Role of Pathogenesis-Related (PR) Proteins in Plant Defense Mechanism

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Abstract

Plant growth and development is often challenged by several abiotic and biotic stresses, such as drought, cold, salinity, wounding, heavy metals, and pathogen attacks, respectively. A plant responds to these threats by activating a cascade of genes, encoding different effectors, receptors, and signaling and protective molecules. Among all, the induction and accumulation of pathogenesis-related (PR) proteins in plants in response to these adverse conditions is very important as PR proteins are an indispensible component of innate immune responses in plants under biotic or abiotic stress conditions. The PR proteins protect the plants from further infection by not only accumulating locally in the infected and surrounding tissues but also in remote uninfected tissues. Induction of PRs has been reported from many plant species belonging to different families suggesting a general role for these proteins in adaptation to biotic or abiotic stress conditions. PR proteins are also involved in hypersensitive response (HR) or systemic acquired resistance (SAR) against infection. Thus, PR proteins have been defined as "proteins encoded by the host plant but induced only in pathological or related situations," the latter inferring situations of nonpathogenic origin. In this chapter, structure, biochemistry, source, regulation of gene expression, and role in defense mechanism of various pathogenesis-related proteins will be discussed.

Keywords

Abiotic stress · PR proteins · Stress response · Biotic stress · Pathogenesis

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A. Singh, I. K. Singh (eds.), *Molecular Aspects of Plant-Pathogen Interaction*,
https://doi.org/10.1007/978-981-10-7371-7_12

12.1 Introduction

Plants, as sessile organisms, are often encountered by various abiotic and biotic stresses affecting their growth and agricultural yield. Plant stress tolerance and susceptibility are governed by a complex exchange of signals and responses collectively known by a general term, cellular stress response occurring under given environmental conditions in plants. So, the key difference between resistant and susceptible plants is the timely recognition of the invading pathogen or stress and the rapid and effective activation of host defense mechanisms.

Cellular stress response is a complex trait that happens due to the balanced coordination of physiological and biochemical alterations at the cellular and molecular level. These alterations could be the physical strengthening of the cell wall or through accumulation of various osmolytes, late embryogenesis abundant (LEA) and PR proteins. Physical strengthening of cell wall is often through lignification, suberization, and callose deposition. Cellular alterations mostly coupled with an efficient antioxidant system and prevent pathogen invasion by producing phytoalexins, phenolic compounds, and PR proteins. Various strategies acquired by plants during cellular stress responses serve the adaptive purpose of protecting a cell against unfavorable environmental conditions, both through short-term mechanisms that minimize acute damage to the overall cell's integrity and through long-term mechanisms which provide the cell a measure of pliancy against similar adverse conditions. For any organism sustainability, the cellular machinery must be activated in response to the various stresses, to ensure that the resources are used when required. Accordingly, plant cells have evolved to perceive different signals from their surroundings, to integrate them, and to respond by modulating the appropriate gene expression that may involve protein phosphorylation, ion fluxes, reactive oxygen species (ROS), and other singling events. A diverse array of plant protectants and defense genes get activated whose products include glutathione S-transferases (GST), peroxidases, proteinase inhibitors, cell wall proteins, hydrolytic enzymes (e.g., chitinases and β-1,3-glucanases), pathogenesis-related (PR) proteins, and phytoalexin biosynthetic enzymes, like phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) (Hammond-Kosack and Jones 1996). Among all, the synthesis and activation of pathogenesis-related (PR) proteins is very critical in response to any stress situation and/or invading pathogen.

During incompatible host-pathogen interactions, the plant's defensive responses restrict the damage caused by the pathogen. Subsequent infection by different types of pathogens is often limited by the defensive responses that is associated with a coordinated and integrated set of metabolic alterations. Further, various novel proteins are synthesized and induced which are collectively known as "pathogenesis-related proteins" (PRs). Pathogen-related proteins (PRs) have been defined as "proteins encoded by the host plant but induced only in pathological or related situations," the latter referring situations of nonpathogenic origin (Antoniw and Pierpoint 1978). PRs have not been identified because of their anti-pathogenic action, but solely because of their easily identification in infected plants. A number of PRs have been reportedly induced in many plant species belonging to various families suggesting a general protective role of PRs against biotic stress (Van Loon 1999). PRs not

