PATHOGENESIS-RELATED PROTEINS: RESEARCH PROGRESS IN THE LAST 15 YEARS

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Summary. An overview of the evolution of knowledge on the nomenclature, classification, induction, occurrence, functions, and role of pathogenesis-related proteins (PRs) is presented, covering generally the last fifteen years. The recommendations for naming and defining PRs are introduced, and the criteria required for the inclusion of new families into PRs are considered. The revision of the previous view on PRs as inducible proteins is argumented, given their constitutive expression in various plant organs and in seeds. Newly-discovered members of PRs families are described, and recent information about functionality of PRs, substantiating their antimicrobial action, is discussed. The biochemical and structural properties, as well as the organ-, tissue-, and cell-localisation, the induction and regulation of PRs are briefly outlined. Recent data about the relevance of PRs to plant development and disease resistance are examined, and the plausible application of engineering of PRs genes for crop improvement is critically commented. The finding that PRs, considered before as plant-specific proteins, are also expressed in other organisms, suggests that these proteins share an evolutionary origin and possess activity essential to the functioning and survival of living organisms.

Key words: PR-proteins: classification, functions, induction, nomenclature, occurrence, role

Abbreviations: GLPs – germin-like proteins; ISR – induced systemic resistance; LTPs – lipid transfer proteins; PRs **-** pathogenesis-related proteins; ROS – reactive oxygen species; SA – salicylic acid; SAR – systemic acquired resistance; TL proteins – thaumatin-like proteins.

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INTRODUCTION

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 proteins" (PRs) is assigned an important role in plant defense against pathogenic constraints and in general adaptation to stressful environment. A large body of experimental data has been accumulated and changing views and concepts on this hot topic have been evolved.

The aim of the present paper is to give a brief overview of the evolution of knowledge on the nomenclature, classification, induction, occurrence, functions, and role of PRs, covering generally the last 15 years.

Terminology

Since their discovery in tobacco leaves hypersensitively reacting to TMV by two independently working groups (Van Loon and Van Kammen, 1970; Gianinazzi et al., 1970), pathogenesis-related proteins (initially named "b" proteins) have focused an increasing research interest in view of their possible involvement in plant resistance to pathogens. This assumption flowed from initial findings that these proteins are commonly induced in resistant plants, expressing a hypersensitive necrotic response (HR) to pathogens of viral, fungal and bacterial origin. Later, however, it turned out that b-proteins are induced not only in resistant, but also in susceptible plant – pathogen interactions, as well as in plants, subjected to abiotic stress factors (Van Loon, 1985, and references therein). Thus, still in 1980 Antoniw et al. coined the term "pathogenesis-related proteins" (PRs), which have been defined as "proteins encoded by the host plant but induced only in pathological or related situations", the latter implying situations of non-pathogenic origin. To be included among the PRs, 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 PRs 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 PRs. These considerations imply that the characteristics of the induction of PRs take priority over other identifying features, such as chemical properties or cellular localization (Van Loon et al., 1994; Van Loon, 1999).

The term "PR-like proteins" was proposed to accommodate proteins homologous to PRs as deduced from their amino acid sequences or predicted from the nucleotide sequence of their corresponding cDNA or gene, but which are induced in developmentally controlled, tissue-specific manner. However, the designation "PR-like proteins" never became popular; some proteins that were originally considered to be

"PR-like" on the basis of sequence homology, have been shown to be strongly induced by infections, and hence are best regarded as genuine PRs (Van Loon et al., 1994; Van Loon, 1999).

Classification of PRs

Originally, five main groups of PRs (PR-1 to PR-5) were characterized by both molecular and molecular-genetic techniques in tobacco, numbered in order of decreasing electrophoretic mobility. Each group consists of several members with similar properties (Bol et al., 1990) (Table 1). Group PR-1 is the most abundant, reaching up to 1-2 % of total leaf proteins. PRs of group 5 share significant amino acid sequence homology with the sweet tasting protein in the fruits of the tropical plant *Thaumatococcus daniellii*, and have been named thaumatin-like (TL) proteins

Table 1. PR proteins induced in Samsun tobacco (NN genotype) by TMV infection (Bol et al. 1990)

