced in 1983, leading to a considerable ***paradigm shift*** [2]: the desired agronomic trait may no longer be selected but ***introduced*** by molecular techniques. The individual produced by transgenesis is called a genetically modified plant (GMP). GMPs are produced by the introduction of DNA fragments carrying the sequence corresponding to the gene encoding the desired trait. The gene can come from the plant, animal or microorganism kingdoms. It can even, depending on the case, be of synthetic origin.

1.1. Broad-Spectrum Resistance Conferred By PRR and Chimeric PRR Transgenes

It has been suggested that the PRR-mediated recognition of PAMPs can be used, in resistance engineering, to confer a ***wider spectrum of disease resistance*** to transgenic plants (Borras-Hidalgo *et al*. 2012). For instance, the *Arabidopsis* EFR, which recognizes the bacterial elongation factor EF-Tu, has been shown to confer resistance against a number of bacteria when transferred into Solanaceae species

(Lacombe *et al*. 2010). It has also been demonstrated that chimeric PRRs may be used to develop a double resistance to both bacteria and fungi (Brutus *et al*. 2010). The combination of different PRRs and chimeric PRRs in a single plant, which recognize several non-self structures, is likely to be the best way to build broad-spectrum and more durable disease resistances.

1.2. Plant Defensins Transformed into Target Plants

Plant defensins have a biotechnological potential because they can be overexpressed in genetically egineered crops, often resulting in enhanced resistance to pathogens as was the case in tobacco, tomato, oilseed rape, rice and papaya (cited in Stotz *et al*. 2009).

Jha and Chattoo (2010) transformed the peptide Rs-AFP2[3] into rice (*Oryza sativa* L. cv. Pusa Basmati). The transgenic plants were tested *in vivo* and *in vitro* against *Magnaporthe oryzae* and *Rhizoctonia solani*, the main causes of rice losses in agriculture, revealing that overexpression of Rs-AFP2 can control the rice blast and sheath blight diseases (Jha and Chattoo 2010). Another example is the transformation of tobacco with the mustard defensin, BjD, which once more validated the potential of these peptide-family members as excellent antifungal agents, as transgenic plants displayed improved resistance towards *F. moniliforme* and *Phytophtora parasitica* (Anuradha *et al*. 2008). More recently, a defensin purified from maize, ZmDEF1, when transformed into tobacco plants, showed increased tolerance against *Phytophtora parasitica* (Wang *et al*. 2011).

1.3. Plant Protease Inhibitors (PI) Transformed into Target Plants

Plant protease inhibitors (PI) are able to protect plants against insect attacks by interfering with the proteolytic activity of insects' digestive gut. This mechanism contributes to defenses against many herbivorous arthropods and microbial pests (Rustgi *et al*. 2017). In particular, serine and cysteine PIs are abundant in plant seeds and storage tissues (Reeck *et al*. 1997) and may contribute to their natural defense system against insect predation. The first PI gene that was successfully transferred artificially to plant species resulting in enhanced insect resistance was isolated from cowpea and encoded the trypsin/trypsin inhibitor CpTI (Cowpea Trypsin Inhibitor) (Hilder *et al*. 1987). Oryzacystatin 1 (OC1) is a well-studied cysteine PI from rice seeds which has been successfully introduced into several different crops like rice (Duan *et al*. 1996), wheat (Altpeter *et al*. 1999), oilseed rape (Rahbe *et al*. 2003) and eggplant (Ribeiro *et al*. 2006). It protects these plant species against beetle attacks and, in some cases, aphids (Sharma *et al*. 2004).

CHAPTER 21

Durability of Plant Resistance to Pathogens and Pests

Abstract: The methods of management of pathogens and pests have been changing during these years, under the pressure of current societal and political demands. To overcome the drawbacks of chemical control, it is possible to mobilize genetic (*i.e*. varieties resistant to diseases) and agronomic controlling methods (cultural practices favoring these resistances or reducing the risks of pressure or development of pests). However, the low durability of genetic resistance, which is linked to the adaptation of pathogens, imposes the need to propose solutions in order to improve the durability of genetic resistance. Resistance is said to be durable when its effectiveness lasts for many years in a large spatial environment, at high pressure from the pathogen, favoring, *a priori*, the selection of virulent variants. The combination of quantitative and qualitative resistance is among the best solutions, but the strategy for deploying the *R* genes is an interesting track to follow.

