

# The molecular initiation and subsequent acquisition of disease resistance in plants

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Interactions between disease resistance (R) genes in plants and their corresponding pathogen avirulence (*Avr*) genes are the key determinants of whether a plant is susceptible or resistance to a pathogen attack. Evidence has emerged that these gene-for-gene interactions in the perception of pathogenic invasions and development of acquired resistance in plants involve different molecular and hormonal transduction pathways, which are still poorly understood. It has become apparent that plants actively produce several phytohormones such as ethylene, jasmonate, salicylic acid, and reactive oxygen intermediates prior to upregulation of R genes. The physiological role of these molecules in plant resistance to pathogens is beginning to attract attention. The use of transgenic plants in recent attempts, including development of mutants with altered R genes, has provided new insights into the mechanisms involved in pathogen perception, signal transduction and subsequent resistance to disease in plants. This review tries to summarize current knowledge of pathogen-related genes in plants, and how they can be used to improve disease resistance in agronomically valuable plants. It also describes the molecular basis of defense mechanisms in plants under pathogen attack.

**Key words:** *Avr*, resistance gene, hypersensitivity, pathogenesis-related proteins, transgenic, plant-defense.

## INTRODUCTION

Being sessile organisms, plants are often exploited as a source of food and shelter by a wide range of parasites including viruses, bacteria, fungi, nematodes, insects and even other plants. However, they have developed remarkable strategies to adapt to environmental changes by using a range of constitutive or inducible biochemical and molecular mechanisms. They exhibit both long- and short-term defense responses to immediate challenges such as pathogen attacks. Nevertheless, a synergic effect of many stresses represents the primary cause of crop loss. The estimated loss caused by pathogens is typically around 10 to 20% (Boyer, 1982). The appropriate response of plant emerges from the perception of an extracellular signal and its transduction between and within plant cells. Specificity of the interactions between plants and pathogens is still an incomprehensible phenomenon with a complicated hierarchy of biological organization. Elucidation of this phenomenon represents an important task of contemporary plant pathology (Scheel, 1998; Nimchuk et al., 2001). However, tremendous opportunities for crop

improvement are likely to arise, as the complete sequencing of *Arabidopsis* plant genome (*Arabidopsis* Genome Initiative, 2000), which is a molecular research model, become a reality. *Arabidopsis* carries around 150 resistance (R) genes, whereas many crop plants may have two to three times this number (*Arabidopsis* Genome Initiative, 2000; Xiao and Jang, 2000). Many R genes, which confer resistance to various plant species against a wide range of pathogens, have been isolated. However, the key factors that switch on and switch off these genes during plant defense mechanisms remain poorly understood (Glazebrook, 2001).

In this review, we summarize recent discoveries in molecular mechanisms of plant responses to pathogen attacks, and aim to make known the potential use of new approaches for research improvement. We point out the gaps in the knowledge of basic defense systems of plants against pathogen attacks. It is not the purpose of this review to cover the whole subject of molecular interactions between plants and pathogens. Rather, we focus on the upregulation of a specific set of plant genes, which harbor conserved domains allowing them to specifically recognize distinct pathogen races, and how these genes can be used to improve disease resistance. An appendix containing a glossary of pathology-related terms used in this text is provided at the end of the report.

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## MOLECULAR BASIS OF PLANT RESPONSES TO PATHOGEN INVASION

At the molecular level, defense systems often depend on the combination of a specific set of dominant R genes in the plant and a corresponding set of dominant avirulence (*Avr*) genes in the pathogen (Keen, 1990). Expression of the *Avr* genes triggers plant defense responses governed by the product of the R gene (for a review, see Bogdanove, 2002). This gene-for-gene resistance strategy underlies the molecular basis of defense systems in plants. The strategy was originally proposed by Flor (1955) when studying resistance to the rust disease of flax. This molecular basis is defined by a single plant R gene for a single pathogen *Avr* gene, hence the name gene-for-gene resistance. According to the terminology involved in gene-for-gene resistance, when the plant is resistant, the pathogen is said to be avirulent and the interaction is said to be incompatible. When the plant is susceptible, the pathogen is said to be virulent and the interaction is said to be compatible. In a given plant-pathogen interaction, it is often possible that more than one specific combination of *Avr* and R genes are operating at the same time, and such different combinations are often co-dominant. These multiple combinations reflect the complexity of defense mechanisms of the plant during pathogen attack. Physiological features such as  $K^+/H^+$  exchange, rapid oxidative burst, hypersensitivity at the site of infection, crosslinking of plant cell walls, synthesis of antimicrobial compounds known as phytoalexins, and induction of pathogen-related proteins such as chitinases and glucanases (Lamb et al., 1989) represent some of the multiple metabolism turnover leading to disease resistance.