only accumulate locally in the infected tissue but are also induced systemically. Thus, PRs are associated with the development of systemic acquired resistance (SAR) or hypersensitive response (HR) against further infection by pathogenic fungi, bacteria, and viruses. HR is characterized by necrotic lesions resulting from localized host cell death at the site of infection (Goodman and Novacky 1994). Moreover, plants respond to pathogen infection by activating defense responses in uninfected parts of the plant. As a result, the whole plant develops resistance to subsequent infections. This systemic acquired resistance (SAR) is a generally occurring phenomenon and often confers broad-based resistance to a variety of different pathogens (Ryals et al. 1996; Delaney 1997) instigating the defensive capacity of plants in response to necrotizing infections. Over the decades, a number of reports depicted the role of different classes of PR proteins during abiotic and biotic stresses and their defense responses in plants; however, the mechanism of action is sparsely described. In this chapter, we will be briefly discussing the biochemistry, source, regulation of gene expression, and mode of action of PR proteins in defense mechanisms.

12.2 PR Proteins: An Overview

PR proteins comprise a huge family of proteins ubiquitous in the plant kingdom. Plant PR proteins were first identified and reported in tobacco plants infected by tobacco mosaic virus (Van Loon and Van Kammen 1970). Later, these proteins were reported in many different plant species. Most plant PR proteins share the common biochemical properties of being acid soluble, low molecular weight, and protease resistance (Leubner-Metzger and Meins 1999; Neuhaus 1999). PR proteins have similar functions, but depending on their isoelectric points, they may be acidic or basic proteins. Most acidic PR proteins are secreted in the extracellular spaces, whereas basic PR proteins are predominantly found in the vacuole (Legrand et al. 1987; Niki et al. 1998) through the signal peptide at C-terminus. However, such localization cannot be generalized for all PR proteins. Though PRs are most abundant in the leaves, they are detected in almost all plant organs including leaves, stems, roots, and flowers. Usually, acidic PRs are upregulated by various signaling molecules like salicylic acid (Yalpani et al. 1991; Sinha et al. 2014) and reactive oxygen species (Chamnongpol et al. 1998), while basic PRs are upregulated by gaseous phytohormone ethylene and methyl jasmonate (Xu et al. 1994) during pathogen attack. Apart from various environmental factors, there are certain internal developmental factors too that trigger the synthesis of these PR proteins.

Based on molecular mass, isoelectric point, localization, and biological activity, the PR proteins have been categorized into 17 families (Table 12.1), including β-1,3-glucanases, chitinases, thaumatin-like proteins, peroxidases, ribosome-inactivating proteins, thionins, nonspecific lipid transfer proteins, oxalate oxidase, and oxalate-oxidase-like proteins (Van Loon and Van Strien 1999) and numbered in the order in which they were discovered. When dealing with a stress-related sequence possibly related with PRs, it is necessary to gather information at both the nucleic acid and the protein level as homologies at the cDNA, or genomic level may be encountered without information on the expression of the encoded protein.

Table 12.1 Classification of pathogenesis-related proteins

Families	Properties	Example
PR-1	Antifungal	Tobacco PR-1a
PR-2	**β**-1,3-Glucanase	Tobacco PR-2
PR-3	Chitinase type I, II, IV, V, VI, VII	Tobacco P, Q
PR-4	Chitinase type I, II	Tobacco "R"
PR-5	Thaumatin-like	Tobacco S
PR-6	Proteinase inhibitor	Tomato inhibitor I
PR-7	Endoproteinase	Tomato P69
PR-8	Chitinase type III	Cucumber chitinase
PR-9	Peroxidase	Tobacco "lignin-forming peroxidase"
PR-10	Ribonuclease-like	Parsley "PR1"
PR-11	Chitinase, type I	Tobacco "class V" chitinase
PR-12	Defensin	Radish Rs-AFP3
PR-13	Thionin	Arabidopsis THI2.1
PR-14	Lipid transfer protein	Barley LTP4
PR-15	Oxalate oxidase	Barley OxOa (germin)
PR-16	Oxalate oxidase-like	Barley OxOLP
PR-17	Unknown	Tobacco PRp27

Though such sequences belong to the PR-type families, they are to be named PR-like proteins (PRLs) and cannot be considered to correspond to pathogen-induced PRs (Van Loon et al. 1994). Among these PRLs, chitinases and β-1,3-glucanases are two important hydrolytic enzymes that accumulate in many plant species after infection by different types of pathogens. These hydrolytic enzymes play the main role of defense reaction against fungal pathogen by degrading cell wall, because chitin and β-1,3-glucan are major structural components of the cell walls of many pathogenic fungi. Some of the tobacco PRs were characterized as chitinases and β-1,3-glucanases (Kauffmann et al. 1987) with potential antifungal activity suggested the group of PRs might be inhibiting pathogen growth and be responsible for the SAR. In spite of their common name, PR proteins show a great diversity in species specificity and in the mechanism of action and do not share any structural relationship among themselves.

