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REVIEW ARTICLE

Plant Defense Proteins

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Plants are compelled to withstand stresses of all kinds, be it biotic, abiotic or anthropogenic as a consequence of their immobility. The initial infection process involving adhesion/recognition events between plants and fungal pathogens is essential for the establishment of pathogenesis (Chakraborty,1988). The extracellular matrix (ECM) is a biologically active part of the cell surface composed of a complex mixture of macromolecules that, in addition to serving a structural function, profoundly affect the cellular physiology of the organism (Roberts, 1989; 1990). During the past two decades it has become evident that the cell wall is a dynamic organization that is essential for cell division, enlargement and differentiation as well as responding to biotic and abiotic stress. ECM is also the source of signals for cell recognition within the same or between different organisms (Brownlee, 2002). Cell walls are natural composite structures, mostly made up of high molecular weight polysaccharides, proteins and lignins (Fry, 2004). Lignins are only found in specific cell types. *Arabidopsis thaliana* cell wall proteins (CWP) that can be involved in modifications of cell wall components, wall structure and signaling as well as interactions with plasma membrane proteins at the cell surface has been reviewed (Jamet *et.al*, 2006). All available *Arabidopsis* cell wall proteome data were screened using bioinformatic tools to select only those proteins containing a signal peptide but devoid of known retention signals for the endomembrane system. These 281 proteins from the database of CWP were assembled in functional classes and subclasses according to the presence of functional domains in the protein.

The defense strategy of plants against stress factors involves a multitude of tools, including various types of stress proteins with putative protective functions, A group of plant-coded proteins induced by different stress stimuli, named "pathogenesis-related (PR) proteins" is assigned an important role in plant defense against pathogenic constraints and in general adaptation to stressful environment (Datta and Muthukrishnan, 1999; Chakraborty, 2005). Such protective plant proteins specifically induced in pathological or related situations have been intensively studied from the agricultural interest. On the other hand, many of the reserve proteins accumulated in seeds and fruits take the constitutive defense function against microbial pathogens and invertebrate pests in addition to their storage function. These inducible or constitutive defense mechanisms of higher plants are relatively conserved in the course of evolution. Accordingly, most plants produce or accumulate similar proteins under certain situations irrespective of their morphological differences.

Terminology

The introduction of polyacrylamide electrophoresis by which proteins could be separated on the basis of their combination and charge was a chief innovation in unearthing PR-proteins - the new protein first observed in tobacco, (*Nicotiana tabacum*) cultivars reacting hypersensitively to tobacco mosaic virus (TMV). Since the discovery of pathogenesis related proteins (initially named as "b" poteins) by two independently investigating groups (Van Loon and Van Kammen, 1970; Gianinazzi *et.al*, 1970) have focused an increasing research interest in view of their possible involvement in plant resistance to pathogens. Since the proteins were induced under specific pathological conditions, they were named pathogenesis-related proteins abbreviated as PRps. By definition, PRps were described as "proteins coded for by the host plant but induced specifically only in pathological or related situations". Later, however, it turned out that this proteins are induced not only in resistant, but also in susceptible plant-pathogen interaction, as well as in plants, subjected to abiotic stress factors. To be included among the PRps, a protein has to be newly expressed upon infection but not necessarily in all pathological conditions. Pathological situations refer to all types of infected states, not just to resistant, hypersensitive responses in which PRps are most common; they also include parasitic attack by nematodes, insects and herbivores. Induction only by abiotic stress conditions is not a sufficient criterion for inclusion as a PRps. These considerations imply that the characteristics of the induction of PRps take priority over other identifying features, such as chemical properties or cellular localization.

Occurrence and properties of PR-proteins

The occurrence of these proteins was not pathogen specific but determined by the type of reaction of the host plant, indicating that these proteins were of host origin. PRps are most often of low molecular weight, selectively extractable in low *p*H, highly resistant to proteolytic degradation/or endogenous proteases and localized predominantly in the intercellular space. PRps were found in small amounts in senescing leaves of flowering plants and in relatively larger quantities when necrosis was more severe. This led to the assumption that these polypeptides were stable proteolytic breakdown products of larger leaf proteins. PRps that have been found in many plant species (Table-1) can be classified into seventeen families.