Group	Acidic PR proteins		Basic PR proteins		Function
	Name	Mol wt (kD)	Name	Mol wt (kD)	
1	1a	15.8	16 kD	16.0	Unknown
	1b	15.5			
	1c	15.6			
2a	2	39.7	Gluc.b	33.0	β-1,3-Glucanase
	N	40.0			
	O	40.6			
	Q'	36.0			
2b	O'	25.0			β-1,3-Glucanase
3	P	27.5	Ch.32	32.0	Chitinase
	Q	28.5	Ch.34	34.0	
4	s1	14.5			Unknown
	r1	14.5			
	s2	13.0			
	r2	13.0			
5a	R	24.0	Osmotin	24.0	Unknown
	S	24.0			Thaumatin-like proteins
5b			45 kD	45.0	Unknown

(Cornelissen et al., 1986). Osmotins of the same group display similarity to TL proteins (Singh et al., 1987). Thereupon, in 1994 a unifying nomenclature for PRs was proposed based on their grouping into families sharing amino acid sequences, serological relationships, and enzymatic or biological activity. By then eleven families (PR-1 to PR-11) were recognized and classified for tobacco and tomato, with the families PR-8 and PR-10 being also present in cucumber and parsley, respectively (Van Loon et al., 1994). Later three novel families (PR-12, PR-13 and PR-14) were recognized in radish, *Arabidopsis* and barley, respectively (Van Loon and Van Strien, 1999) (Table 2). Germins and germin-like proteins (GLPs) have been classified as PR-15 and PR-16; PR-16 has been isolated from hot pepper during the resistance response to bacterial and viral infection (Park et al., 2004 b; personal communication of Van Loon cited therein).

Criteria used for the inclusion of new families into PRs are that (a) the protein must be induced by a pathogen in tissues that do not normally express it, and (b) induced expression must occur in at least two different plant-pathogen combinations, or expression in a single plant-pathogen combination must be confirmed independently in different laboratories (Van Loon and Van Strien, 1999).

Table 2. Recognized and proposed families of pathogenesis-related proteins (Van Loon, Van Strien, 1999)

Family	Type member	Properties
PR-1	Tobacco PR-1a	unknown
PR-2	Tobacco PR-2	β-1,3-glucanase
PR-3	Tobacco P, Q	chitinase type I, II, IV, V, VI, VII
PR-4	Tobacco "R"	chitinase type I, II
PR-5	Tobacco S	thaumatin-like
PR-6	Tomato Inhibitor I	proteinase-inhibitor
PR-7	Tomato P_{6g}	endoproteinase
PR-8	Cucumber chitinase	chitinase type III
PR-9	Tobacco "lignin-forming peroxidase"	peroxidase
PR-10	Parsley "PR1"	"ribonuclease-like"
PR-11	Tobacco class V chitinase	chitinase type I
PR-12	Radish Rs-AFP3	defensin
PR-13	*Arabidopsis* THI2.1	thionin
PR-14	Barley LTP4	lipid-transfer protein

Biochemical and structural characteristics. Cellular and tissue localisation.

PRs are distinguished by specific biochemical properties. They are low-molecular proteins (6-43 kDa), extractable and stable at low pH (< 3), thermostable, and highly resistant to proteases (Van Loon, 1999). The structure of a PR-1 family member (tomato PR1-b) was solved by nuclear magnetic resonance and found to represent a unique molecular architecture. The protein contains four α-helices and four β-strands arranged antiparallel between helices. The tight packing of the α-helices on both sides of the central β-sheet (α–β–α sandwich structure) results in a compact, bipartite molecular core, which is stabilized by hydrophobic interactions and multiple hydrogen bonds (Fernández et al., 1997). This compact structure probably determines the high stability of PRs and their insensitivity to proteases.

PRs have dual cellular localisation – vacuolar and apoplastic, the apoplast being the main site of their accumulation (Van Loon, 1999). Apart from being present in the primary and secondary cell walls of infected plants, PRs are also found in cell wall appositions (papillae) deposited at the inner side of cell wall in response to fungal attack (Benhamou et al., 1991; Jeun, 2000). Interestingly, they are detected in the cell walls of invading fungal pathogens and in the space formed between cell walls and invaginated plasma membrane of fungi (Jeun, 2000; Jeun and Buchenauer, 2001).

Acidic and basic PRs are identified, each of these counterparts having both apoplastic and vacuolar localisation (Buchel and Linthorst, 1999). Earlier data show that acidic tobacco PR-1 are localized in the apoplast, whereas basic tobacco PR-1 accumulate in the vacuole (Bol et al., 1990). This may be valid for one PR family (PR-1) in a host plant, such as tobacco, but cannot be generalized as a differential localization feature of acidic and basic proteins in plants.