PR proteins unveil diverse functions within the plant. Many PRs exhibit antifungal activity (Caruso et al. 1996) though few of the PR proteins also show antibacterial, insecticidal, nematicidal, and antiviral activity (Edreva 2005). PR proteins thus have a critical role in disease resistance, seed germination, and plant facilitation to adapt to the environmental stress.

12.2.1 Plant Chitinases

Chitinases (E.C. 3.2.1.14) are widely distributed across plant, animal, fungi, and bacteria kingdoms. These enzymes catalyze the cleavage of a bond between C1 and C4 of two consecutive N-acetyl-D-glucosamine monomers of chitin which is a

common component of fungal cell walls and of the exoskeleton of arthropods (Bartnicki-Garcia 1968). Usually the plant chitinases are endo-chitinases capable of degrading chitin as well as inhibit fungal growth (Schlumbaum et al. 1986; Broekaert et al. 1988). Chitinases are either localized in the vacuole (Class I) or outside the cell (Class III) (Neuhaus et al. 1996). Many reports strongly indicated that chitinases, together with β-1,3 glucanases, play critical role in the plant defense response against fungal pathogens (Abeles et al. 1971).

Plant chitinases have been classified into seven classes, class I through VII, based on their primary structures. Certain isoforms of chitinases are induced by particular elicitors, and only few isoforms have antifungal activities, while some isoforms have shown another role like antifreeze activity (Sela-Buurlage et al. 1993; Yeh et al. 2000). Class I chitinases have a cysteine-rich N-terminal chitin-binding domain (CBD) that is homologous to havein, a chitin-binding lectin from the rubber tree (Suarez et al. 2001). Class II chitinases are similar to class I but they lack the N-terminal CBD. Class III chitinases are unique in structure and belong to the PR-8 family and family 18 of glycosyl-hydrolases. Class III chitinases are more closely related to the bacterial chitinases and generally have lysozyme activity. Class IV, V, VI, and VII chitinases belong to the PR-3 family of proteins (Meins et al. 1994).

Various biotic and abiotic factors have been known to induce chitinases in the plant. Various studies reported the inhibitory effect of plant chitinases on fungal growth by demonstrating on the growth of chitin-containing fungi (Mauch et al. 1988a, b). Various studies showed that chitinase expression is induced against phytopathogen systems, and resistant varieties have stronger upregulation than susceptible varieties in the sugar beet (Nielsen et al. 1992), wheat (Anguelova-Merhar et al. 2001), and tomato (Lawrence et al. 2000). Transformation of chitinase genes was performed in tobacco (Brogue et al. 1991), grapevine (Yamamoto et al. 2000), rice (Datta et al. 2001), and peanut (Rohini and Rao 2001), and enhanced disease resistance has been achieved. Overexpression studies have been made with chitinase genes, alone or together with β-1,3-glucanase genes in a number of plant species, and in most cases, the resulting transgenic plants exhibited enhanced levels of fungal disease resistance or delayed symptom development as compared to the control plants (Zhu et al. 1994; Jach et al. 1995; Jongedijk et al. 1995). However, several studies showed that plants transformed with either chitinase or β-1,3-glucanase gene alone did not exhibit resistance to certain pathogens or showed less resistance. Plant chitinases alone are unable to effectively degrade harder chitin structures of fungi as they usually affect only the hyphal tip, but when coexpressed with β-1,3-glucanase, these two enzymes act synergistically against fungal pathogen.