## SIGNAL PERCEPTION AND DEFENSE ACTIVATION

The perception of inductive signals by plants has received considerable attention mainly as a consequence of recent data on clones and genes encoding signaling components, which can be accessed at [www.pgfsun.ucdavis.edu/niblrrs/At\\_Rgenes/](http://www.pgfsun.ucdavis.edu/niblrrs/At_Rgenes/). The first process in signal transduction is the perception of an extracellular signal and its transmission via the plasma membrane, resulting in accumulation of intracellular signaling molecules and induction of a phosphorylation/dephosphorylation cascade, a cue system for the activation of R gene expression. Plant recognition of pathogens is mediated by large families of highly polymorphic R genes (Dangl and Jones, 2001; Jones, 2001). The products of these genes function to recognize directly or indirectly the products of pathogen-encoded *Avr* genes (Nimchuk et al., 2001).

The majority of the identified R genes encode for intracellular proteins containing a predicted nucleotide

binding site (NBS) followed by a series of leucine-rich repeats (LRR) at their C termini ([http://pgfsun.ucdavis.edu/niblrrs/At\\_Rgenes/](http://pgfsun.ucdavis.edu/niblrrs/At_Rgenes/)), although some R genes are notably different (Xiao et al., 2001). NBS-LRR resistance proteins generally contain one of two types of N-terminal domains. These are either a domain that has homology with the Toll and Interleukin-1 Receptor proteins (TIR) or a predicted coiled-coil domain (CC).

Studies of resistance proteins have indicated that the highly variable LRR domains determine recognition of the pathogen *Avr* products (Dodds et al., 2001; Ellis et al., 1999; Jia et al., 2000), whereas the more conserved TIR-NBS or CC-NBS regions are believed to propagate the perception signal (Tao et al., 2000). A favored model based on genetic data postulates that R proteins (products of R genes) act as receptors for pathogen-encoded *Avr* proteins to initiate a signal, which activates plant defense responses to arrest pathogen propagation.

Evidence for direct interaction between an R protein and an *Avr* protein came from yeast two-hybrid analysis, which demonstrated binding of the tomato bacterial speck R gene product (Pto) to the corresponding *AvrPto* avirulence gene product of *Pseudomonas syringae* pv. *tomato* (Scofield et al., 1996; Tang et al., 1996). Since then, several R genes have been genetically engineered into susceptible plants to produce transgenic plants, which exhibit disease resistance. Table 1 shows examples of improved resistance to pathogen attacks, obtained through overexpression of different R genes in transgenic plants.

The R genes and genes encoding signal transduction proteins possess loci at their downstream sequences for production of pathogenesis-related proteins (PRs), enzymes involved in the generation of phytoalexins and protection of plants from oxidative stress, tissue repair, and lignification. Eleven pathogenesis-related protein families from different plant species have been characterized and classified according to sequence similarities (Van Loon, 1997), as illustrated in Table 2. They were found to play different protective roles against pathogens.

Despite these significant insights into R gene structure, much remains to be elucidated. Interestingly for example, the NBS-LRR class of R genes represents only 1% of the *Arabidopsis* genome (Ellis et al., 2000). This indicates the probable involvement of many other gene families in defense responses.