Table 1. Plant species in which PR-proteins have been identified

Plant species	Family
Arabidopsis thaliana	Cruciferae
Brassica nigra	Cruciferae
Brassica napus	Cruciferae
Camellia sinensis	Theaceae
Chenopodium amaranticolor	Chenopodiaceae
Chenopodium quinoa	Chenopodiaceae
Citrus sinensis	Rutaceae
Glycine max	Papilionaceae
Lablab purpureus	Papilionaceae
Gomphrena globosa	Amaranthaceae
Picea abies	Pinaceae

On the basis of similarities in molecular weights, amino acid composition, and serological properties, and confirmed by nucleotide sequencing of corresponding cDNAs, the 10 major acidic PRps of tobacco were grouped into five families, designated PR-1 to PR-5. This classification has set a standard for other plant species, in which PRps with properties homologous to the tobacco PRps are now similarly designated by these family numbers.

To accommodate further classes of PRps with different properties, additional families were adopted. The criteria for inclusion of new families of PRps were (i) protein(s) must be induced by a pathogen in tissues that do not normally express the protein(s), and (ii) induced expression must have shown to occur in at least two different plant-pathogen combinations, or expression in a single plant-pathogen combination must have been confirmed independently in different laboratories. Each PR-family is numbered and the individual family members are assigned lower case letters in the order in which they are described. In accordance with the recommendations of the Commission for Plant Gene Nomenclature, PR-genes are designated as *ypr*, followed by the same suffix as of the family. Later on three more peptides, which were capable of inducing defense responses of plants, were identified. Based on the grouping of PRps into plant-wide families sharing amino acid sequences, serological relationship, enzymatic or biological activity seventeen families of PRps have been recognized (Table 2).

Table 2. Recognized and proposed families of pathogenesis-related proteins

Family	Type member	Properties	Gene symbols
PR-1	Tobacco PR-1a	Antifungal?14-17kD	*ypr1*
PR-2	Tobacco PR-2	Class 1,II and III endo-β 1,3-glucanase, 25-35kD	*ypr2*
PR-3	Tobacco P,Q	Class I, II, IV, V, VI and VII endochitinases about 30kD	*ypr3*
PR-4	Tobacco R	Antifungal, *win*-like proteins endochitinase activity, 13-19kD	*ypr4*
PR-5	Tobacco S	Antifungal, thaumatin-like Proteins, osmoins, zeamatins	*ypr5*
PR-6	Tomato inhibitor I	Proteinase inhibitor, 6-13kD	*ypr6*
PR-7	Tomato P	Endoproteases	*ypr7*
PR-8	Cucumber chitinase	Class III chitinase	*ypr8*
PR-9	Tobacco"lignin-forming peroxidase"	Peroxidase	*ypr9*
PR-10	Parsley PR-1	Ribonucleases	*ypr10*
PR-11	Tobacco classV Chitinase	Endochitinase Class I	*ypr11*
PR-12	Radish Ps-AFP3	Plant defensins	*ypr12*
PR-13	*Arabidopsis* THI2.1	Thionins	*ypr13*
PR-14	Barley LTP4	Nonspecific lipid transfer Proteins (ns-LTPs)	*ypr14*
PR-15	Barley OxOa	Oxalate oxidase	*ypr15*
PR-16	Barley OxOLP	Oxalate-oxidase-like proteins	*ypr16*
PR-17	Tobacco PRp27	Unknown	*ypr17*

Besides proteins newly defined mRNAs (cDNAs) are often considered as additional members of the existing families where shown to be induced by pathogens or specific elicitors. Thionins and defensins both families of small basic, cysteine-rich polypeptides, qualify for inclusion as new families of PRps. However, because PRps are generally defined by their occurrence as protein bands on gels, and classified within each family once the protein has been characterized, cDNA or genomic sequences without information on the corresponding protein cannot be fitted in to the adopted nomenclature. Thus for naming it is necessary to gather information at both the nucleic acid and the protein level when dealing with a stress-related sequence falling within the definition of PRps. Conversely, homologies at the cDNA or genomic level may be encountered without information on the expression or characteristics of the encoded protein. Such sequences obviously belong to the PR-type families, but yet cannot, be considered to correspond to pathogen-induced PRps and named accordingly. In more than a few situations, it is difficult to distinguish PRps from related proteins/ mRNAs that are present in some organs or appear during specific developmental stages. Homologous proteins/ mRNAs in healthy tissues in which no induction by pathogen infection has yet been demonstrated, are to be termed PR-like proteins (PRLs) and their genes *yprl*.