12.2.2 Plant β-1,3-Glucanases

Plant β-1,3-glucanases belong to the PR-2 family of pathogenesis-related proteins and reportedly play an important role in plant defense responses to pathogen infection. These enzymes have been identified across plants, yeasts, actinomycetes, bacteria, fungi, insects, and fish (Pan et al. 1989). These enzymes catalyze the cleavage of the

β-1,3-glucosidic bonds in β-1,3-glucan (Simmons 1994) which is another major structural component of the cell walls of many pathogenic fungi (Adams 2004). β-1,3-Glucanases play an important role in plant defense and other physiological functions such as cell division and cell elongation (Fulcher et al. 1976), fruit ripening (Meins et al. 1992), pollen germination and tube growth (Meikle et al. 1991), fertilization (Ori et al. 1990), somatic embryogenesis (Helleboid et al. 2000), seed germination (Buchner et al. 2002), and flower formation (Akiyama et al. 2004). The role of plant β-1,3-glucanases as an important component of plant defense mechanisms against pathogens has been well documented (Legrand et al. 1987; Cordero et al. 1994). It has been postulated that β-1,3-glucanases hydrolyze fungal cell walls, which consequently causes the lysis of fungal cells when defending against fungi. On the pathogen encounter, β-1,3-glucanases also cause the formation of oligosaccharide elicitors, which elicit the production of other PR proteins or low molecular weight antifungal compounds, such as phytoalexins (Klarzynski et al. 2000).

β-1,3-Glucanase genes have been reported from a wide range of plant species, and many studies have shown that the synthesis of β-1,3-glucanases is stimulated by pathogen infections (Alonso et al. 1995; Roulin et al. 1997) and can change during plant development (Wyatt et al. 1991). Different plant species may have different β-1,3-glucanase genes, and a single plant species may have various copies of β-1,3-glucanase genes. Plant β-1,3-glucanases were classified into two major classes I and II and two minor classes based on their amino acid sequence, structural properties, and cellular localizations (Beerhues and Kombrink 1994). Generally, β-1,3-glucanases are stress regulated, but a few β-1,3-glucanases are exclusively developmentally regulated and do not show a stress-related regulation (Bucciaglia and Smith 1994; Sharma 2013). β-1,3-Glucanases are usually expressed at low concentration in plants, but when plants are challenged by fungal, bacterial, or viral pathogens, β-1,3-glucanases enzyme accumulate dramatically (Castresana et al. 1990; Lusso and Kuc 1995). Class I β-1,3-glucanases and class I chitinases showed synergistic effect in pathogen defense. Class I β-1,3-glucanase accumulated only at the site of tobacco mosaic virus (TMV) infection in tobacco plants, while class II and III β-1,3-glucanases accumulated both at the site of infection and systemically (Vögeli-Lange et al. 1994; Livne et al. 1997). Many reports showed that the transcript levels of glucanases accumulated after infected with pathogens, such as barley infected by powdery mildew (Ignatius et al. 1994), maize infected with *Aspergillus flavus* (Lozovaya et al. 1998), pepper infected with *Xanthomonas campestris* pv. *vesicatoria* and *Phytophthora capsici* (Jung and Hwang 2000), wheat infected with *Fusarium graminearum* (Li et al. 2001), chickpea infected with *Ascochyta rabiei* (Pass.) Labr. (Hanselle and Barz 2001), and peach infected with *Monilinia fructicola* (Zemanek et al. 2002). β-1,3-Glucanases and other PR protein induction in the plant can also occur due to some elicitors, including fungal β-glucan, chitin, chitosan, glycoproteins, and N-acetylchito oligosaccharides (Chang et al. 1992; Kaku et al. 1997), or by other factors, for example, salicylic acid-induced accumulation of mRNAs of class II and III β-1,3-glucanases in wild-type tobacco plants (Ward et al. 1991), abscisic acid (ABA) in tobacco (Rezzonico et al. 1998),

and methyl jasmonate, ethylene, and gibberellin A3 in tomato seeds and leaves (Wu and Bradford 2003). Stress factors like wounding, drought, exposure to heavy metals, air pollutant ozone, and ultraviolet radiation can also upregulate β-1,3-glucanases in some plants (Thalmair et al. 1996; Fecht-Christoffers et al. 2003).