## PATHOGEN-INDUCED SIGNALING PROMOTERS

The levels of different transcriptional expressions during plant responses to pathogen attack are regulated by cis- and trans-acting elements. Cis-acting elements involved in pathogen-induced R gene expression have been analyzed extensively. Two groups of pathogen-inducible

**Table 1:** Improved disease resistance in transgenic plants overexpressing defense related R genes.

R Gene	Identity	Transgenic host plants	Effects observed in transgenic plants	Reference
<i>Hml</i>	NADPH-dependent HC toxin reductase	Maize	Controls resistance to the fungus <i>Cochliobolus carbonum</i> race 1	Johal and Briggs, 1992
N	Interleukin-1 mammalian like protein	Tobacco	Confers resistance to tobacco mosaic virus (TMV)	Whithman et al., 1994
<i>Cf-9</i>	Elicitor	Tobacco	Resistance to <i>Cladosporium fulvum</i>	Jones et al., 1994
<i>Flax L</i> <sup>6</sup>		Maize	Resistance to <i>Melampsora lini</i>	Ellis et al., 1995
<i>PTO</i>	Serine-threonine protein kinase	Tomato	Confers resistance to <i>Pseudomonas syringae</i> pv tomato	Martin et al., 1993
<i>RPS2</i>	Leucine-rich repeat protein	<i>Arabidopsis</i>	Confers resistance to <i>Pseudomonas syringae</i> pv tomato	Bent et al., 1994
<i>RPM1</i>	Leucine Zip-like protein	<i>Arabidopsis</i>	Confers resistance to <i>Pseudomonas syringae</i> pv tomato	Grant et al., 1995
<i>Xa7</i>		Rice	Confers resistance to <i>Xanthomonas oryzae</i> pv <i>oryzae</i> race 6	Yang et al., 2000
<i>Pita</i>	Neutral zinc metalloprotease	Rice	Confers resistance to <i>Magnaporthe grisea</i>	Jia et al., 2000
<i>Rar1</i>	Homologous to the yeastSGT1 protein: positive regulator of E3 ubiquitin ligase	Barley	Confers resistance to powdery mildew	Kitagawa et al., 1999
<i>EDS1</i>	Lipase like protein	<i>Arabidopsis</i>	Mediates the down stream signaling of known TIR-type	Feys et al., 2001
<i>PAD4</i>	Lipase like protein	<i>Arabidopsis</i>	Mediates the down stream signaling of known TIR-type	Feys et al., 2001

cis-acting elements, the GCC-like elements (Ohme-Tagaki et al., 2000) and the W boxes (Rushton et al., 1996; Eulgem et al., 2000) are among the best characterized in the context of pathogen defense mechanisms. The cis-acting elements with pathogen-inducible R gene functions fall into three groups: the W boxes, the GCC-like boxes, and the box D. The binding site for WRKY (W box) or AP2/ERF (GCC-like box) transcription factors can be sufficient to confer pathogen inductibility. Systematic DNA-binding studies have shown that nucleotides flanking the W boxes and the GCC-like box specify the DNA-protein interactions and subsequent gene activation (Rushton et al., 2002). The pathogen-inducible systemic promoter could have major applications, first as molecular markers, and second, in the engineering of crops with increased disease resistance. Systemic promoters could be used to better characterize the function of a gene or a mutation within a

pathogen-inducible gene. Moreover, the use of defined regulatory sequences may allow highly restricted expression of the desired product, which could be accumulated exclusively at the sites of attempted pathogen invasion. Promoter analysis using transient expression assays may be a promising way to characterize several distinct cis-acting elements and the cloning of related transcription factors. This approach could be used to assess the regulatory pattern of promoters in transgenic plants.

