BULG. J. PLANT PHYSIOL., 2001, 27(1–2), 101–109

REVIEW

THE COMPLEXITY OF PATHOGEN DEFENSE IN PLANTS

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Received June 07, 2001

Summary. Plants defend themselves against pathogen attack by activating a multicomponent defense response. In host defense, pathogen invasion is recognized by proteins encoded by plant disease resistance (R) genes that bind specific pathogen-derived avirulence (Avr) proteins. In nonhost resistance, specific pathogen or plant cell wall derived exogenous or endogenous elicitors are recognized. A complex signalling network, involving cytosolic Ca^{2+} and H^+ ions, reactive oxygen intermediates, jasmonate, salicylic acid, ethylene, triggers the induction of the defense mechanisms. Defense genes encode pathogenesis-related proteins, such as glucanases, chitinases; enzymes involved in the biosynthesis of phytoalexins; the enzymes of oxidative stress protection, tissue repair, lignification, and others.

Key words: plant defense, systemic acquired resistance, salicylic acid, jasmonate, pathogenesis-related proteins

Plants are exploited as a source of food and shelter by a wide range of parasites, including viruses, bacteria, fungi, nematodes, insects and even other plants. Plants lack a circulating adaptive immune system to protect themselves against pathogens. They have evolved other mechanisms of antimicrobial defense which are either constitutive or inducible. Plants are resistant to most pathogens in their environment, as they are not host plants for particular pathogen or are host plants, but harbor resistance genes, allowing them to recognize specifically distinct pathogen races (Scheel, 1998). Two types of plant resistance response can be distinguished: nonhost and host or race/cultivar specific resistance response. In both cases, the biochemical processes in-

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volved in pathogen resistance are very similar (Somssich and Hahlbrock, 1998). Resistance in plants is manifested by the inability of the pathogen to grow or multiply and spread and often takes the form of a hypersensitive reaction (Agrois, 1988). 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 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 (Hammon-Koasack and Jones, 1996; Baker et al., 1997; Fritig et al., 1998). These local responses often trigger nonspecific resistance throughout the plant, known as systemic acquired resistance, providing durable protection against challenge infection by a broad range of pathogens (Ryals et al. 1996; Sticher et al., 1997; van Loon, 1997; Fritig et al. 1998). The metabolic alterations in localized acquired resistance include: cell wall reinforcement by deposition and crosslinking of polysaccharides, proteins, glycoproteins and insoluble phenolics; stimulation of secondary metabolic pathways, some of which yield small compounds with antibiotic activity (the phytoallexins) but also defense regulators such as salicylic acid, ethylene and lipid-derived metabolites; accumulation of broad range of defense-related proteins and peptides (Hahn, 1996; Fritig et al., 1998).

Understanding of the plant response to the pathogen attack has advanced rapidly in recent years. Bacterial and fungal pathogenicity factors have been isolated, and mechanisms utilized by the plant to recognize the pathogen and initiate a plethora of defense mechanisms have been identified. The present review is focused on recent advances in the study of molecular mechanisms and components involved in pathogen defense in plants.

Pathogen recognition

Activation of inducible defenses is triggered by a specific recognition of pathogen invasion by plants. Perception in host specific resistance involves receptors with high degrees of specificity for pathogen strains, which are encoded by constitutively expressed defense resistance (R) genes, located either on the plasma membrane or in the cytosol (Edreva, 1991; Martin, 1999; McDowell and Dangl, 2000). Large repertoires of distantly related individual R genes with diverse recognitional specificities are found within a single plant species (Ellis et al., 2000). Individual R genes have narrow recognition capabilities and they trigger resistance when the invading pathogen expresses a corresponding avirulence (Avr) gene. Avr genes from different pathogen classes are structurally very diverse and have different primary functions in the biology of these organisms. Specific recognition of the aggressor by the plant requires the presence of matching Avr and R genes in the two species and is thought to be mediated by ligand receptor binding (Glazebrook, 1999). Over 20 R genes with recog-