12.3 Role of PR Proteins in SAR and HR

PRs are most common in hypersensitive responses but appear to contribute to SAR also. An induced systemic resistance (ISR) can be induced by nonpathogenic rhizobacteria, considerably modified the relationships between necrotic lesion formation, PRs, and SAR. ISR induction with these rhizobacteria shows no symptoms in plants; however, this resistance is independent of the production of salicylic acid (SA) by the plant and is not associated with the accumulation of PRs (Pieterse et al. 1996; Van Loon et al. 1998). This indicates that plants can substantially enhance their defensive capacity against variety of pathogens in either SA-dependent or independent way (Pieterse and Van Loon 1999). SAR is mainly SA-dependent (Ryals et al. 1996) while ISR is SA-independent. Until now the mechanism involved in ISR has been unclarified. At least in *Arabidopsis*, similar to SAR, ISR depends on the functioning of the *npr1* gene, which in turn distinguishes ISR from the JA- and ethylene-dependent inducible defense response pathway effective against *Alternaria brassicicola*, which is independent of *npr1*. PRs are often associated with SAR, but not with ISR, which have led to hypothesize that PR accumulation is not a prerequisite for the induction of resistance, but they contribute to the protective state (Van Loon 1997). The JA- and ethylene-dependent pathway induced by, and effective against, *A. brassicicola* involves increase in SA, JA, and ethylene levels resulting in detection of PRs in the infected plants. The differential expression of various PRs determines the extent of the plant's response and its effectiveness to inhibit further infection. Recent report shows that SA-dependent expression of PR-1, PR-2, and PR-5 is required for increased protection against the biotrophic fungus *Peronospora parasitica* in *Arabidopsis*, whereas SA-independent but JA-dependent induction of PR-3 and PR-4 is associated with the induced resistance against the necrotrophic fungi *A. brassicicola* (Penninckx et al. 1996), *Botrytis cinerea* (Thomma et al. 1998), and *Fusarium oxysporum* f.sp. *matthiolae* (Bohlmann et al. 1998). These results suggest that PRs appear to contribute differentially to the induced resistance against different pathogens.

12.4 Signaling Involved in Pathogen-Induced Expression of PRs

The pathogen-activated PR gene expression plays a critical role in plant defense against pathogens. Regulation of PR gene expression has always been a highly active research area since the time PR proteins were discovered. However, the signaling behind the

pathogen-induced PR gene expression is still poorly understood in plants. This is partly due to the complexity of environmental stimuli and stimulation by phytohormones that can induce the expression of various PR genes (Brederode et al. 1991).

12.5 Signals and Putative Receptors Involved in PR Gene Expression

During plant-pathogen interactions, a number of molecules derived from pathogens can serve as elicitors for PR gene induction such as chitin fragments and glucans from fungal cell wall, extracellular glycoproteins/peptides from few fungal species, oligosaccharides and harpins from bacteria, and Avr proteins derived from bacterial and fungal pathogens (Boller and Felix 1996). Though a large number of signals are known to induce PR gene expression, no receptors have been unambiguously established for these signal molecules. For example, β-glucan elicitor (GE) is released from *Phytophthora sojae* cell wall by β-1,3-glucanase from soybean and reportedly induces phytoalexin biosynthesis (Darvill and Albersheim 1984). Recently, a GE-binding protein (GEBP) has been purified from soybean whose antiserum partially inhibited the binding of GE to soybean membrane proteins and reduced the phytoalexin accumulation elicited by GE. Another set of elicitors is the polypeptide encoded by pathogen avirulence *(avr)* genes. Any pathogen containing a particular *avr* gene is recognized by the corresponding resistance *(R)* gene of the host plant and activates a variety of defense responses in the host, including increased PR gene expression. In the past decade, a number of *R* genes and *avr* genes have been isolated. For example, an elicitor encoded by the *avr9* gene from *Cladosporium fulvum*, a fungal pathogen of tomato, rapidly activated the transcription of glucanase and chitinase genes in plants carrying the cognate *R* gene *Cf9* (Ashfield et al. 1994; Wubben et al. 1996). Another fungal elicitor, NIP1 protein from the barley pathogen *Rhynchosporium secalis* is known to activate PR genes (Rohe et al. 1995). The only evidence that an Avr protein directly interacts with an R gene product comes from the study of bacterial speck disease in tomato. Tomato plants having *R* gene *Pto*, encoding cytoplasmic kinase, are resistant to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* carrying the *avrPto* gene (Martin et al. 1993). The Pto and AvrPto proteins showed a highly specific association in yeast too (Scofield et al. 1996; Tang et al. 1996). The results confirmed Pto as the receptor for the AvrPto protein.