Presently, PRs are established in all plant organs – leaves, stems, roots, flowers (Van Loon, 1999), being particularly abundant in the leaves, where they can amount to 5-10% of total leaf proteins. Thus, the original claiming that PRs occurrence is limited to photosynthetizing tissues (Asselin et al., 1985) has been abolished. The application of sensitive immunological techniques allowed the detection of PRs in roots of tobacco and tomato plants inoculated with the fungal pathogens *Chalara elegans* and *Fusarium oxysporum*, respectively (Tahiri-Alaoui et al., 1990; Benhamou et al., 1991), as well as in lupine and birch roots exposed to abiotic stress (Utriainen et al., 1998; Przymusinski et al., 2004).

In the leaves PRs are present in mesophyll and epidermal tissues. They are also localized in the abscission zone of leaves and inflorescence, abscission zone at the stem-petiole junction, and vascular tissue of stems and petioles (Del Campillo and Lewis, 1992; Eyal et al., 1993). In inflorescences PRs are detected in sepals, pedicels, anthers, pistils, stigmata and ovaries (Van Loon, 1999; Buchel and Linthorst, 1999). In seeds of maize, sorghum, oat, barley, and wheat a group of PRs is established,

commonly named permatins, characterized as PR-5 thaumatin-like proteins (Vigers et al., 1991). Linusitin from flax seeds is referred to the same group (Anžlovar et al., 1998). Noteworthy, specific cell types, such as cultured plant cells, are highly active in PRs expression (Singh et al., 1987).

Induction

Besides the known PRs inducers of biotic origin (pathogens, insects, nematodes, herbivores) (Fidantsef et al., 1999; Van Loon, 1999; Robert et al., 2001; Reiss and Horstmann, 2001; Schultheiss et al., 2004), a new type of biotic inducers, *Orobanche* weeds, has been reported in tobacco (Joel and Portnoy, 1998). Pathogen-derived elicitors are potent PRs inducers. Well-characterized are glucan and chitin fragments derived from fungal cell walls, fungus-secreted glycoproteins, peptides, and proteins of elicitin family (Münch-Garthoff et al., 1997; Honée et al., 1998; Zhou, 1999; Kombrink et al., 2001; Edreva et al., 2002). Protein products of avirulence genes in fungi and bacteria are capable of PRs inducing (Staskawicz et al., 1995; Hennin et al., 2001). Earlier data pointed that cell wall splitting enzymes, such as polygalacturonases, induced PRs accumulation (Pierpoint et al., 1981). Later it has been shown that polygalacturonases release biologically active pectic fragments from plant cell walls, named endogenous elicitors (Mc Neil et al., 1984), capable of inducing a set of defense responses in plants, including PRs accumulation (Boudart et al., 1998). 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 PRs induction. A special class of PRs inducers are hormones (ethylene, jasmonates, abscisic acid, kinetin, auxins) (Edreva, 1990, 1991, and references therein; Tamás et al., 1997; Pääkkönen et al., 1998; Van Loon, 1999; Buchel and Linthorst, 1999; Fujibe et al., 2000). Recently, the dissipation of the proton gradient across the plasma membrane, provoked by the fungal toxin fusicoccin, activator of the plasma membrane H^+-ATPase, was reported to induce PRs (Schaller et al., 2000). Reactive oxygen species (ROS)-mediated PRs-formation has largely been recognized (Anderson et al., 1998; Surplus et al., 1998; Grant and Loake, 2000; Schulteiss et al., 2004).

Besides being induced by a wide array of environmental/external cues, PRs synthesis can be triggered by internal plant developmental stimuli. Fraser (1981) was the first to report the formation of a set of PRs in leaves of healthy tobacco plants as they reached the flowering and senescing stage. Similar data are reported by Hanfrey et al. (1996) for senescing *Brassica napus* leaves. The presence of PRs in different flower parts, their appearance in abscission zones (Lotan et al., 1989; Buchel and Linthorst, 1999), as well as their relation to seed germination (Vögeli-Lange et al., 1994) and somatic embryogenesis (Kragh et al., 1996) point that they are developmentally con-

trolled. It is noteworthy that developmentally-induced PRs are accumulated in an organ and tissue-specific manner (Ma et al., 1996; Van Loon and Van Strien, 1999; Ekramoddulah et al., 2000; Kombrink et al., 2001).