Keywords: Durability of plant resistance to pathogens, Gene pyramiding, Gene rotation, Quantitative resistance, Qualitative resistance, *R*-gene deployment strategy.

INTRODUCTION

The different attack and defense strategies implemented by plants and their pathogens result from a long co-evolution between interacting organisms and can be described according to the concept of the ***arms race***. Indeed, ***in natural ecosystems***, a population of pathogens having acquired the capacity to bypass the basal defenses of a given plant will be able to colonize it.

In standardized agrosystems, the slow coevolution between plants and their pathogens is greatly accelerated through agricultural practices. In fact, breeders generally favor the introgression of major resistance genes into varieties. However, these are cultivated within large homogeneous plots, with a low crop rotation. The selection pressure exerted on pathogens by cultivated plants is therefore much stronger than in natural ecosystems. Under these conditions, microorganisms have the capacity to adapt quickly to the selection pressures to which they are subjected. Numerous cases of monogenic resistance breakdown

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have been reported, among which we can cite the cases of powdery mildew resistance in cereals (Hovmøller *et al*. 2000), grapevine downy mildew resistance in *Vitis vinifera* (Peressotti *et al*. 2010), and tomato mosaic virus (ToMV) resistance in tomato (Lanfermeijer *et al*. 2005).

This permanent coevolution as well as the rapid adaptive molecular changes that it implies in pathogens, imposes to the plant breeder (geneticist) a ***race against time*** to constantly identify new sources of resistance. The transfer of efficient genes, into commercial cultivars, has been accelerated, thanks to molecular and genetic engineering technologies.

The question of the durability of resistance can be considered as a problem of adaptive response in populations of pathogens to the selection exercised by resistant hosts (McDonald and Linde 2002). Strategies to maximize sustainability should therefore both limit the selection of virulent pathogen genotypes and reduce the size of the pathogen populations (Mundt *et al*. 2002).

1. MECHANISMS FOR OVERCOMING SPECIFIC RESISTANCE

Durability of resistance is defined as the time between the introduction of the cultivar into the landscape and the time when the frequency of virulent pathotypes reaches a predefined threshold (van den Bosch and Gilligan 2003). When a resistance has been overcome, it loses its effectiveness following pathogen adaptation. Such an adaptation of pathogenic populations to the resistance gene, found in host plants, occurs when individuals virulent for this resistance become progressively more frequent. In other words, there is evolution of the genetic structure of pathogen populations due to selection forces, resulting in the resistance gene then becoming ineffective.

The circumvention of a specific resistance is dependent on the size and the genetic structure of the pathogen population, as well as on the pathogen biological characteristics such as its regime of reproduction. The parasite's ability to evolve is governed by the five classic evolutionary forces: mutation, genetic drift, migration, recombination and selection (McDonald and Linde 2002). Mutation and sexual recombination ***generate genetic diversity (new alleles)*** in pathogenic populations. In particular, the mutation is directly responsible for overcoming specific resistances. Genetic drift, selection and migration influence the ***distribution of genetic diversity (change in allele frequencies)*** (Table **1**).

Table 1. Evolutionary forces involved in the adaptation of pathogens (adapted from Hossard *et al*. 2010).

Evolutionary Force	Mechanism of Action	Consequence
Mutation	(Random) change in the nucleotide sequence of certain genes	Birth of new alleles in the population
Genetic drift	Random fluctuation in the frequency of alleles in a population of limited numbers	Allele extinction or fixation
Migration	Flow of genes or genotypes between populations of a pathogen	Movement of virulent mutant genotypes between populations
Sexual recombination	Reassortment of alleles (*i.e*. Modification of allele associations)	Modification of global (multilocus) genetic diversity in the pathogen population
Selection	Higher or lower reproduction of a given genotype	Selection of the most successful pathogens