12.6 Different Pathways for PR Genes' Activation by Pathogens

Plants often exhibit increased production of reactive oxygen species (ROS), salicylic acid (SA), ethylene, and jasmonates upon infection by pathogens (Hammond-Kosack and Jones 1996; Yang et al. 1997). These molecules may serve as secondary signals to activate plant defense, and many of these reportedly work as inducers for PR gene expression. For example, SA induces acidic PR genes that are normally

activated during SAR, whereas ethylene and jasmonates are known to induce proteinase inhibitors, defensin, thionin, and basic PR proteins (Brederode et al. 1991; Ward et al. 1991; Epple et al. 1995; Donnell, et al. 1996; Penninckx et al. 1996). However, the involvement of secondary messengers in the PR gene induction is uncertain in majority of studies. Cross talks are often common between signaling pathways mediated by these secondary messengers. The use of signaling pathway mutants would be supportive in clarifying the roles of these secondary messengers in plant defense responses against pathogen attacks.

12.7 Functions and Relevance of PR Expression in Disease Resistance

In the last decades, proteinase, peroxidase, ribonuclease, and lysozyme activities were assigned to PR-7, PR-9, PR-10, and PR-8, respectively. Also, membrane-permeabilizing functions are characteristic of defensins (PR-12), thiols (PR-13), lipid transfer proteins (LTPs, PR-14), and of osmotins and thaumatin-like proteins (PR-5). Multiple enzymatic, structural, and receptor functions are reported in germins (PR-15) and germin-like proteins (PR-16) (Van Loon and Van Strien 1999; Bernier and Berna 2001; Selitrennikoff 2001; Park et al. 2004a, b). Besides this, some PRs also exhibited antibacterial, insecticidal, nematicidal, and antiviral action, though an important common feature of most PRs is their antifungal effect. Their hydrolytic, proteinase inhibitory, and membrane-permeabilizing ability made them toxic to pathogens. Thus, hydrolytic enzymes (β-1,3-glucanases, chitinases, and proteinases) can effectively weaken and decompose fungal cell walls, containing glucans, chitin, and proteins, while PR-8 can damage gram-positive bacteria due to lysozyme activity (Van Loon and Van Strien 1999; Selitrennikoff 2001). The defensive functions of PRs against pathogens can be attributed to a number of their ingenious properties; their constitutive expression in seeds and plant organs, high fungitoxicity of seed osmotins and thaumatin-like proteins (Vigers et al. 1992; Abad et al. 1996), their accumulation in plant cell wall appositions formed against pathogen invasions (Jeun 2000; Jeun and Buchenauer 2001). Inspite of all studies and reports, the defensive mechanism of PR function against pathogen attack is still unclear. The protective role of PRs is supported by following evidences:

(a) *Transcript accumulation of PRs in pathogen-tolerant and susceptible plants*. Recently, the differential responses of resistant/susceptible plants were reported in tomato plants, inoculated with *Cladosporium fulvum* (Wubben et al. 1996), *Phytophthora infestans*-infected potato (Tonón et al. 2002), *Venturia inaequalis*-inoculated apple (Poupard et al. 2003), *Pseudomonas syringae*-infected grapevine (Robert et al. 2001), *Xanthomonas campestris* pv. *vesicatoria*, and TMV-Po-infected hot pepper (Park et al. 2004a, b).
(b) *The plants with high natural disease resistance constitutively express PRs*. This correlation has been proved in many pathosystems, such as apple-*Venturia*

inaequalis (Gau et al. 2004), tomato-*Alternaria solani* (Lawrence et al. 2000), and potato-*Phytophthora infestans* (Vleeshouwers et al. 2000).

(c) *Overexpressing PRs in transgenic plants results in increased resistance to pathogens*. Tobacco overexpressing *PR1a* gene showed increased tolerance to *Peronospora tabacina* and *Phytophthora parasitica* var. *nicotianae* (Alexander et al. 1993). Similarly, overexpression of thaumatin-like PR-5 in transgenic rice and orange plants showed increased tolerance to *Rhizoctonia solani* and *Phytophthora citrophthora*, respectively (Datta et al. 1999, Fagoaga et al. 2001); transgenic potato overexpressing PR-2 and PR-3 had improved resistance to *Phytophthora infestans* (Bachmann et al. 1998); transgenic carrot overexpressing PR-2 and PR-3 genes, coding for β-1,3-glucanase and chitinase, respectively, showed increased resistance to several fungal pathogens; and the transgenic tomato simultaneously expressing tobacco β-1,3-glucanase and chitinase genes had improved resistance to fungal pathogens (Melchers et al. 1998).