Induction

Besides the known PRps inducers of biotic origin (pathogen, insects, nematodes, herbivores), a new type of biotic inducers, *Orobanche* weeds, has been reported in tobacco. Pathogen-derived elicitors are potent PRps inducers. Well characterized are glucan and chitin fragments derived from fungal cell walls, fungus-secreted glycoproteins, peptides and proteins of elicitin family (Edreva *et.al*, 2002). Protein products of avirulence genes in fungi and bacteria are capable of PRps induction. Chemicals, such as salicylic, polyacrylic and fatty acids, inorganic salts as well as physical stimuli (wounding, UV-B radiation, osmotic shock, low temperature, water deficit and excess), are involved in PRps induction. A special class of PRps inducers are hormones (ethylene, jasmonates, abscisic acid, kinetin, auxins). Reactive oxygen species (ROS)-mediated PRps formation has largely been recognized (Schultheiss *et.al*, 2003).

PRps synthesis can also be triggered by internal plant developmental stimuli. The presence of PRps in different flower parts (Fraser, 1981), their appearance in abscission zones as well as their relation to seed germination and somatic embryogenesis point that they are developmentally controlled. It is noteworthy that developmentally-induced PRps are accumulated in an organ and tissue specific manner (Kombrink *et.al*, 2001).

Functional Properties of PR-proteins

Elucidation of the biochemical properties of the major, pathogen-inducible PRps of tobacco and subsequent cloning of their cDNAs and/ or genes revealed proteins with substantial similarity to the classical PRps, which are mostly acidic and extracellular proteins, the homologous counterparts are mostly basic and localized intracellularly in the vacuole. As far as it has been possible to deduce, they possess the same type of enzymatic activities, but their substrate specificity and specific activity may be rather different (Kauffmann *et.al*, 1987; Legrand *et.al*, 1987).

PRps are, as such, a collective set of novel proteins which a plant produces in reaction to a pathogen mainly in incompatible interactions and thus impedes further pathogen progress. The "related situations" in which PRps were found to be induced, seem to prove the point: application of chemicals that mimic the effect of pathogen infection or induced some aspects of the host response, as well as wound responses that give rise to proteins that are also induced during infections, can induce both PRps and acquired systemic resistance (SAR). Few of the inducible acidic PRps associated with SAR have been shown to possess significant anti-pathogenic activity (VanLoon, 1997).

The occurrence of homologous PRps as small multigene families in various plant species belonging to different families, their tissue-specificity during development and consistent localization in the apoplast as well as in the vacuolar compartment and their differential induction by endogenous and exogenous signaling compounds suggest that PRs may have important functions extending beyond their apparently limited role in plant defense. During the hypersensitive reaction cellular damage and death is a major stress to the plant, as exemplified by high increases in abscisic acid and ethylene. It is possible, therefore, that PRps are stress proteins directed to alleviate harmful effects of cellular degradation products on thus far untouched neighboring cells.

Both acidic and basic PRps may be induced by high concentrations of ethylene (Lotan *et.al*, 1989) or physiological necrosis (Edreva, 1990), plasmolysis or wounding. Such induction in the absence of pathogenic attack might be taken to indicate protection of cellular structures, either physically to stabilize sensitive membranes or macromolecules, or chemically to keep potentially harmful saprophytic microorganisms on tissue surfaces or in intercellular spaces in check. In virtually any natural stress condition e.g., heat, cold, drought, osmotic stress, water logging, anaerobiosis, metal toxicity, etc., plants are known to react by the synthesis of novel, and sometimes partly overlapping, sets of proteins (Wasternack and Parthier 1997). The various conditions under which PRps occur are reminiscent of those under which heat-shock proteins (HSP) are induced. These proteins are ubiquitous in living organisms and associated with the acquisition of thermotolerance to otherwise lethal temperatures, but a causal connection is not evident.

Interestingly, the promoters of all three tobacco PR-1 genes that are expressed, as well as of a different type of PR in parsley, contain a heat shock regulatory element (Somssich *et.al*, 1988) but the proteins are not induced to detectable levels by heat shock. Nevertheless, PRps might have an analogous function, though quite different, chaperonin-like function: unlike PRps, HSP are intercellular proteins that do not accumulate during heat shock. However, the specific occurrence of individual PRps in some floral organs, but not in others, points to other, more specific roles.