PRs in plants are coded by a small multigene family. Since their discovery, regulation of PRs has been a highly active research area. Putative plasma membrane-localized receptors of PRs inducers are suggested, and secondary signals of PRs induction, such as salicylic acid (SA), jasmonic acid and ethylene, are established. Many of these secondary signals are well-known inducers of PRs expression (Durner et al., 1997; Surplus et al., 1998; Anderson et al., 1998; Zhou, 1999; Cameron et al., 2000; Poupard et al., 2003). Cross-talks are common between signaling pathways mediated by these secondary messengers. Thus, SA-independent/jasmonate dependent, and *vice-versa* pathways of PRs induction have been demonstrated (Mitsuhara et al., 1998; Fidantsef et al., 1999). It has been proven that PRs synthesis is regulated at transcriptional level; the exact mechanisms of transcriptional regulation have been ones of the most active fields of PR gene studies. Several *cis*- regulatory elements in PR-promotors mediating PR gene expression have been identified. These include W-box, GCC box, G box, MRE-like sequence, SA-responsive element (SARE) (Zhou, 1999). New mutants are developed providing clues into the better understanding of the regulation of PRs (Delaney, 2000). PRs are synthesized following a long lag period (no less than 8 h) (Matsuoka and Ohashi, 1986); the synthesis proceeds *in situ*, i.e. PRs are not translocated from the site of their induction to other plant parts, as proven by elegant grafting experiments (Gianinazzi et al., 1982).

Occurrence

Being first detected in tobacco, PRs have subsequently been identified in numerous monocotyledonous and dicotyledonous plants across different genera, and hence can be considered as ubiquitously distributed in plant kingdom. PRs are distinguished by species specificity, thus allowing their application as genetical markers in taxonomical, phylogenetical and evolutionary studies. By using PRs patterns the origin of *N.tabacum* from the wild progenitors *N.sylvestris* and *N.tomentosiformis* was confirmed (Ahl et al., 1982).

As discussed above, PRs were considered as inducible proteins elicited by environmental and developmental stimuli. However, increasing body of data points to their occurrence as constitutive components in different plant organs and in seeds, irrespective of stress conditions (Vigers et al., 1991; Buchel and Linthorst, 1999), this suggesting a possible role of preformed defense barrier. Moreover PRs, classified as plant-specific proteins, were surprisingly detected in other organisms, where they are constitutively expressed. Comparative analysis of PR-1 type protein reveals that related sequences have been found in yeasts, insects and vertebrates. At the protein level, the yeast sequences show 25-39 % identity and up to 51 % similarity with

the PR-1 type member. c DNA cloning of the major allergen in the venom of the white-face hornet wasp revealed sequence similarity with tobacco PR-1a. Similar sequences were also revealed in venom proteins of other vespids and fire ants, as well as in fruit fly, being up to 30 % homologous with tobacco PR-1a. A related protein family in vertebrates has been described as cystein-rich secretory proteins (CRISPs), having up to 46 % similarity with tobacco PR-1a. The amino acid sequence GHYTQVVW is a particularly well-conserved region in the two groups of proteins, suggestive of the functional role of this domain (Van Loon and Van Strien, 1999, and references therein).

Functions

The assumption that PRs are devoided of enzymatic functions was challenged by Legrand et al. (1987) detecting chitinase activity in four members of group 3 tobacco PRs. The same research team established β-1,3-glucanase activity in four members of group 2 tobacco PRs (Kauffmann et al. 1987). Later on chitinase activity was detected in PR-4, PR-8 and PR-11, PR-4 being referred to as chitin-binding proteins. Proteinase, peroxidase, ribonuclease and lysozyme activities were established in PR-7, PR-9, PR-10 and PR-8, respectively. PR-6 was assigned proteinase-inhibitory properties. Membrane-permeabilizing functions are characteristic of defensins, thiols and lipid-transfer proteins (LTPs), referred to as PR-12, PR-13 and PR-14, respectively, and of osmotins and thaumatin-like proteins (PR-5). Multiple enzymatic, structural and receptor functions are detected in "do-all" germins and germin-like proteins referred to as PR-15 and PR-16, respectively (Van Loon and Van Strien, 1999, and references therein; Van Loon, 2001; Selitrennikoff, 2001; Bernier and Berna, 2001; Park et al., 2004 a, b).