nition-specificity for defined Avr genes have been isolated from seven plant species, including both monocots and dicots (Milligan et al., 1998; Rossi et al., 1998; Martin, 1999). These genes are effective against bacterial, viral, and fungal pathogens and against both nematodes and aphides species (Mi gene from tomato) (Martin, 1999). In spite of the great diversity in lifestyles and pathogenic mechanisms of disease-causing organisms, R genes were found to encode proteins with certain common motifs. Five classes R proteins are now recognized: intracellular protein kinases; receptor-like protein kinases with an extracellular leucine-rich repeat (LRR) domain; intracellular LRR proteins with a nucleotide binding site (NBS) and a leucine zipper (LZ) motif; intracellular NBS-LRR proteins with a region with similarity to the Toll and interleukin-1 receptor (TIR) proteins from Drosophila and mammals; and LRR proteins that encode membrane bound extracellular proteins. Interestingly, the NBS-LRR class of R genes represents as much as 1% of the *Arabidopsis* genome (Ellis et al., 2000). Plant R genes encode proteins that both determine recognition of specific Avr proteins and initiate signal transduction pathways leading to complex defense responses (Zhou et al., 1998; del Pozo and Estelle, 1999; Martin, 1999). Despite these significant insights into R gene structure, much remains to be elucidated about the molecular mechanisms by which R proteins recognize and transduce this information in the plant cell.

In addition to gene for gene recognition mediated by R and Avr genes, nonhost resistance is achieved through the recognition of specific pathogen or plant cell wall derived signal molecules, termed exogenous or endogenous elicitors, respectively. These elicitors are often low-molecular-weight compounds that are either synthesized as such or are liberated from polymeric precursors during infection (Somssich and Hahlbrock, 1998). The chemical structure of different elicitors is of great variety, such as glycoproteins, peptides and oligosaccharides. Some proteinaceous elicitors are directly produced by bacterial or fungal pathogens, whereas biologically active oligosaccharides are released from pathogen and plant cell walls by hydrolases secreted by the two organisms. Complex and largely unresolved perception systems exist for these elicitors on the plant cell surface that activate multiple intracellular defense signaling pathways.

In conclusion, the multicomponent response of plants to pathogens in host and nonhost resistance appears to be activated by ligand/receptor interactions, in which Avr gene and pathogen or plant surface-derived elicitors serve as ligands for plasma membrane located or cytosolic receptors.

Signal transduction

Receptor-mediated recognition at the site of infection initiates cellular and systemic signaling processes that activate multicomponent defense responses at local and sys-

themic levels, resulting in rapid establishment of local resistance and delayed development of systemic acquired resistance (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 (McDowell and Dangl, 2000). Ion fluxes subsequently induce extracellular production of reactive oxygen intermediates, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl free radical ($OH^\bullet$), catalyzed by a plasma membrane-located NADPH oxidase and/or apoplastic-localized peroxidases (Somssich and Hahlbrock, 1998). The initial transient reactions are, at least in part, prerequisites for further signal transduction events resulting in a complex, highly integrated signalling network that triggers the overall defensive response (Fig. 1). The role of calcium is shown in experiments with calcium channel inhibitors, which, preventing increases of cytosolic calcium concentrations, delaye the development of the hypersensitive response. Heterotrimeric GTP-binding proteins and protein phosphorylation/dephosphorylation are probably involved in transferring signals from the receptor to calcium channels that activate downstream reactions (Legendre et al. 1992). The changes in ion fluxes trigger localized production of reactive oxygen intermediates and nitric oxide, which act as second messengers for hypersensitive response induction and defense gene expression (Piffanelli et al., 1999). Synergistic interactions between reactive oxygen intermediates, nitric oxide and salicylic acid have been postulated (McDowell and Dangl, 2000). Other components of the signal network are specifically induced phospholipases, which act on lipid-bound unsaturated fatty acids within the membrane, resulting in the release of linolenic acid, which serves as a substrate for the production of jasmonate, methyl jasmonate and related molecules via a series of enzymatic steps.

Oxidative burst is a central component of plants‘ defense machinery (Lamb and Dixon, 1997; Alvarez et al., 1998). Reactive oxygen intermediates have been associated with apoptosis of mammalian cells, indicating a role in cell death during the hypersensitive response in plants (Heath, 1998; Richberg et al., 1998). This analogy is supported by the identification of a plant equivalent to the mammalian NADPH oxidase complex that produces respiratory burst in neutrophils (Keller et al., 1998). The burst of H_2O_2 production at the plant cell surface drives rapid peroxidase-mediated oxidative cross-linking of structural proteins in the cell wall, thereby reinforcing this physical barrier against pathogen ingress (Scheel, 1998). Additionally, low doses of reactive oxygen metabolites act as signals for the induction of detoxification mechanisms involving superoxide dismutases and glutathione-S-transferase, and activation of other defense reactions in neighbouring cells. As in mammalian phagocytosing cells, superoxide radicals are first generated and then rapidly converted to hydrogen peroxide and oxygen, probably by extracellular superoxide dismutase (Scheel, 1998).

