Plant Innate Immunity: Current Status and Future Prospects (Postprint)

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Abstract

Plant diseases caused by pathogenic microorganism infections inflict tremendous losses on crop yield and quality, posing a significant threat to national food security. While chemical pesticides can effectively control crop diseases, their extensive application causes severe environmental pollution. Through long-term interactions, plants and pathogenic microorganisms have evolved mechanisms of mutual recognition and co-evolution. A profound understanding of the molecular mechanisms underlying recognition and interaction between crops and pathogenic microorganisms can accelerate molecular breeding of resistant varieties, thereby reducing chemical pesticide usage. Over the past decade, research on the molecular mechanisms of plant immune recognition and pathogen virulence has achieved remarkable progress, gradually establishing evolutionary models of plant-pathogen molecular interactions. These advances have laid the foundation for crop disease resistance research and functional analysis of pathogen virulence, while providing novel strategies and insights for improving plant disease resistance through modern biotechnology. This article reviews major advances in plant innate immunity research and explores important future research directions.

Full Text

Abstract

Crop diseases caused by pathogenic microorganisms inflict substantial losses on agricultural yield and quality, posing a major threat to national food security. While chemical pesticides effectively control crop diseases, their extensive application causes severe environmental pollution. Through long-term co-evolution, plants and pathogenic microbes have developed intricate mechanisms for mutual recognition. A deep understanding of the molecular mechanisms underlying recognition and interaction between crops and pathogenic microorganisms can accelerate molecular breeding for disease-resistant crop varieties, thereby

reducing chemical pesticide usage. Over the past decade, significant progress has been made in elucidating the molecular mechanisms of plant immune recognition and microbial pathogenicity, leading to the development of evolutionary models for plant-pathogen molecular interactions. These achievements have laid the foundation for research on crop disease resistance and functional analysis of pathogenicity factors, while providing novel strategies and approaches for improving plant disease resistance through modern biotechnology. This review summarizes major advances in plant innate immunity research and discusses important future research directions.

Keywords: plant disease, chemical pesticides, plant immunity, microbial pathogenicity

Concept and Research History of Plant Innate Immunity

Human understanding of plant immunity originates from agricultural practice. Farmers long observed that plants, like humans and animals, could become sick, though the underlying causes remained unknown. In 1846, Mile Joseph proposed that pathogenic microbial infection causes plant disease, initiating the field of plant pathology. By the late 19th century, plant pathologists recognized that some plants resist disease due to inherent resistance traits. However, the concept of plant innate immunity only emerged in the late 20th century following the discovery of numerous disease resistance genes in plants, which advanced our understanding of plant defense mechanisms.

Innate immunity refers to a heritable defense system that does not depend on acquired responses. This system recognizes "non-self" substances and triggers defense reactions characterized by broad-spectrum rejection of foreign materials and prevention of pathogen invasion. Plant innate immunity activated by pathogen recognition operates at two levels: specific recognition mediated by disease resistance genes, and broad-spectrum recognition mediated by cell surface receptors. In 1955, Harold Flor discovered the correlation between flax rust disease and the absence of plant resistance genes, revealing specific recognition of pathogens by plants and proposing the "gene-for-gene" hypothesis. In this model, plants with specific resistance genes recognize only those pathogens carrying corresponding avirulence genes, triggering a strong hypersensitive response (cell death at infection sites). This feature resembles animal adaptive immunity, yet these resistance genes are germline-encoded and heritable rather than acquired postnatally, distinguishing them from animal adaptive immunity. Later research revealed that plants can also perceive conserved microbial "non-self" molecules, such as chitin and peptidoglycan, through cell surface receptors, initiating responses including metabolic changes, accumulation of defense hormones (e.g., ethylene, salicylic acid), reactive oxygen production, and expression of pathogenesis-related genes to block pathogen invasion. This recognition does not cause hypersensitive cell death and resembles animal innate immunity in both recognition features and induced defense responses.