An important common feature of most PRs is their antifungal effect; some PRs exhibited also antibacterial, insecticidal, nematicidal, and – as recently shown – antiviral action. Toxicity of PRs can be generally accounted for by their hydrolytic, proteinase-inhibitory and membrane-permeabilizing ability. Thus, hydrolytic enzymes (β-1,3-glucanases, chitinases and proteinases) can be a tool in weakening and decomposing of fungal cell walls, containing glucans, chitin and proteins, while PR-8 can disrupt gram-positive bacteria due to lysozyme activity (Van Loon and Van Strien, 1999; Van Loon, 2001; Selitrennikoff, 2001). Last year PR-10 (named Ca PR-10), induced in hot pepper (*Capsicum annuum*) by incompatible interactions with TMV-Po and *Xanthomonas campestris* pv. *vesicatoria*, was shown to function as a ribonuclease. Data are presented that subsequent phosphorylation of Ca PR-10 increases its ribonucleolytic activity to cleave invading viral RNAs, and this activity is important to its antiviral pathway *in vivo*. Antibiotic activity of Ca PR-10 is also exerted against oomycete fungi (Park et al., 2004 a). Proteinase-inhibitory properties of PR-6 may confer anti-insect and anti-nematode effects, inactivating the proteins secreted by

these parasites in the invaded plant tissues. Plasma membrane-permeabilizing ability proper to PR-5, PR-12, PR-13 and PR-14 contributes to plasmolysis and damage of fungal and bacterial pathogens, inhibiting their growth and development (Vigers et al., 1992; Abad et al., 1996; Van Loon and Van Strien, 1999, and references therein; Van Loon, 2001; Selitrennikoff, 2001). This effect may be due to electrostatic interactions of PRs with membrane components, leading to conformatial changes, dissipation of membrane gradient, and formation of pores in membranes (Abad et al., 1996; Cheong et al., 1997; Anžlovar et al., 1998). Multifaceted functionality of PR-15 and PR-16, including cell wall remodeling ability, can be directed against pathogens and may have protective role (Park et al., 2004 b).

Interestingly, it was recently found that among the identified plant allergens 23% belong to the group of PRs. So far, plant-derived allergens have been identified with similarities to PR families 2, 3, 4, 5, 10 and 14 (Hoffmann-Sommergruber, 2001). This property may confer defensive functions displayed by the plant in hostile environment.

Apart from the above-described mechanisms of direct break-down or damage of pathogens, PRs can operate in a distinct pathway involving the hydrolytic release of chitin and glucan fragments from fungal cell walls. These oligosaccharides are endowed with elicitor activity and can induce a chain of defense reactions in the host plant (Ham et al., 1991; Lawrence et al., 2000; Kombrink et al., 2001). The peroxidase activity of PR-9 can contribute to the rigidification and strengthening of plant cell wall in response to pathogen attack (Lagrimini et al., 1987).

The defensive functions of PRs against pathogens are presumably corroborated by data about their constitutive expression in seeds and plant organs; high fungitoxicity of seed osmotins and thaumatin-like proteins has been established (Vigers et al., 1992; Abad et al., 1996). Protective role of PRs can also be inferred from their accumulation in plant cell wall appositions formed against pathogen ingress, as well as from their release into fungal structures penetrating plant tissues (Benhamou, 1991; Jeun, 2000; Jeun and Buchenauer, 2001).

The cell-, tissue-, organ- and development-specific expression pattern of PRs suggests important functions beyond defense against pathogens. Thus, basic tobacco glucanase PR-2d functions developmentally in seed germination by weakening the endosperm, thus allowing the radicle to protrude (Vögeli-Lange et al., 1994). Chitinases homologous to PR-3 and PR-4 act as morphogenetic factors in carrot embryogenesis (Kragh et al., 1996), and several PRs accumulate upon the transition of plants to flowering and senescence (Fraser, 1981; Hanfrey et al., 1996), also suggestive of a developmental role. Basic PR-5 (osmotins) are abundantly induced in tobacco and tomato cells in response to osmotic stress, thus contributing to osmotic adaptation (Singh et al., 1987). These findings raise the question as to whether PR genes evolved primarily to limit damage by invading pathogens, or were adapted from other functions to serve an accessory protective role (Van Loon and Van Strien, 1999).