Interactions among these five evolutionary forces ultimately determine the genetic structure, and hence the evolutionary potential, of pathogen populations. These interactions can be explained through the following examples:

a. ***Example 1, interaction between mutation and selection:*** Mutation may produce mutant alleles at the avirulence locus of the pathogen resulting in a few virulent individuals, but if the plant doesn't exert a selection pressure on wild-type susceptible pathogen individuals, by means of its plant receptor (*R*-gene), then the virulent pathogen genotypes may never increase to a detectable frequency.

b. ***Example 2, interaction between mutation/selection and migration:*** Virulent pathogen mutants that originate and increase in frequency in a field containing a resistant cultivar may never cause a widespread epidemic if gene flow among fields is very low as a result of an effective quarantine.

c. ***Example 3, interaction between mutation/selection/migration and genetic drift:*** Highly virulent genotypes that are distributed over long distances may never become established in some locations because they experience extinctions as a result of genetic drift.

Nevertheless, according to McDonald and Linde (2002), ***selection remains the main evolutionary force***, which leads to overcoming resistance, *via* an increase in the frequency of mutant alleles, once the mutation for virulence has appeared. This selection of virulent pathotypes results in cycles of ***boom and bust*** (explosion and extinction). The cycle begins with the large-scale deployment of a

References

Abbas, MST (2018) Genetically engineered (modified) crops (*Bacillus thuringiensis* crops) and the world controversy on their safety. *Egypt J Biol Pest Control,* 28, 52.
[http://dx.doi.org/10.1186/s41938-018-0051-2]

Acevedo-Garcia, J, Kusch, S & Panstruga, R (2014) Magical mystery tour: MLO proteins in plant immunity and beyond. *New Phytol,* 204, 273-81.
[http://dx.doi.org/10.1111/nph.12889]

Agrawal, GK, Rakwal, R, Jwa, NS & Agrawal, VP (2001) Signalling molecules and blast pathogen attack activates rice *OsPR1a* and *OsPR1b* genes: A model illustrating components participating during defense/stress response. *Plant Physiol Biochem,* 39, 1095-103.
[http://dx.doi.org/10.1016/S0981-9428(01)01333-X]

Aharoni, A & Vorst, O (2002) DNA microarrays for functional plant genomics. *Plant Mol Biol,* 48, 99-118.
[http://dx.doi.org/10.1023/a:1013734019946]

Albrecht, V, Ritz, O, Linder, S, Harter, K & Kudla, J (2001) The NAF domain defines a novel protein-protein interaction module conserved in Ca^{2+}-regulated kinases. *EMBO J,* 20, 1051-63.
[http://dx.doi.org/10.1093/emboj/20.5.1051]

Ali, S, Ganai, BA, Kamili, AN, Bhat, AA, Mir, ZA, Bhat, JA, Tyagi, A, Islam, ST, Mushtaq, M, Yadav, P, Rawat, S & Grovera, A (2018) Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiol Res,* 212–213, 29-37.
[http://dx.doi.org/10.1016/j.micres.2018.04.008]

Ali, Z, Abul-faraj, A, Li, L, Ghosh, N, Piatek, M, Mahjoub, A, Aouida, M, Piatek, A, Baltes, NJ, Voytas, DF, Dinesh-Kumar, S & Mahfouz, MM (2015) Efficient Virus-Mediated Genome Editing in Plants Using the CRISPR/Cas9 System. *Mol Plant,* 8, 1288-91.
[http://dx.doi.org/10.1016/j.molp.2015.02.011]

Altpeter, F, Diaz, I, McAuslane, H, Gaddour, K, Carbonero, P & Vasil, IK (1999) Increased insect resistance in transgenic wheat stably expressing trypsin inhibitor CMe. *Mol Breed,* 5, 53-63.
[http://dx.doi.org/10.1023/A:1009659911798]

Alves, MS, Soares, ZG, Vidigal, PMP, Barros, EG, Poddanosqui, AMP, Aoyagi, LN, Abdelnoor, RV, Marcelino-Gumaraes, FC & Fietto, LG (2015) Differential expression of four soybean bZIP genes during *Phakopsora pachyrhizi* infection. *Funct Integr Genomics,* 15, 685-96.
[http://dx.doi.org/10.1007/s10142-015-0445-0]

Anderson, CM, Wagner, TA, Perret, M, He, ZH, He, D & Kohorn, BD (2001) WAKs: cell wall-associated kinases linking the cytoplasm to the extracellular matrix. *Plant Mol Biol,* 47, 197-206.

