

P. Narayanasamy



**Molecular Biology in Plant
Pathogenesis and Disease
Management:
Disease Development**

Volume 2



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Molecular Biology in Plant Pathogenesis
and Disease Management

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Disease Development

Volume 2

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*Dedicated to the Memory
of My Parents
for their Love and Affection*

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Preface

The discovery of the structure of DNA followed by the introduction of recombinant DNA technology has provided significant impetus to the applications of molecular techniques widely in biological sciences including plant pathology. Molecular techniques have been very useful in understanding the phenomenon of plant pathogenesis (disease development) and for elucidating the intricacies of the interactions between microbial plant pathogens and the host plants at cellular and molecular levels. Molecular genetic tools allow the identification and functional analysis of genes involved in the interplay of pathogens and their host plants.

The interaction of products of pathogenicity genes and host defense genes determine the progress of pathogenesis either leading to development of disease in susceptible plants or disease suppression in resistant plants that can perceive the presence of pathogen and activate their defense genes that encode various antimicrobial compounds or form formidable barriers arresting the invasion of host tissues by the pathogen concerned. Successful pathogens have mechanisms that specifically counteract and dismantle plant defense components, resulting in avoidance of host's surveillance system, prevention of activation of plant defenses and development of pathways to suppress manifestation of host defense responses. Various kinds of effector proteins secreted by fungal and bacterial pathogens create conditions favoring pathogen colonization of host tissues to varying degrees. On the other hand, viral pathogens even with such small genomes that carry information for the synthesis of viral nucleic acid and other structural components, are able to deplete efficiently the resources of infected plants to their advantage. Enormous genetic data required for epidemiological analysis for studying the patterns of disease occurrence and development in populations of plants under natural conditions have been gathered using molecular techniques. By determining pathogen gene frequencies, changes within and among populations, as a result of both natural selection of genes and population increases and spread, could be assessed. Molecular epidemiological approaches have been applied for tracking disease outbreaks, for development of prediction models to determine the sources of inoculum and for understanding evolution of pathogen virulence. It may be expected that novel methods of managing crop diseases more effectively may become available, by studying the signal pathways involved in plant pathogenesis and determining the vulnerable genetic targets for intervention in the life cycles of plant pathogens.

This book presents updated and comprehensive information in an easily understandable style, on the molecular biology of plant-pathogen interactions in three volumes: (1) Microbial plant pathogens, (2) Molecular biology of plant disease development and (3) Molecular biology in crop disease management. The usefulness and effectiveness of molecular techniques to establish the identity of pathogens precisely, to have a better understanding of the intricacies of the success or failure of pathogen infection respectively in compatible and incompatible plant species and to develop more effective crop disease management systems is highlighted with suitable examples. Appendices containing protocols included in appropriate chapters will be useful for students, teachers and researchers of various departments offering courses and pursuing research programs in molecular biology in general and plant pathology in particular.

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Chapter 1

Introduction

When the microbial pathogens reach an appropriate host plant species and if favorable environmental conditions prevail, they develop rapidly resulting in the derangement of various physiological functions of the host, followed by the development of characteristic macroscopic symptoms. The pathogen suppresses innate natural disease resistance (NDR) mechanisms by producing various enzymes or toxic metabolites resulting in tissue necrosis or dissolution of cell walls releasing the cell contents that may serve as nutrient source. The fungal physiology was the subject of great interest during the first half of the 20th century. Although several enzymes produced by fungal pathogens were described, their role in pathogenesis and virulence of the pathogen could not be established for most of the enzymes using physiological and biochemical techniques. The discovery of the structure of DNA by Watson and Crick (1953) was hailed as the momentous achievement of the last century in biological sciences. The introduction of recombinant DNA technology later, was considered as an important milestone leading to breath-taking accomplishments in molecular genetics. The effectiveness of molecular cloning and other molecular approaches to address the basic questions regarding plant pathogenesis was demonstrated by different researchers during the past three decades. The dramatic revelation of the process of infection by *Agrobacterium tumefaciens* leading to production of tumors in plants has been the torch bearer lighting the path for the others attempting to understand the intricacies of interactions, at cellular and molecular levels, between fungal, bacterial and viral pathogens and their host plants. Applications of various molecular techniques that have widened information base for studying different phases of disease development in individual plants in vitro and the factors influencing disease incidence on populations of plants and spread in vivo are discussed in two chapters of the volume 2.

1.1 Disease Development in Individual Plants

Molecular genetic tools have allowed the identification and detailed functional analysis of genes involved in the interplay of microbial plant pathogens and their host plants. A pathogen has to overcome a series of barriers during different phases of

pathogenesis. The involvement of conserved signal networks in disease initiation by microbial pathogens with contrasting infection strategies in different host plant species has been indicated (Lee et al. 2003; Talbot 2004). Different genes are involved in signal events which accompany and control all stages of infection and colonization processes.