Important Advances in Molecular Biology of Plant Innate Immunity

Molecular Models of Plant Innate Immunity

The discovery of disease resistance genes and pattern recognition receptors has established a solid foundation for understanding the molecular recognition mechanisms and biological significance of plant innate immunity. Over the past decade, research using the model system of *Arabidopsis thaliana* and *Pseudomonas syringae* has revealed the main components of plant innate immunity. Plants rely primarily on two classes of immune receptors for molecular recognition of pathogens: pattern recognition receptors (PRRs) localized on the cell surface, and intracellular immune receptors (NB-LRR proteins/resistance genes). When pathogens infect plants, conserved molecular patterns on their surfaces (pathogen/microbe-associated molecular patterns, PAMPs/MAMPs) are recognized by plant PRRs, initiating the first layer of immune response known as PAMP-triggered immunity (PTI). Successful pathogens secrete numerous effector proteins into the apoplast or plant cells to interfere with PTI and facilitate infection. Some of these effectors are recognized by plant NB-LRR proteins, activating a second layer of immune response called effector-triggered immunity (ETI). ETI is often accompanied by hypersensitive cell death at infection sites and accumulation of salicylic acid (SA), which further induces systemic acquired resistance (SAR) in neighboring plant cells.

PAMPs are highly conserved across different microbes and are typically essential for microbial life processes. Consequently, PTI activated by PRR recognition of PAMPs provides broad-spectrum resistance against most pathogens, making it the preferred target for modern molecular breeding. In contrast, NB-LRR proteins recognize only their corresponding effectors specifically, rendering ETI highly specific and important for specialized breeding programs.

Mechanisms of Plant Innate Immune Receptor Recognition and Activation

Plant PRRs include receptor kinases and receptor proteins. Receptor kinases typically contain an extracellular ligand-sensing domain, a transmembrane domain, and an intracellular protein kinase domain, whereas receptor proteins possess only extracellular and transmembrane domains without an intracellular kinase domain. Many receptor kinases and receptor proteins form receptor complexes with other receptor-like kinases to sense PAMPs and initiate immune signal transduction. Pathogen-derived molecular patterns often serve as bridges, inducing dimerization of PRRs themselves or with other receptor-like kinases.

Compared to direct PAMP recognition by PRRs, NB-LRR proteins recognize effectors through multiple mechanisms. Some NB-LRR proteins recognize pathogen effectors through direct protein-protein interactions, such as rice Pi-ta recognizing rice blast fungus Avr-Pita and *Arabidopsis* ATR1 recognizing downy mildew RPP1. More commonly, NB-LRR proteins recognize effectors

indirectly through two distinct models: (1) the guard model, where NB-LRR proteins monitor effector attacks on virulence targets, activating ETI by sensing modifications or alterations of these targets (e.g., *Arabidopsis* RPM1 and RIN4 recognition of *Pseudomonas* AvrRpm1); and (2) the decoy model, where plants have evolved decoy targets that mimic authentic virulence targets, luring effectors to attack the decoys instead, with NB-LRR proteins activating ETI by sensing modifications to these decoys. The key difference lies in that authentic virulence targets are essential for effector pathogenicity, whereas decoy targets are not.

Plant Hormones Play Important Roles in Innate Immunity

Plant hormones are crucial regulators of plant growth and development and also play significant roles in disease resistance regulation. Salicylic acid, jasmonic acid, ethylene, and brassinosteroids form a complex regulatory network with plant immune signaling pathways that involves both synergistic and antagonistic interactions. Current research indicates that salicylic acid primarily mediates resistance against biotrophic pathogens, while jasmonic acid and ethylene mainly mediate resistance against necrotrophic pathogens. Brassinosteroids exhibit both synergistic and antagonistic effects with PTI. Additionally, gibberellins, cytokinins, and auxin also participate in the regulation of plant immune signaling networks. These hormones help coordinate the balance between plant development and immunity.