Most of the inducible, defense-related genes are regulated by signal pathways involving one or more of the three regulators jasmonate, ethylene and salicylic acid (Delaney et al., 1994; Sticher et al., 1997; Van Loon, 1997; Reymond and Farmer, 1998;

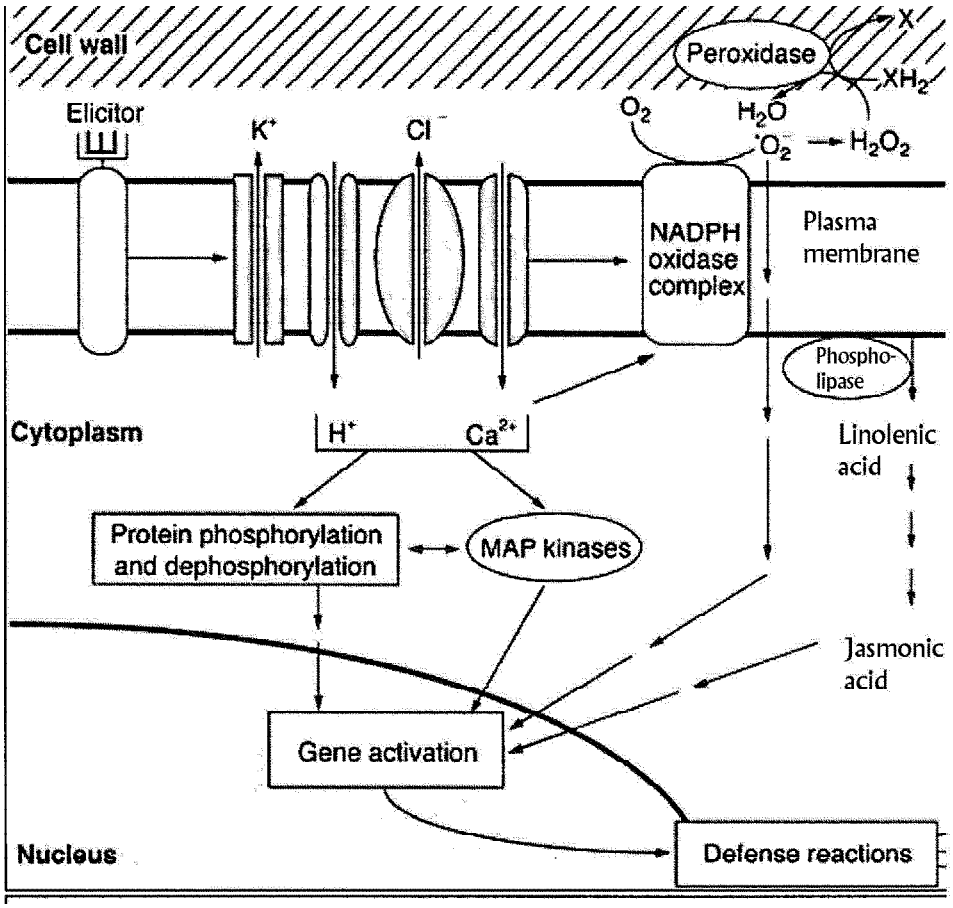

Fig. 1. Major components of the signal-transduction chain from elicitor perception to gene activation. Recognition of the elicitor by its plasma membrane receptor stimulates transient influxes of H^+ and Ca^+, and effluxes of K^+ and Cl^-. These jon fluxes are prerequisite for the activation of specific MAP (mitogen-activated protein) kinases and for the generation of reactive oxygen intermediates (the oxidative burst). Phosphorylation and dephosphorylation of some proteins is also observed. Binding of the elicitor stimulates also the generation of jasmonic acid via a membrane associated phospholipase. However, activation of jasmonate pathway is not essential for initiating the various defense responses (Somssich and Hahlbrock 1998).

Knoester et al., 1988; Ananieva and Ananiev, 1999). The exact role of ethylene as a defense regulator is not clear but it has been shown that this hormone preferentially induces basic pathogenesis-related proteins. Jasmonate is essential for the defense of tomato against tobacco hornworn larvae and for defense of *Arabidopsis* against fungal pathogens as *Phytum mastophorum* and the fly *Bradysia* (Reymond and Farmer, 1998). Jasmonate and ethylene co-operate to regulate the expression of many genes and at least some jasmonate-inducible genes are not inducible in plants unable