The fungal pathogenicity genes necessary for disease development may not be essential for the completion of pathogen life cycle. The success of a pathogen in initiating infection depends primarily on its ability to perceive and respond to signals generated by the plant especially in the early stages of infection (recognition) and also at later stages involving different types of cells and tissues. Identification of pathogenicity determinants with certainty has not been possible in several pathogen–host interactions, because of functional redundancy of genes thought to be involved in pathogenesis (Gold et al. 2001; Yoder and Turgeon 2001). The fungal pathogens appear to have broadly two types of mechanisms and the associated genes that specifically counteract and dismantle plant defense components and mechanisms that assist the pathogens to avoid or suppress activated plant defenses. The effects of preformed antimicrobial compounds (phytoanticipins) and compounds that are synthesized postinfectiously (phytoalexins) have to be countered by the invading pathogen. The presence or absence of saponins has been correlated with disease development, as in the interaction of *Gaeumannomyces graminis* with oat and wheat. The mutant strains (*ava1*) are unable to detoxify avenacin and hence they cannot infect oat producing avenacin A, while they were pathogenic on wheat which does not naturally produce saponins (Papadopoulou et al. 1999). The pathogenesis-related (PR) proteins are both preformed and induced antifungal proteins that are suggested to play a role in providing protection by direct toxicity to the invading fungal pathogen and also by release of fungal elicitors that can potentially activate plant defenses (Selitrennikoff 2001).

Successful fungal pathogens seem to have evolved mechanisms that help them avoiding plant detection system and preventing induction of plant defenses or they may develop pathways to suppress the manifestation of these responses. The *Phytophthora* GIPs have been indicated to prevent the release of fungal elicitors and consequently prevent the activation of plant defense systems (Rose et al. 2002). Another group of genes expressed in planta are involved in the establishment and maintenance of infection and they are not directly associated with acquisition of nutrients from the host plant. The genes *CLTA1* in *Colletotrichum* spp. (Dufrense et al. 2000) and *CIH1* in *C. lindemuthianum* (Perfect et al. 2000) have been reported to prevent elicitation of host defenses.

Bacterial pathogens employ diverse strategies to undermine plant defenses and target core components of plant resistance such as hypersensitive response (HR)-based programmed cell death (PCD), cell wall-based defenses, jasmonic acid (JA) signaling and the expression of defense genes (Abramovitch and Martin 2004). The type III secretion system (TTSS) is an essential virulence system employed by many Gram-negative bacterial pathogens to inject the effector proteins into host cells. The TTSS is important to pathogenicity of bacterial pathogens belonging to the genera *Pseudomonas*, *Xanthomonas*, *Ralstonia*, *Erwinia* and *Pantoea*. These pathogens

colonize the intercellular spaces (apoplast) of plants and are generally capable of eliciting plant cell death at some stage of disease development. As most of them are host-specific, they exhibit compatibility with the plant hosts. The TTSS pathway is encoded by the *hrp* and *hrc* genes. Some of the effector proteins possess double functions by enhancing virulence in compatible interactions and also by acting as avirulence genes the products of which elicit defense-related responses in incompatible interactions (Alfano and Collmer 2004).

Plant viruses have comparatively small genomes. Nevertheless, they adopt effective strategies to infect the plant hosts as severely as the bacterial and fungal pathogens. In addition, the viral pathogen can also infect their natural vectors (exhibiting propagative relationship) involved in the transmission of viruses from plant to plant. There are no detectable physiological functions such as respiration in viruses. But they are able to bring the host's synthetic machinery under their control by effectively suppressing the defense mechanisms. Plant virus genomes carry genes for synthesis of coat protein, movement protein, replicase and virus-coded nonstructural proteins such as HC-Pro. It is intriguing and equally amazing to note that with such small genomes, the viruses deplete efficiently the resources of infected plants to their advantage to reach high titers and induce various kinds of external and internal symptoms characteristic of virus infection (Narayananamy and Doraiswamy 2003).

1.2 Disease Development in Populations of Plants

Molecular biological approaches have provided large quantities of genomic data for microbial plant pathogens that are useful for epidemiological analyses. New information about genetic sequences and gene expression has formed the basis for predictions about epidemic features and outcomes and for understanding host resistance and pathogen evolution. Microarrays have opened up the possibility of analyzing expression simultaneously for thousands of genes. These tools can be expected to contribute to plant disease epidemiology by providing necessary information about which resistance or pathogenicity genes are present in individuals or population. In addition, it is possible to identify genes other than those directly involved in resistance and virulence important in epidemics, the role of phenotypic status of hosts and pathogens and the role of the status of the environmental metagenome. Gene expression within individual organisms and in populations of organisms can be assessed, based on studies on models of group dynamics derived from population biology and ecology. By determining pathogen gene frequencies, changes within and among populations as a result of both natural selection of genes and population increases and spread can be assessed for tracking disease outbreaks (Zwankhuizen et al. 1998), for development of predictions about sources of inoculum (Cortesi and Milgroom 2001) and for understanding the evolution of pathogen virulence (Escriu et al. 2003). Improved diagnostic systems and genomic data for all microbial pathogens causing economically important diseases on various crops have to

be developed to determine precisely the abundance of pathogen and its strains in a range of environmental settings. The presence of pathogenicity genes and other genes related to toxin production and epidemiological features has to be detected to determine more reliably the genotypes in a population responsible for disease incidence, especially when the infection is still at very low levels, in order to eradicate the pathogen before it becomes well established (Garrett et al. 2006).

The importance and utility of the molecular techniques for gaining a deeper insight into the intricacies of plant–pathogen interactions may not have any lasting value unless it translates into real impacts in crop protection. It is considered that novel methods of managing crop diseases more efficiently may be developed in the near future based on the findings that conservation of signal pathways involved in pathogenesis by a diversity of microbial pathogens may be vulnerable generic targets for intervention in the life cycles of plant pathogens. The effectiveness and applications of molecular techniques for developing suitable disease management systems for various crops grown in different agroecosystems are discussed in six chapters included in volume 3 of this treatise.

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