Until now the functions of the most abundant PRs family, PR-1, remain obscure. A direct inhibitory effect of basic tomato and broad bean PR-1 family members against fungal pathogens (*Phytophthora infestans* and *Uromyces fabae*, respectively) has been demonstrated by *in vitro* and *in vivo* experiments (Niderman et al., 1995; Rauscher et al., 1999), but the mode of action as well as the cellular and molecular targets of PR-1 proteins are still unknown. Comparative "screening" of PR-1-type proteins from various plant taxa indicates that PR-1 family is highly conserved in plants. Moreover, as already mentioned, it is related to sequences present in yeast, insects and vertebrates. The corresponding proteins in insects are major venom allergens presumably directed to other organisms. It may be speculated that related protein family in vertebrates encodes lytic enzymatic and antimicrobial activity. The widespread occurrence of PR-1 family suggests that these proteins share an evolutionary origin and possess activity essential to the functioning and surviving of living organisms (Van Loon, 2001).

Relevance of PRs to disease resistance.

Presently, it is still difficult to assign a causative role of PRs in plant resistance to pathogens. The reason for this is that the numerous data on PRs as disease resistance factor are mostly of correlative character. Four lines of supporting evidence can be outlined.

a) Stronger accumulation of PRs in inoculated resistant as compared to susceptible plants. Besides previous data, substantiating this statement (Van Loon, 1985, and references therein), differential responses of resistant/susceptible plants were recently reported in tomato plants, inoculated with *Cladosporium fulvum* (Wubben et al., 1996); *Phytophthora infestans*-infected potato (Tónon 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., 2004 a, b), etc. Additional resistance gene(s) against *Cladosporium fulvum* present on the *Cf-9* introgression segment have been shown to be associated with strong PR-protein accumulation (Laugé et al., 1998). In some pathosystems mRNAs for certain PRs members accumulate to similar levels in compatible and incompatible interactions, but the maximum level of expression is reached much faster in the latter (Van Kan et al., 1992).

b) Important constitutive expression of PRs in plants with high level of natural disease resistance. This correlation was observed in several 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), the last authors proposing PR mRNAs as molecular marker in potato breeding programs.

c) Significant constitutive expression of PRs in transgenic plants overexpressing PR genes accompanied by increased resistance to pathogens. Thus, increased toler-

ance to *Peronospora tabacina* and *Phytophthora parasitica* var. *nicotianae* was demonstrated in tobacco overexpressing PR1a gene (Alexander et al., 1993). Transgenic rice and orange plants overexpressing thaumatin-like PR-5 possessed increased tolerance to *Rhizoctonia solani* and *Phytophthora citrophthora*, respectively (Datta et al., 1999; Fagoaga et al., 2001), while transgenic potato overexpressing PR-2 and PR-3 had improved resistance to *Phytophthora infestans* (Bachmann et al., 1998). PR-2 and PR-3 genes, coding for β-1,3-glucanase and chitinase, respectively, confer resistance of carrot to several fungal pathogens. The simultaneous expression of tobacco β-1,3-glucanase and chitinase genes in tomato plants results in increased [resistance to fungal pathogens] (Melchers et al., 1998). On the contrary, silencing of PR-1b gene in barley facilitates the penetration of the fungal pathogen *Blumeria graminis* f. sp. *hordei* in the leaves (Schultheiss et al., 2003).

d) Accumulation of PRs in plants in which resistance is locally or systemically induced. Generalizing this broad research area it can be stated that PRs are recognized as markers of the systemic acquired resistance (SAR), and PRs genes are involved in the list of the so-called SAR-genes (Ward et al., 1991). It has largely been demonstrated that SAR and the accompanying set of PRs are induced by different pathogens, as well as by a range of chemicals predominantly in a salicylic acid-dependent pathway; SAR is active against a broad spectrum of pathogens. Some SAR-inducing chemicals, such as benzothiadiazole (BTH), β-aminobutyric acid (BABA) or 2,6-dichloroisonicotinic acid (DCINA) are harmless commercially supplied compounds and have promising practical application as novel tools in plant protection (Van Loon, 1997; Kuč, 2001; Edreva, 2004 and references therein).