(d) *Accumulation of PRs in plants with locally or systemically induced resistance*. As discussed before, PRs are identified as markers of the systemic acquired resistance (SAR). SAR and the associated set of PRs are induced by different pathogens and various chemicals predominantly in a salicylic acid-dependent pathway. It is important to note that the direct role of PRs in disease resistance is being suggestive by their high expression in resistant or SAR-expressing plants, as well as transgenic resistant plants exhibiting high antimicrobial activity (Rauscher et al. 1999; Tonón et al. 2002; Anand et al. 2004).

12.8 Transcriptional Regulation of PR Gene Expression

The most active area in PR gene research is to study its transcriptional regulation. Several *cis*-regulatory elements mediating pathogen-induced PR gene expression have been identified and characterized through traditional promoter deletion analysis coupled with mutagenesis of putative regulatory elements, gain-of-function studies with synthetic promoters, and DNA-fingerprinting analysis (Yang et al. 1997; Rushton and Somssich 1998). Many of these elements are W-box (consensus TTGACC or TGAC-[N]x-GTCA), GCC-box (consensus AGCCGCC), MRE-like sequence (consensus A[A/C]C[A/T]A[A/C]C), G-box (consensus CACGTG), and SA-responsive element (SARE, with a consensus of TTCGACCTCC). Among all, the GCC-box and W-box have been extensively studied and have a wide role in PR gene regulation (Hart et al. 1993; Ohme-Takagi and Shinshi 1995). Defense responses mediated by ethylene are often associated with GCC-box and are known to confer ethylene-induced transcription of the tobacco *gln2* gene encoding a β-1,3-glucanase (Ohme-Takagi and Shinshi 1995). EREB clones (1–4) were identified in tobacco cDNA library with radiolabeled GCC-box as probe (Ohme-Takagi and Shinshi 1995). EREBP transcripts are induced by ethephon, a compound known to

release ethylene upon its degradation that suggests that ethylene further induces the expression of EREBP genes. The EREBP-1 gene was reportedly induced by *Pseudomonas* bacteria and SA, suggesting a role of this gene in plant defense (Zhou et al. 1997; Horvath et al. 1998). The direct correlation of the EREBP proteins with a disease-resistance pathway was confirmed by the study of the signaling pathway mediated by the tomato gene *Pto* (Zhou et al. 1997). Phosphorylation also plays an important role in the activation of PR gene expression during pathogen attacks (Raz and Fluhr 1993). Another highly conserved *cis*-element, W-box, is present in parsley *PR1-1* and *PR1-2* (both encoding the PR1 protein), tobacco *CHN50* (encoding a class I basic chitinase), asparagus *AoPR1* (encoding the PR10 protein), potato *PR-10a* (encoding the PR10 protein), and maize *PRms* (encoding the PR1 protein). Besides PRs, W-box is also present in the promoter of other pathogen inducible genes such as the potato glutathione S-transferase gene *prp1* and the grape phytoalexin synthesis gene *Vst1*, suggesting a broader role for this element in pathogen-induced gene expression (Rushton and Somssich 1998). Three parsley cDNAs encoding W-box-binding proteins were identified by using a south-western screening (Rushton et al. 1996). These proteins are termed WRKY family proteins and contain the consensus sequence, WRKYGQK. Since the in vivo function of the cloned transcription factors is still to be worked upon, so rigorous tests on transgenic plants with altered expression of the transcription factor genes are required to establish their roles in PR gene expression and defense responses. In addition, it is necessary to answer few questions. How different signals affect PR gene expression? Do different signals converge on the same transcription factor? Do these transcription factors interact? Answering these questions help us in better understanding of cross talks between different signaling pathways.

12.9 Conclusions

PR proteins and their homologues are generally responsible for the defense against various stresses including pathogen attacks, wounding, use of chemicals, and pollutants. However, many PR proteins (members of PR 1, 2, 3, 4, 5, 8, 10, and 14 families) have demonstrated allergenicity, but the allergenicity is also guided by several environmental factors like the use of chemical inducers in agriculture and environmental pollutants. Recent reports have documented their critical importance as preservative agents in food industry and for producing disease-resistant plants by genetic engineering. Various studies have revealed that transgenic plants overexpressing PR genes mediate host plant resistance to phytopathogenic fungi. Such genetically modified (GM) plants with enhanced expression of PR proteins may be associated with increased allergenicity and toxicity, thus raising a serious question for their commercial acceptability, so different strategies are adopted to monitor the transformed crops for their allergenicity.

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