#### **HYPERSENSITIVE CELL DEATH AND DEFENSE MECHANISM**

The hypersensitive response is characterized by localized cell and tissue death at the site of infection (Van Loon, 1997). As a result, the pathogen remains confined

**Table 2.** Pathogenesis-related protein (PRs) families in plants and their putative functions.

Protein family	Reporter protein activity	Targeted pathogen sites or components
PR-1	Pathogenesis-related protein 1 precursor	Membrane
PR-2	1,3- $\beta$ -glucanase	Cell wall glucan
PR-3	Endochitinase	Cell wall chitin
PR-4	Endochitinase	Cell wall chitin
PR-5	Osmotin	Membrane
PR-6	Proteinase inhibitor	Proteinase
PR-7	Proteinase	Not defined
PR-8	Endochitinase	Cell wall chitin
PR-9	Peroxidase	*
PR-10	RNase	Pathogen-RNA
PR-11	Endochitinase	Cell wall chitin

\*Peroxidase exerts indirect antimicrobial activity by catalyzing oxidative crosslinking of protein and phenolics in the plant cell wall, leading to reinforcement of physical barrier (Odjakova and Hadjiivanova, 2001).

to necrotic lesions near the site of infection. A ring of cells surrounding necrotic lesions become fully refractory to subsequent infection, known as localized acquired resistance (Fritig et al., 1998). These local responses often trigger non-specific resistance throughout the plant, which leads to a systemic acquired resistance, providing durable protection against infection by a broad range of pathogens (Sticher et al., 1997). The metabolic alterations in acquired resistance include: cell wall reinforcement, stimulation of secondary metabolic pathways, which yield molecular compounds with antibiotic activity, and defense regulators such as salicylic acid, ethylene and lipid-derived metabolites (Fritig et al., 1998; Hahn, 1996).

Receptor-mediated recognition at the site of infection initiates cellular and systemic signaling processes that activate multicomponent defense responses at local and systemic levels. The local resistance occurs rapidly while the development of systemic acquired resistance is delayed (Scheel, 1998). The earliest reactions of plant cells include changes in plasma membrane permeability leading to calcium and proton influx, and potassium and chloride efflux, which subsequently induce extracellular production of reactive oxygen intermediates such as superoxide, hydrogen peroxide, and hydroxyl free radicals (McDowell and Dangl, 2000). The localized production of reactive oxygen intermediates and nitric oxide act to induce hypersensitive responses and expression of disease defense genes (Piffanelli et al.,

1999). Other components of the signal network are the induction of phospholipases (PLPs), which act on lipid-bound unsaturated fatty acids within the membrane, resulting in the releasing of jasmonate, methyl jasmonate and related molecules. Recent evidence confirmed the potential role of jasmonate, ethylene and salicylic acid in the signal pathways leading to up-regulation of pathogen defense-related genes in plants (Ananieva and Ananiev, 1999; Reymond and Farmer, 1998).

## HORMONAL PATHWAYS IN PLANT DEFENSE

Phytohormones such as ethylene and jasmonate have been found to synergistically induce the expression of plant-defense genes in response to different pathogen attacks (Penninckx et al., 1998). Exogenous application of methyl jasmonate to *Arabidopsis* plants reduces disease development after infection by several fungi, such as *A. brassicicola*, *B. cinerea*, and *P. cucumerina* (Thomma et al., 2000). Lorenzo et al. (2003) have demonstrated that ERF1 (Ethylene response factor 1), a downstream component of the ethylene-signaling pathway (Solano et al., 1998), is an early ethylene- and jasmonate-responsive gene. They suggest that ERF1 may be a common component of both ethylene and jasmonate signaling pathways. In order to investigate the involvement of ERF1 in the regulation of the expression of pathogenesis-related genes, Lorenzo et al. (2003) performed a transcriptome analysis of Col-0 and 35S-ERF1 transgenic plants using *Arabidopsis* GeneChip from "Affymetrix", which allows the simultaneous monitoring of 8000 genes. They reported about 164 genes, which were constitutively expressed, 77 genes inducible and 70 genes repressed after 6 h of treatment with jasmonate and Ethylene (Lorenzo et al., 2003). Most research has focused on understanding how relevant genes are upregulated during pathogen-defense mechanism (Table 3). However, plant responses to pathogen attack also involve the downregulation of several genes (Table 4). In potato for example, the mRNA and protein levels of Rubisco are drastically reduced by pathogen infection or elicitor treatment (Table 4). In persley, the expression of several genes involved in cell proliferation and cell-cycle regulation as well as flavanoid biosynthesis, are repressed to a large extent during defense responses (Somssich and Hahlbrock, 1998). Reports of Lorenzo et al. (2003) confirmed the combination of positive and negative regulatory mechanisms of pathogen-acquired resistance in plants. The high number of defense-related genes whose expression was upregulated by ERF1 is fully consistent with their previous results (Berrocal-Lobo et al., 2002) demonstrating that overexpression of ERF1 in transgenic plants confers resistance to several pathogens. In summary, different types of phytohormonal signaling pathways are involved in plant defense responses during

pathogen attack. These results suggest that hormonal transcription factors are key elements in the integration of signal transduction for the regulation of defense-related genes.