Prospects for Plant Innate Immunity Research

Important Unresolved Scientific Questions in Plant Innate Immunity Research

Research on PRR activation, maintenance of active states, and deactivation regulation requires deeper investigation. The connecting molecules between pattern receptors and downstream signaling kinases remain to be identified. The isolation and characterization of novel PAMPs from important crop pathogens and the identification of new pattern recognition receptors in major crops represent important directions for crop PRR immunity research. The signaling mechanisms following NB-LRR protein recognition of pathogen effectors have long been a challenging and hot topic in plant intracellular receptor immunity research. Moreover, NB-LRR proteins are abundant in plants—*Arabidopsis* has approximately 150, tomato about 350, and rice at least 400—yet only a handful have been functionally characterized. Whether these numerous NB-LRR proteins participate in disease resistance remains unclear. Although some NB-LRR genes have been successfully introduced into crops and enhance immune responses, the resistance they mediate typically loses effectiveness against specific physiological races within 3–5 years in field conditions, indicating that NB-LRR-mediated resistance can be readily overcome by rapid pathogen evolution. Therefore, in-depth study of immune signal transduction mechanisms

following NB-LRR recognition of pathogen effectors is crucial for maintaining and extending the effectiveness of NB-LRR proteins in the field.

Current understanding of plant-pathogenic bacteria interactions is relatively advanced, whereas research on plant-pathogenic fungus interactions remains in early stages. Compared to the dozens of effectors secreted by pathogenic bacteria, pathogenic fungi can secrete hundreds of effectors to facilitate infection and nutrient acquisition. Some effectors function outside plant cells, while others enter plant cells to manipulate physiological and immune responses. Key unresolved questions include the mechanisms of fungal virulence factors and effectors, their regulation of other microbes, the molecular mechanisms of effector entry into plant cells, and the molecular basis for broad host ranges of biotrophic fungi.

Soil-borne diseases caused by root-infecting pathogens, such as cotton Verticillium wilt, cause enormous yield losses in China, yet effective control measures remain lacking. Research on soil pathogen infection, pathogenicity, life cycle regulation, and the complex interactions among root pathogens, beneficial microbes, and host plants constitutes an important area in plant innate immunity.

Furthermore, most current research focuses on binary interactions between plants and single pathogens under controlled laboratory conditions, which differs substantially from natural systems involving multiple species and environmental factors. Given that disease development results from complex environmental conditions, future research must incorporate environmental factors and other organisms into studies of multi-species interactions.

The Necessity of Model Plant Research and Transition to Crop Studies

Research using model plants offers the distinct advantage of establishing large-scale mutant libraries with easily isolatable genes, enabling high-throughput screening and genetic manipulation. Model plant research has dramatically accelerated the molecular dissection of plant innate immunity mechanisms. Consequently, model plant disease resistance research will remain essential for the foreseeable future. Many important resistance genes from model plants function similarly in crops—for example, soybean Rpg1 is homologous to *Arabidopsis* RPM1, both recognizing the effector AvrB from bacterial spot disease. Studies on the signaling mechanism in *Arabidopsis* led to the discovery of the important negative immune regulator RIN4, establishing the "guard model." Subsequently, RIN4 homologs were identified in soybean, where they mediate recognition of bacterial spot pathogens carrying AvrB through mechanisms similar to *Arabidopsis* RIN4.

Several pattern recognition receptors have been successfully isolated from *Arabidopsis*, including FLS2 (recognizing bacterial flagellin), EFR (recognizing elongation factor), and CERK1 (recognizing chitin and glucan). Notably, heterologous expression of *Arabidopsis* EFR in tobacco and tomato enhances bacterial

resistance. More promisingly, EFR expression in monocot crops such as rice and wheat also enhances resistance to some bacterial pathogens, demonstrating that PRR-mediated pathogen recognition and immune responses are highly conserved across species. This conservation raises expectations for PRR applications in crop resistance improvement.

In recent years, increasing numbers of *Arabidopsis* resistance genes have been introduced into crops, proving effective in enhancing disease resistance. For example, the broad-spectrum resistance gene BRT1 from *Arabidopsis* increases soybean resistance to rust when transferred into soybean, while NPR1, a key regulator in the SA pathway, enhances citrus resistance to Huanglongbing when introduced into citrus. Additionally, important resistance genes from other model plants like tomato and rice have been used for crop improvement. These examples underscore the importance of model plant research and the potential of transferring key resistance genes to food and economic crops.