to produce or sense ethylene (Reymond and Farmer, 1998). During systemic acquired response salicylic acid levels rise throughout the plant. Defense genes such as pathogenesis-related genes are expressed, and the plant becomes more resistant to pathogen attack. Plants that cannot accumulate salicylic acid due to the presence of transgene that encodes salicylic acid-degradating enzyme develop hypersensitive response after challenge to avirulent pathogens, but do not exhibit systemic expression of defense genes and do not develop resistance to subsequent pathogen attack (Glazebrook, 1999). *Arabidopsis* mutants, compromised in their ability to respond to jasmonate or to produce salicylic acid, have been used to demonstrate that the two pathways are utilized differentially against contrasting modes of attack (McDowell and Dangl, 2000). The ethylene-jasmonate-dependent pathway is activated by pathogens that kill plant cells to obtain nutrients. In contrast, salicylic acid-dependent response is triggered by a pathogen that obtains nutrients from living plant tissue. It has been also suggested that ethylene-jasmonate and salicylic acid pathways are mutually inhibitory. Such cross-talk probably implies a capacity for a selective defense against specific types of parasites.

Changes in gene activity

Activation of signal transduction network after pathogen recognition results in reprogramming of cellular metabolism, involving large changes in gene activity. Plants contain many defense related proteins. In addition to resistance R genes and genes encoding signal transduction proteins, they possess downstream defense genes, such as pathogenesis-related proteins (PRs), enzymes involved in the generation of phytoalexins, the enzymes of oxidative stress protection, tissue repair, lignification, and others. It should be stressed that many of these genes are involved in secondary metabolism, such as shikimate and phenylpropranoid pathways (Somssich and Hahlbrock, 1998). Induction of defense gene transcripts is observed sometimes in the attacked cells, but mostly in surrounding plant tissues (Scheel, 1998). Accumulation of the pathogenesis-related proteins represents the major quantitative change in protein composition that occurs in noninoculated plant parts that, upon challenge, exhibit acquired resistance (Van Loon, 1997). Eleven pathogenesis-related protein families from different plant species have been characterized and classified according to sequence similarities (Fritig et al., 1998), although additional pathogen-induced proteins with potential anti-pathogenic action keep being described (Table 1). Within one family several members may share similar biological activities but differ substantially in other properties such as substrate specificity, physicochemical properties or subcellular localization. The inducible pathogenesis-related proteins are mostly acidic proteins that are secreted into the intercellular space (Van Loon, 1997). In addition, basic pathogen-related proteins occur at relatively low levels in the vacuole.

Table 1. Pathogen-related proteins in plants

Pathogen-related family	Activity	Pathogen target
PR-1	?	Membrane?
PR-2	1,3-β-glucanase	Cell wall glucan
PR-3	Endochitinase	Cell wall chitin
PR-4	Endochitinase	Cell wall chitin
PR-5	?	Membrane
PR-6	Proteinase inhibitor	Proteinase
PR-7	Proteinase	?
PR-8	Endochitinase	Cell wall chitin
PR-9	Peroxidase	*
PR-10	RNAase	?
PR-11	Endochitinase	Cell wall chitin
Unclassified	α-Amylase	Cell wall glucan
	Polygalacturonase Inhibitor (PGI)	Polygalacturonase

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

Most pathogen-related proteins have a damaging action on the structures of the parasite: PR-1 and PR-5 interact with the plasma membrane, whereas β-1,3-glucanases (PR-2) and chitinase (PR-3, PR-4, PR-8 and PR-11) attack β-1,3-glucans and chitin, which are components of the cell walls in most higher fungi. Pr-5 proteins are thought to create transmembrane pores and have therefore been named permatins. Chitinases can also display lysosyme activity and hydrolyze bacterial peptidoglycan. Microbial proteinases involved in pathogenesis are completely inhibited by a tobacco proteinase inhibitor. Plants also synthesize inhibitors of fungal polygalacturonases considered as pathogenicity factors. The PR-10 family has sequence similarity to ribonucleases and is the only family consisting of cytoplasmic proteins. In addition to pathogenesis-related proteins, small peptides with antimicrobial activity, such as thionins, defensins and lipid transfer proteins, also accumulate in infected plants and are probably components of the induced defense system (Bergey et al., 1996; Broekaert et al., 1997; Fritig et al., 1998).

The reprogramming of cellular metabolism comprises not only positive, but also negative regulatory mechanisms. For example, in potato the mRNA and protein levels of Rubisco are drastically reduced by pathogen infection or elicitor treatment. In parsley, the expression of several genes in cell proliferation and cell-cycle regulation and as well flavanoid biosynthesis, are repressed to a large extent during defense response (Somssich and Hahlbrock, 1998).

In conclusion, the complex picture of pathogen defense in plants is beginning to be elucidated, but a lot remains still unclear. A better understanding of the mechanisms of plant defense against pathogens might lead to improved strategies for enhancement of disease resistance in economically important plant species.

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