The relative ineffectiveness of PRps in determining resistance to pathogens does not preclude an involvement in defense. As first proposed (Mauch and Staehelin,1998) acidic, extracellular PRps might be involved in recognition processes, releasing defense-activating signal molecules from the walls of invading pathogens. This would hold particularly for chitinases and glucanases that could liberate elicitor-type carbohydrate molecules from fungal and bacterial cell walls. β-1,3-glucanase induced in soybean seedlings by infection or chemical stress releases

elicitor-active fragments from cell wall preparations of the fungus *Phytophthora megasperma* f.sp. *glycinea* (Ham *et.al*, 1991). Such elicitors could help stimulate defense responses in adjacent cells and thus accelerate and enhance these reactions, as well as induce acquired resistance to further infection.

Demonstration that the PR-2 family are β-1,3-endoglucanases and the PR-3,-4,-8, and -11 families consist of chitinases with or without lysozyme activity, immediately suggested that these PRps are directed against cell walls of fungi and bacteria Homology of the thaumatin-like proteins of the PR-5 family with a bifunctional l-amylase/trypsin inhibitor from maize seeds seemed consistent with a role in protection against phytophagous insects. However, no proteinase inhibitor activity has been demonstrated for PR-5 proteins.Resistance to insect attack is taken to be conferred primarily by wound-inducible proteinase inhibitors, which have now been grouped into the family PR-6.

A role of PRps as specific internal signal generating enzymes would be consistent both with their occurrence in specific organs and with their induction during the development and in response to stressful pathogen infections. The major chitinase of bean leaves first described (Boller *et.al*, 1983) to be induced by ethylene and located in the vacuole, appears to be also induced in abscission zones at the stem petiole-junction (Del and Lewis, 1992) together with a PR-1-like protein, two isoforms of β-1,3-glucanase, other chitinases, and a thaumatin-like protein. However, the natural substrates for chitinases in higher plant cell walls remain to be determined. The tobacco PR-2 glucanases vary 250-fold in specific activity on laminarin (Kauffmann *et.al*, 1987) and their relative activities on different substrates vary greatly, suggesting that their normal actions may be diverse. Expression studies of PR-2d in transgenic tobacco suggest that this protein functions developmentally in seed germination by weakening the endosperm, thus allowing the radicle to protrude (Vögeli-Lange *et.al*,1994).

The occurrence of almost all types of PRps in various floral tissues also suggests specific physiological functions during flower development rather than a role in general defense against pathogen invasion. This notion is supported by the presence in floral organs of additional PR-like proteins, glucanases (Coté *et.al*, 2002) and thaumatin-like proteins. In petunia flowers, chitinase activity is localized in the petals (about 15%) and stigma (about 85%). In the stigma it increases about five-fold following anther dehiscence, strongly suggesting that the chitinase has a specific function in reproduction.

Major interest has been devoted to plant hydrolases, β-1,3-glucanases (E.C. 3.2.1.39) (PR-2) and chitinases (E.C. 3.2.1.14) (PR-3), as they are capable of cleaving fungal cell walls resulting in pathogen growth inhibition, and moreover, the products of the hydrolysis can act as elicitors of further defence responses (Boller, 1995). Both β-1,3-glucanases and chitinases are highly abundant proteins in plants involved in diverse physiological and developmental processes. They can act either alone or in combination strengthening their antifungal activity (Mauch *et.al*, 1988). Their accumulation is not restricted only to resistant plants but is often observed in compatible plant-pathogen interactions or even non-pathogenic combination.

Activation of natural weapons before infection, called systemic acquired resistance (SAR) is initiated by pathogens, pathogen- or pathogen-derived elicitors, as well as a number

of chemical compounds. Among the main defence genes, which are switched on in response to pathogen infection, belong those encoding pathogenesis related (PR) proteins. The association of PRps with SAR, but not with ISR, has led to the hypothesis that accumulation of PRps is not a pre-requisite for the induction of resistance, but that PRps contribute to the protective state. SAR is dependent on the accumulation of SA, but not JA or ethylene. It appears that only when increases in the levels of any of these signals occur, PRps become detectable in the infected plants. The observations indicate that individual PRps are induced to various extents by these different signals. Consequently, the mixture of signals released or produced upon microbial stimulation appears to determine the magnitude of the plant's response and its effectiveness to inhibit further infection.