Andolfo, G, Donato, AD, Chiaiese, P, Natale, AD, Pollio, A, Jones, JDG, Frusciante, L & Ercolano, MR (2019) Alien domains shaped the modular structure of plant NLR proteins. *Genome Biol Evol,* 11, 3466-77.
[http://dx.doi.org/10.1093/gbe/evz248]

Andreu, AB, Guevara, MG, Wolski, EA, Daleo, GR & Caldiz, DO (2006) Enhancement of natural disease resistance in potatoes by chemicals. *Pest Manag Sci,* 62, 162-70.
[http://dx.doi.org/10.1002/ps.1142]

Anuradha, TS, Divya, EK, Jami, ESK & Kirti, EPB (2008) Transgenic tobacco and peanut plants expressing a mustard defensin show resistance to fungal pathogens. *Plant Cell Rep,* 27, 1777-86.
[http://dx.doi.org/10.1007/s00299-008-0596-8]

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature,* 408, 796-815.
[http://dx.doi.org/10.1038/35048692]

Araújo, ACde, Fonseca, FCDA, Cotta, MG, Alves, GSC & Miller, RNG (2019) Plant NLR receptor proteins and their potential in the development of durable genetic resistance to biotic stresses. *Biotechnology Research and Innovation,* 3, 80-94.
[http://dx.doi.org/10.1016/j.biori.2020.01.002]

Arruda, RL, Paz, ATS & Bara, MTF (2016) An approach on phytoalexins: function, characterization and biosynthesis in plants of the family Poaceae. *Ciência Rural, Santa Maria,* 46, 1206-16.
[http://dx.doi.org/10.1590/0103-8478cr20151164]

Arya, P, Kumar, G, Acharya, V & Singh, AK (2014) Genome-Wide Identification and Expression Analysis of NBS-Encoding Genes in *Malus x domestica* and expansion of NBS genes family in Rosaceae. *PLoS One,* 9, e107987.
[http://dx.doi.org/10.1371/journal.pone.0107987]

Asai, T, Tena, G, Plotnikova, J, Willmann, MR & Chiu, W-L (2002) MAP Kinase Signalling Cascade in Arabidopsis Innate Immunity. *Nature,* 415, 977-83.
[http://dx.doi.org/10.1038/415977a]

Ashfield, T, Egan, AN & Pfeil, BE (2012) Evolution of a Complex Disease Resistance Gene Cluster in Diploid Phaseolus and Tetraploid Glycine. *Plant Physiol,* 159, 336-54.
[http://dx.doi.org/10.1104/pp.112.195040]

Ashrafi, H, Hill, T, Stoffel, K, Kozik, A, Yao, J, Chin-Wo, SR & Van Deynze, A (2012) *De novo* assembly of the pepper transcriptome (*Capsicum annuum*): a benchmark for *in silico* discovery of SNPs, SSRs and candidate genes. *BMC Genomics,* 13, 571.
[http://dx.doi.org/10.1186/1471-2164-13-571]

Atkinson, NJ (2011) Plant molecular response to combined drought and nematode stress. PhD thesis, University of Leeds. http://etheses.whiterose.ac.uk/2131/

Atkinson, NJ, Dew, TP, Orfila, C & Urwin, PE (2011) Influence of Combined Biotic and Abiotic Stress on Nutritional Quality Parameters in Tomato (*Solanum Lycopersicum*). *J Agric Food Chem,* 59, 9673-82.
[http://dx.doi.org/10.1021/jf202081t]

Aylward, J, Steenkamp, ET, Dreyer, LL, Roets, F, Wingfield, BD & Wingfield, MJ (2017) A plant pathology perspective of fungal genome sequencing. *IMA Fungus,* 8, 1-15.
[http://dx.doi.org/10.5598/imafungus.2017.08.01.01]

Aziz, A, Poinssot, B, Daire, X, Adrian, M, Bézier, A, Lambert, B & Joubert, JM (2003) Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola. Mol Plant Microbe Interact,* 16, 1118-28.
[http://dx.doi.org/10.1094/MPMI.2003.16.12.1118]

Baggs, E, Dagdas, G & Krasileva, K (2017) NLR diversity, helpers and integrated domains: making sense of the NLR IDentity. *Curr Opin Plant Biol,* 38, 59-67.