It is essential to underline, that PRs members induced in resistant or SAR- expressing plants, as well as PRs from transgenic resistant plants exhibit high antimicrobial activity (Enkerli et al., 1993; Anfoka and Buchenauer, 1997; Rauscher et al., 1999; Tonón et al., 2002; Anand et al., 2004), this suggesting their direct role in disease resistance.

In contrast to the above data, a view has been developed that PRs are rather related to the severity of symptom expression than to resistance. This assumption flows from experiments with PVY-infected potato (Naderi and Berger, 1997), PVY-, PVX-, and CMV-infected tobacco (Röhring, 1998), viroid-infected tomato (Camacho Henriquez and Sänger, 1982), etc. The strong induction of PRs in tobacco leaves by PVY and necrosis-inducing abiotic factors (Edreva, 1990) is in line with this idea. It may be speculated that the induction of PRs accompanying the symptom expression could also have a protective role of "last barrier", impeding the full destroyment of stressed plants by both internal and environmental constraints.

In the last years it was surprisingly established that PRs are not synthesized during the expression of a newly-reported "induced systemic resistance" (ISR) in *Arabidopsis* (Pieterse et al., 1996), as well as in crop plants (Hoffland et al., 1995; Reitz et al., 2001; Siddiqui and Chaukat, 2004). ISR is induced during root coloniza-

tion by non-pathogenic rhizobacteria of *Pseudomonas* spp., and is effective against a broad range of pathogens. A novel signaling pathway, salicylic acid-independent but jasmonate- and ethylene-dependent, is engaged in the control of ISR (Pieterse et al., 1998). Besides by non-pathogenic *Pseudomonas* bacteria, ISR is also elicited by growth-promoting *Bacillus* spp. (Kloepper et al., 2004; Silva et al., 2004), with this approach having a beneficial effect in field conditions. The non-involvement of PRs in ISR is indicative of the diversity of plant defense strategies, pointing that PRs are only one of the multiple means employed by plants against environmental cues.

Applications: brief overview

Experimental evidences substantiated the utility of PRs genes to develop disease resistance in transgenic plants. This practical aspect of PRs gene research resulted in the release of agronomically important crops resistant to various diseases of economical interest. One promising strategy is based on the exploitation of the genes encoding antifungal hydrolases, such as β-1,3-glucanase and chitinase, which are associated with SAR-response in plants. Increased resistance of tomato against fungal pathogens was achieved by simultaneous expression of a class I chitinase and β-1,3-glucanase (PR-3 and PR-2 family, respectively) from tobacco. Transgenic tomato plants expressing either of these genes alone were less protected. Field evaluation of transgenic carrot plants containing the same genes has shown a high level of resistance against major fungal pathogens of carrots. An important feature is that the majority of the transgenic lines which had resistance to one pathogen exhibited significant resistance to the other pathogens (Melchers et al., 1998). The constitutive overexpression of tobacco class I PR-2 and PR-3 transgenes in potato plants enhanced their resistance to *Phytophthora infestans*, the causal agent of late blight (Bachmann et al., 1998). Similar results about the effectiveness of the co-expression of chitinase and β-1,3-glucanase in plant disease resistance are reported by Kombrink et al. (2001). *Brassica napus* transgenic plants, constitutively expressing a chimeric chitinase gene, display field tolerance to fungal pathogens (Grison et al., 1996). Increased resistance to crown rust disease in transgenic Italian ryegrass expressing the rice chitinase gene was demonstrated (Takahashi et al., 2005). Gene-engineering of PR-5 is another promising strategy for improvement of crop disease resistance, based on the potent plasmolyzing and antifungal effect of this PRs family. Thus, overexpression of the cloned rice thaumatin-like (PR-5) gene in transgenic rice plants enhanced the environmental friendly resistance to *Rhizoctonia solani* causing sheath blight disease (Datta et al., 2001). Overexpression of a pepper basic pathogenesis-related protein 1 gene in tobacco plants enhances resistance to heavy metal and pathogen stresses (Sarowar et al., 2005). For biotechnological purposes PR genes are transferred from novel sources, such as the insectivorous sundew (*Drosera rotundifolia* L.) (Matušikova et al., 2004). Cautions however must be taken before releasing of

PRs transgenic crops, by assuming that some PRs members display allergenic properties (Hoffmann-Sommegruber, 2001).

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. This research encouraged the application of PRs 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 molecular targets involved in PRs induction are a challenge for both fundamental and applied studies.

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