In *Arabidopsis*, SA-dependent expression of PR-1, PR-2 and PR-5 is required for increased protection against the biotrophic fungus *Perenospora parasitica*, whereas SA-independent but JA-dependent induction of the plant defensin gene *pdf1.2*, as well as 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 oxysporium* f.sp. *matthiolae* (Epple *et.al*, 1998). These results suggest that the SA- and JA-dependent defense pathways in *Arabidopsis* contribute to resistance against distinct microbial pathogens. As a result, PRps and similarly induced antimicrobial proteins appear to contribute differentially to the induced resistance against different pathogens.

Biosynthesis

Biosynthesis of PRps in sugar beet has been intensively investigated both on plants infected by pathogens and treated with synthetic inducers of SAR: salicylic acid and its functional derivates 2,6-dichloroisonicotinic acid (INA) and benzo-1,2,3-thiadiazole-7-carbothioic acid S-methyl ester (BTH). BTH was shown to induce resistance to a number of fungal and viral pathogens, e.g. *Arabidopsis*, wheat, tobacco. Analysis of extracellular fluid isolated from BTH-treated sugar beet leaves revealed the accumulation of acidic and basic proteins displaying both chitinase and b-1,3-glucanase activities indicating the ability of BTH to activate defence reactions in sugar beet. However, in contrast to there was no increase in accumulation of transcripts encoding three chitinase isozymes (including Ch4) and β-1,3-glucanase Glu2 in sugar beet leaves following the treatment with INA, compound similar to BTH, even though the INA pretreatment completely inhibited the development of *Cercospora beticola*. Recently, the BTH capability of inducing SAR to root pathogens was shown on cucumber plants against *Pythium* damping-off as well as to *Phytophthora* root rot and even to root-parasitic weed *Orobanche cumana*, suggesting that BTH-induced or potentiated defence mechanism might be of more general character. Similarly, the cross-activity of defence responses against diverse pathogens has been demonstrated on rhizomania-diseased sugar beet and *Heterodera schachtii* root nematodes.

Cellular and tissue localization

Localization of the major, acidic PRps in the intercellular space of the leaf seems to guarantee contact with invading fungi or bacteria before these are able to penetrate. In localization studies *in planta*, labelling for β-1,3-glucanases and chitinases was especially pronounced over fungal cell walls confirming their role in plant defence. With most of the investigations devoted to leaf tissues. In roots, expression of defence genes was studied on

infection by pathogens, arbuscular mycorrhizal fungi, antagonistic fungus *Trichoderma harzianum* or non-pathogenic bacterium *Pseudomonas fluorescens* and differences in the expression of distinct classes of chitinases and β-1,3-glucanases were reported in dependence on the particular microbial inducer. Antimicrobial proteins extracted from *Exobasidium vexans* inoculated resistant tea varieties were electrophoretically resolved on SDS-gels, analysed by EDAS and characterized immunologically after probing with PAb-chitinase (Chakraborty *et.al*, 2004, 2005; Sharma and Chakraborty, 2004). Induction of resistance in tea varieties against blister blight pathogen was attempted with a few abiotic inducers (Chakraborty *et.al*, 2005a) Salicylic acid and hexaconazole treated leaves of *Camellia sinensis* (L.) O. Kuntze were reacted with polyclonal antibodies of chitinase (PR-3) and labeled with FITC, bright apple green fluorescence was observed in the epidermal and mesophyll tissues (Sharma and Chakraborty, 2005). Similar results of localization of PR-3 proteins have been reported in potato leaves infected by *Phytophthora infestans*.