**Table 3.** Defense related genes upregulated by ethylene and jasmonate treatment, or by pathogen attack.

Gene name	Features of encoded polypeptide	Accession number
ASP	Cys-rich antifungal protein 1	CAA63009
ERF1	Ethylene response factor 1	AF076277
CHI	Putative endochitinase	AAB64047
OSL3	Osmotin precursor	CAB39936
SRG2At	$\beta$ -glucosidase	CAA57943
ChiB	Basic endochitinase	BAA82810
AtSS-2	Strictosidine synthase	AAB40594
PME	Pectin methylesterase	AAB82640
LOX1	Lipoxygenase 1	AAA32827
BBE	Berberine bridge enzyme	AAD25759
TSA1	Trp synthase	AAC49117
ASA	Anthranilate synthase	AAA32738
InGPS	Indole-3-glycerol phosphate synthase	AAA60380
RbohD	Respiratory burst oxidase protein D	AAC39479
HMZ1	Ferrochelataase 1	P42049
p9a	Peroxidase	CAA07352
NIT4	Nitralase	AAA19628
PrxCb	Peroxidase	CAA50677
JR3	IAA-Ala hydrolase	AF081067
TPx2	Peroxiredoxin	AAD28243
GLP5	Germin-like protein 5	U75198
GST11	Glutathione S-transferase	AAC32912
RKC1	Receptor-like protein kinase	AAC95354
CYP71A13	Cytochrome P450	AAC02748
RLK	Receptor Ser/Thr kinase-like protein	CAA16797
JIP	Jasmonate-inducible protein isolog	AAB63634

**Table 4.** Genes constitutively repressed under pathogen attack.

Gene	Encoded polypeptide	Accession number
Thi2.1	Thionin	AAC41678
ARF	Auxin response factor-like	AAD20695
ICK1	Cyclin-dependent kinase inhibitor	AAC49698
RBFA	Putative ribosome-binding factor	CAA18852
AtMYB76	R2R3-MYB transcription factor	CAB09231
VSP	Vegetative storage protein	BAA22095
Sqp1.1	Squalene epoxidase	CAA06772
SPL3	Squamosa-promoter binding protein-like 3	CAB56585
SPL10	Squamosa-promoter binding protein-like 10	CAB56589
MYB	Putative MYB transcription factor	AAD23043

## ENGINEERING PLANT RESISTANCE TO PATHOGENS

The cloning of R genes has opened doors for producing disease-resistant crop plants. Transgenic plants that resist pathogen attacks can be engineered either by insertional mutagenesis or by map-based cloning methodology.

Using the insertional mutagenesis method of transformation, a fragment of DNA is inserted into the coding region or regulatory region of a gene, which results in disruption of gene expression. After this, one proceeds to the cloning of the plant DNA that flanks the integrated insertional mutagen by plasmid rescue. The cloned plant DNA can then be used as a hybridization probe to isolate the gene by screening a lambda or cosmid library constructed from the wild-type plant. The final test is to introduce the cloned gene by transforming sensitive plants and examining them in order to determine whether they have become disease-resistant. The most commonly used DNA molecules for insertional mutagenesis are transposons (Baker et al., 1986) and T-DNA from the Ti plasmid.