Plant Innate Immunity Research in the Post-Genomic Era

With advances in high-throughput sequencing, an increasing number of species have been fully sequenced, with genome announcements published at a rate approaching 100 species per month. Genome-based functional studies, such as genome-wide association analysis (GWAS), can efficiently identify genes associated with specific traits. Resequencing pathogen genomes enables rapid identification of variations in potential effectors or other virulence genes, providing effective means to differentiate physiological races. Transcriptome analysis before and after pathogen infection also serves as an important method for discovering key resistance genes and signaling pathways. Due to increased sequencing throughput and reduced costs, these technologies are now applied to microbiome studies previously difficult to conduct. The Agricultural Microbiome Project, hailed as the second green revolution for food crop production, aims to decipher relationships between microbial communities and host resistance, seeking to improve microbial composition under specific conditions and enhance plant resistance while reducing pesticide and fertilizer use—all predicated on sequencing-based understanding of microbial populations.

While genomics and transcriptomics offer advantages in studying plant immune signaling, proteomics and metabolomics effectively identify proteins and metabolites that execute defense functions. The application of ion trap and breakthrough orbitrap technologies has enabled proteomic analysis with resolution and throughput exceeding currently used transcriptomic techniques. Proteomic data have been compared with transcriptomic data to analyze systematic gene expression regulation and modification, greatly expanding our understanding of defense protein expression regulation under pathogen infection. Metabolomics development helps elucidate how plants alter metabolic pathways to affect pathogen infection, an area currently understudied. Furthermore, functional genomics analysis has promoted synthetic biology applications in plant innate immunity research, such as artificially modifying resistance gene pro-

moter regions or redesigning novel genes and gene clusters to introduce large DNA fragments conferring resistance to multiple pathogens, thereby enhancing resistance gene effectiveness.

New technologies have also accelerated breeding processes and improved efficacy. For example, genome editing has enabled simultaneous knockout of multiple powdery mildew susceptibility genes in wheat, conferring powdery mildew resistance. Host-induced gene silencing (HIGS) technology has been used in cotton to express small RNAs targeting important virulence genes of *Verticillium dahliae*, silencing the fungal genes and enhancing cotton resistance.

Considerations on Crop Disease Control and Resistance Improvement

Modern society' s increasing connectivity accelerates species dispersal across regions, introducing threats from emerging pathogens. Weakly pathogenic microbes may overcome plant innate resistance by acquiring virulence factors from other species (e.g., through horizontal gene transfer). Additionally, climate change promotes emerging diseases. Insects such as planthoppers from Southeast Asia previously could not overwinter in China, but rising temperatures now allow them to survive and transmit new diseases. Airborne fungal spores present even greater control challenges, as disease outbreaks in one region can rapidly spread to others. For instance, the new wheat stem rust race Ug99 that emerged in Central Africa around 2000 overcame resistance in nearly all major wheat cultivars and spread to most of West Asia and Europe within a decade. Therefore, alongside resistance breeding and research, establishing robust prediction and forecasting systems for emerging diseases is essential.

As chemical pesticide use faces increasing public criticism despite remaining the most effective disease control method, the economic losses from plant diseases remain substantial. Transferring disease and pest resistance genes into crops can reduce yield losses and increase farmer income. A USDA Economic Research Service analysis indicates that every 10% increase in insect-resistant corn cultivation raises net profits by 2.3%. Moreover, cultivation of disease-resistant, insect-resistant, and herbicide-tolerant crops benefits the environment. Rational application of theoretical discoveries in plant disease resistance mechanisms to crop improvement offers significant economic and environmental benefits, prompting major agricultural biotechnology companies to invest heavily in pesticide and seed R&D. Leading global agricultural biotechnology companies such as Syngenta and Monsanto invest hundreds of millions of dollars annually, with recent R&D expenditures exceeding $800 million per year.

Future efforts should strengthen basic research to elucidate molecular mechanisms of plant disease resistance and pathogen virulence, particularly for major crop diseases, while continuing model plant studies. Basic research findings should be rapidly translated into breeding programs, incorporating new biotechnologies to transform traditional breeding concepts and integrate biological control with host resistance. Since single resistance genes often lose effectiveness

quickly in field conditions, synthetic biology approaches should be employed to introduce resistance gene clusters into crops, extending R gene durability. Additionally, integrating resistance loci into shared databases will accelerate utilization of novel resistance resources.

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