Subcellular localization of PR-1 proteins was studied in roots of resistant *Nicotiana tabacum* cv. *Xanthi* uninfected or infected *in vitro* by the black root rot fungus *Chalara elegans*, using polyclonal or monoclonal antibodies raised against PR-1 protein. In healthy tobacco roots, the PR-1 proteins were found to be present in low amounts in intercellular space material, over cell walls and over secondary thickening of xylem vessels. All these cell compartments were significantly enriched in the PR-1 proteins in infected tobacco root tissues. Their accumulation over the cell walls of inter- and intracellular hyphae of *C. elegans* colonizing tobacco roots may reflect an eventual role of these proteins, in association with other PR-proteins like β–1,3-glucanases and chitinases in directly hindering hyphal growth of the pathogen. Transmission electron microscopic observations of tea leaf tissues treated with salicylic acid and labeled with PAb-chitinase and PAb-β-1,3-glucanase revealed intense labeling corresponding systemic accumulation of both the PRps (PR-2 and PR-3) in treated plants. Accumulation of the PRps in treated tea plants was observed in cell walls and extracellular spaces.

Ribosome inactivating proteins (RIPS)

Ribosome-inactivating proteins or RIPs are divided into two groups. Type 1 RIPs are single A-chain molecules (30 kDa) exhibiting the ribosome-inactivating activity. Type 2 RIPs additionally contain a lectin B-chain (30 kDa) connected to the active A-chain via a single disulfide bond. The lectins of most type 2 RIPs specifically recognize galactose or N-acetylglucosamine. The antifungal activity of this protein was synergistically enhanced in combination with either a basic class IV glucanase or basic class II chitinase from barley seeds. The basis of the antifungal activity of this RIP may rely on its inhibitory effect on fungal ribosomes, although it has not been demonstrated that this occurs *in vivo*. Better documented is the antiviral activity of several type 1 RIPs. When purified RIPs are applied on plants together with viruses, they drastically suppress virus multiplication and symptom development. It is supposed that RIPs enter cell together with the viruses and exert their adenosine glycosidase activity in the cytosol to affect either host ribosomes or possibly viral RNA.

Applications

Plant protection is a major challenge to agriculture worldwide. One of the effective strategies for disease resistance in plants has been the incorporation of disease resistant genes into commercially acceptable cultivars. Experimental evidences substantiated the utility of

PRps genes to develop disease resistance in transgenic plants. This practical aspect of PRps gene research resulted in the release of agronomically important crops resistant to various diseases of economical interest. The most attractive initial candidates for manipulation of the single gene defense mechanism approach are genes encoding chitinases or β-1,3-glucanases because these two enzymes hydrolyze chitin and β-1,3-glucans which are structural components of the cell walls of several fungi. Chitinase gene from *Rhizopus oligosporus* has been shown to operate as an antifungal system in transgenic tobacco. Transgenic cucumber harboring the rice chitinase genes exhibited enhanced resistance against gray mold, *Botrytis cinerea*. While it is clear that it is possible in several cases to alter the expression of chitinase transgenes to generate plants with increased resistance to the pathogen, it is not clear whether constitutively expressed chitinase alone is responsible for the reduction of disease symptoms as observed in the case of tobacco and canola. Introduction of bacterial chitinase gene from *Serratia marcescens* in transgenic tobacco cells showed up to an eightfold increase in amount of chitinase protein in the plants and conferred resistance to *Rhizoctonia solani*. Expression of β-1,3-glucanase in transgenic tobacco plants was shown to result in enhanced resistance to *Alternaria alternata*. The constitutive over expression of tobacco class 1 PR-2 and PR-3 transgenes I potato plants enhanced their resistance to Phytophthora infestans, the causal agent of late blight. *Brassica napus* transgenic plants, constitutively expressing a chimeric chitinase gene, display field tolerance to fungal pathogens. Combined expression of PR-2 (β-1,3-glucanase) and PR-3 (chitinase) gives effective protection against fungal infection as they have been shown to act synergistically.

Conclusion

Increasing amount of data enlarged the knowledge on the relevance of PRs to important plant performances, such as development, disease resistance and general adaptation to stressful environment. As defense related proteins usually provide a plant with resistance to stresses, varieties that are apt to intensively induce such proteins are agriculturally important and are drawing much attention of plant breeders. The knowledge gained by such studies also provides a base for the development of novel agrochemicals for disease control and also for the development of disease-resistant crops by regulating the system in plants through genetic manipulation which encouraged the application of PR genes in gene-engineering technologies for crop improvement. However, fundamental aspects of PRs gene studies remain little understood, particularly the exact mechanisms of gene regulation; thus, the receptors, signal transducing cascades and moleclular targets involved in PRs induction are a challenge for both fundamental and applied studies.

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