In the map-based cloning which is also known as positional cloning, one needs to determine the chromosomal location of the gene of interest. The restriction fragment length polymorphism (RFLP) is the most frequently used method for linking the chromosomal position to a particular trait. In this case, co-inheritance of the disease resistance trait with one specific RFLP DNA probe defines the approximate location of the R gene on the chromosome. The linkage between several specific

DNA probes and the R gene allows one to use the DNA probe as a starting point to seek, or to land on the R gene. The gene will be identified in the next step after screening for coding sequences (e.g., cDNA) within the specific region using either a yeast artificial chromosome (YAC) vector or a bacterial artificial chromosome (BAC) vector. The final test can be done after transferring the candidate cDNA fragments into susceptible plants by looking for the acquisition of disease resistance in the transgenic plants.

Resistance of plants to pathogen attacks entails a concerted action of many gene products, and thus, alteration in the expression of multiple genes is required. In this respect, transcription factors may be excellent targets of genetic engineering for improving disease resistance in plants. Transcription factors generally regulate a group of genes rather than a single gene. Therefore, manipulating a single transcription factor could have the same effect as manipulating a set of specific genes within the plant. As highlighted above, transgenic plants allow the targeted expression of pathogen-related genes *in vivo* and are therefore an excellent system to assess the function of/and tolerance conferred by the encoded proteins. Another purpose for using transgenic plants is to improve disease resistance in agronomically valuable plants.

## CONCLUSIONS AND PERSPECTIVES

The defense response in plants appears to be activated by ligand/receptor interactions, in which *Avr* gene and pathogen or plant surface-derived elicitors serve as ligands for receptors located in the plasma membrane or in the cytosol.

Cloning an R gene is an intensive and time-consuming task. However, one can expect many more R genes to be cloned if discoveries in this area are accessible worldwide. Knowing the gene structure will lead to a better understanding of the pathogen-host interactions and the availability of cloned R genes will increase the potential for engineering disease resistance in plants.

Unfortunately, up to date, the functions of many proteins and other molecules that interact with R genes during defense mechanisms are largely unknown. Therefore, additional proteins are likely to participate in *Avr* perception and subsequent signal transduction. The genetic approaches utilized so far to isolate and characterize disease-related genes may need to be complemented by biochemical studies to understand fully their functions (Kotchoni and Shonukan, 2002). Many of the identified genes, which are associated with pathogen-defense mechanisms, are still at descriptive level and only the functions of few have been established. The production of mutants using an antisense-RNA approach could turn out to be a powerful technique in understanding the potential role of a gene product during

defense mechanisms (Kotchoni and Bartels, 2001). This approach could elucidate certain aspect of disease resistance in plants. A combination of novel approaches including molecular techniques and genetics will provide insights into pathogen-defense mechanism and subsequent disease resistance in years to come.

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## APPENDIX: GLOSSARY OF PATHOLOGY-RELATED TERMS USED IN THE TEXT

**Disease necrosis:** A common, slow-developing disease symptom caused by necrotrophic pathogens. The molecular basis of necrosis and its relationship to the rapid hypersensitive response during incompatible interactions are not known.

**Ethylene:** A molecular plant growth regulator involved in response to abiotic stresses, fruit ripening, leaf senescence, and plant tolerance/resistance to pathogens

**Induced systemic resistance:** A long-lasting and broad-spectrum resistance induced throughout the plant cells as a result of prior exposure to pathogen and abiotic stresses.

**Jasmonic acid:** Jasmonic acid and methyl jasmonate are plant growth regulators derived from the octadecanoid-signaling pathway elicited by wounding and insect chewing. They are required for signal transduction leading to resistance to insects and certain fungal pathogens.

**Local resistance:** A local plant defense observed in response to infection by avirulent or virulent pathogens.

**Pathogenesis-related (PR) proteins:** Proteins induced throughout in the plant in response to pathogen infection, which are associated to systemic acquired resistance. Some PR proteins such as chitinases and glucanases exhibit antifungal activity *in vitro*.

**Phytoalexins:** Anti-microbial compounds produced by plants in response to infection.

**Salicylic acid:** Is an endogenous messenger for the activation of multiple resistance responses against pathogens and abiotic stresses

**Systemic acquired resistance:** A long-lasting, broad-spectrum resistance induced throughout the plant cells by prior local infection of necrotic pathogens or pretreatment of plant with certain chemicals such as salicylic acid.

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