[http://dx.doi.org/10.1016/j.pbi.2017.04.012]

Bai, Y, Kissoudis, C, Yan, Z, Visser, RGF & van der Linden, G (2018) Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. *Plant J,* 93, 781-93.
[http://dx.doi.org/10.1111/tpj.13800]

Baillo, EH, Kimotho, RN, Zhang, Z & Xu, P (2019) Transcription Factors Associated with Abiotic and Biotic Stress Tolerance and Their Potential for Crops Improvement. *Genes (Basel),* 10, 771.
[http://dx.doi.org/10.3390/genes10100771]

Baker, RJ & Briggs, KG (1984) Comparison of grain-yield of uniblends and biblends of 10 spring barley cultivars. *Crop Sci,* 24, 85-7.

Bakker, PAHM, Doornbos, RF, Zamioudis, C, Berendsen, RL & Pieterse, CMJ (2013) Induced Systemic Resistance and the Rhizosphere Microbiome. *Plant Pathol J,* 29, 136-43.
[http://dx.doi.org/10.5423/PPJ.SI.07.2012.0111]

Balagué, C, Lin, B, Alcon, C, Flottes, G, Malmström, S, Köhler, C, Neuhaus, G, Pelletier, G, Gaymard, F & Roby, D (2003) HLM1, an Essential Signaling Component in the Hypersensitive Response, Is a Member of the Cyclic Nucleotide-Gated Channel Ion Channel Family. *Plant Cell,* 15, 365-79.
[http://dx.doi.org/10.1105/tpc.006999]

Balandín, M, Royo, J, Gómez, E, Muniz, LM, Molina, A & Hueros, G (2005) A protective role for the embryo surrounding region of the maize endosperm, as evidenced by the characterisation of *ZmESR*-6, a defensin gene specifically expressed in this region. *Plant Mol Biol,* 58, 269-82.
[http://dx.doi.org/10.1007/s11103-005-3479-1]

Balbi, V & Devoto, A (2008) Jasmonate signalling network in *Arabidopsis thaliana*: crucial regulatory nodes and new physiological scenarios. *New Phytol,* 177, 301-18.
[http://dx.doi.org/10.1111/j.1469-8137.2007.02292.x]

Ballini, E, Morel, JB, Droc, G, Price, A, Courtois, B, Notteghem, JL & Tharreau, D (2008) A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol Plant Microbe Interact,* 21, 859-68.
[http://dx.doi.org/10.1094/mpmi-21-7-0859]

Banerjee, A & Roychoudhury, A (2015) WRKY Proteins: Signaling and Regulation of Expression during Abiotic Stress Responses. *ScientificWorldJournal,* 807560
[http://dx.doi.org/10.1155/2015/807560]

Barbary, A, Djian-Caporalino, C, Marteu, N, Fazari, A & Caromel, B (2016) Plant genetic background increasing the efficiency and durability of major resistance genes to root-knot nematodes can be resolved into a few resistance QTLs. *Front Plant Sci,* 7, 56-64.
[http://dx.doi.org/10.3389/fpls.2016.00632]

Barka, EA, Eullaffroy, P, Clément, C & Vernet, G (2004) Chitosan Improves Development, and Protects *Vitis Vinifera* L. Against *Botrytis Cinerea. Plant Cell Rep,* 22, 608-14.
[http://dx.doi.org/10.1007/s00299-003-0733-3]

Barreto-Bergter, E, Sassaki, GL & de Souza, LM (2011) Structural analysis of fungal cerebrosides. *Front Microbiol,* 2, 239.
[http://dx.doi.org/10.3389/fmicb.2011.00239]

Barrett, LG, Kniskern, JM, Bodenhausen, N, Zhang, W & Bergelson, J (2009) Continua of specificity and virulence in plant host–pathogen interactions: Causes and consequences. *New Phytol,* 183, 513-29.

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