Proteomics Applied To Plant Defense Mechanism: Role of Pathogenesis-Related Proteins in Disease Defense

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Abstract: The production of Pathogenesis–Related Proteins (PR-Proteins) in response to abiotic and biotic stresses is very clearly understood and is consider as a fundamental response for plant protection. Enormous PR-Proteins were identified and divided in to 17 functional families on the basis of their biological, structure and phylogenetic activities. PR-Proteins show numerous effects within the plant and possess antimicrobial activity, and can be considered as a part of defense system in plants. In present study, the structural and biochemical properties, as well as cell, tissue & organ localization, the induction and regulation of PR-Proteins were concisely outlined and critically commented. This review provides the finding that PR-Proteins share an evolution based origin and retain activity necessary for proper functioning and endurance of living entities in biotic or abiotic stresses. Cloning and characterization of the genes encoding PR-Proteins will be responsible for better understanding of their regulation and will raise their potential of biotechnological applications in disease resistance strategies.
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Introduction

Plants react to pathogen attacks, environmental or mechanic stress by triggering or upregulating a wide range of genes (Baker et al. 1997). The most significant event in all stages of the defense response is the increased expression of PR-Proteins, a diverse group of proteins with numerous functions that represent a widespread plant defensive mechanism against a variety of pathogens (Hammond-Kosack and Jones, 1996). The attack of pathogens, such as bacteria, fungi, and viruses, normally induce these PR-Proteins in plants. Induction of PR-Proteins is a renowned mechanism in numerous plant species, suggesting a significant role for these proteins in plant's defense system (Van Loon and Van Strien, 1999). Accumulation of PR-Proteins plays a key role against pathogens as they are responsible for improved resistance of plant against a pathogenic attack (Sexton and Barbara, 2006).

Ever since the first Pathogenesis-Related protein was identified in tobacco leaves against tobacco mosaic virus (Van Loon et al. 1994), many other PR-Proteins have been documented as chitinases, endoproteinase, glucanases, peroxidases, and proteinase inhibitors in different species of plants. Previously, PR-Proteins were characterized and grouped into 14 families on the basis of their primary structure, enzymatic activities and serological relationships (Van Loon and Van Strien, 1999). Currently, new members of PR-Proteins have been added and categorized into 17 functionally and structurally different groups or families (Edreva, 2005; Van Loon et al. 2006). These PR-Proteins occur extensively in plants from monocot to dicots.

Classification of PR-Proteins

Presently, a huge number of PR-proteins are characterized and classified into 17 families on the basis of their structures and functions (Christensen et al. 2002). Most of them have antifungal properties against pathogenic fungi, although some PR-proteins have enzymatic or inhibitory activity such as glucanases (PR-2), chitinases (PR-3, PR-8 & PR-11), proteinase inhibitors (PR-6), peroxidases (PR-9) and ribonuclease-like (PR-10) proteins. Formerly, it was thought that the variable functions of these molecules make them perfect tool in plant defense mechanisms against ''all comers''. In addition, less studied PR-Proteins are of PR-4 family. Up to now, although their effect in inhibiting the pathogen hyphal growth and spore germination has been reported, their interaction and exact mechanism of action with molecular targets of pathogen are still to be discovered (Caruso et al. 1996).

Family PR-1

PR-1 proteins are produced in huge amount after pathogen invasion and are antifungal *in vitro* as well as in planta (Niderman et al. 1995). This class of proteins has been found in *Arabidopsis thaliana*, barley, maize, rice, tobacco, wheat and several other plants (Agrawal et al. 2000). These proteins have antifungal properties even in a very minute concentration against a wide range pathogenic fungi

infecting plants comprising *Phytophthora infestans*, *Erysiphe graminis* and *Uromyces fabae* (Niderman et al. 1995). Molecular masses of PR-1 proteins is 17 kDa and they are cysteine-rich proteins. Though the exact way of action of PR-1 proteins is yet to be discovered. Helothermine (PR-1-like Protein), toxin from Heloderma horridum horridum (Mexican beaded lizard) work together with channel proteins present in membrane of target cells, limits Ca^{2+} release (Morrisette et al. 1995). It is still suspected whether antifungal activity of PR-1 proteins plant is through this method or not.

Table 1: Classification and Properties of PR-Protein Families (modified from Van Loon et al. 2006)

Family	Size (kDa)	Type Member	Organism	Properties	Microbial Target	Gene Symbols
PR-1	15	Tobacco PR-1a	*Brassica napus*	Antifungal	Unknown	*Ypr1*
PR-2	30	Tobacco PR-2	*Castanea sativa*	β -1,3-glucanase	B-1,3 glucanase	*Ypr2, (Gns2('Glb')*
PR-3	25-30	Tobacco P, Q	*Citrus jambhiri*	Chitinase type I, II, IV, V, VI, VII	Chitin	*Ypr3, Chia*
PR-4	15-20	Tobacco R	*Nicotiana tabacum*	Chitinase type I, II	Chitin	*Ypr4, Chid*
PR-5	25	Tobacco S	*Nicotiana tabacum*	Thaumatin-like	Membrane	*Ypr5*
PR-6	8	Tomato Inhibitor I	*Lycopersicon esculentum*	Proteinase-inhibitor	*	*Ypr6, (Pis')(Pin')*
PR-7	75	Tomato P_{69}	*Theobroma cacao*	Endoproteinase	*	*Ypr7*
PR-8	28	Cucumber chitinase	*Cucumis sativus*	Chitinase type III	Chitin	*Ypr8, Chib*
PR-9	35	Tobacco 'lignin-forming peroxidase'	*Nicotiana tabacum*	Peroxidase	*	*Ypr9, Prx*
PR-10	17	Parsley 'PR1'	*Gossypium arboretum*	'Ribonuclease-like'	*	*Ypr10*
PR-11	40	Tobacco class V chitinases	*Nicotiana tabacum*	Chitinases, type I	Chitin	*Ypr11, Chic*
PR-12	5	Radish Rs-AFP3	*Raphanus sativus*	defensin	Membrane	*Ypr12*
PR-13	5	*Arabodopsis* THI2.1	*Arabidopsis thaliana*	Thionin	Membrane	*Ypr13, Thi*
PR-14	9	Barley LTP4	*Hordeum vulgare*	lipid-transfer protein	Membrane	*Ypr14, Ltp*
PR-15	20	Barley OXOa (germin)	*Hordeum vulgare*	Oxalate oxidase	*	*Ypr15*
PR-16	20	Barley OxOLP	*Hordeum vulgare*	'Oxalate oxidase-like'	*	*Ypr16*
PR-17	7	Tobacco PRp27	*Nicotiana tabacum*	Unknown	*	*Ypr17*

*No *in vitro* antimicrobial activity

Family PR-2

On the basis of the amino acid sequences, this family has been divided into three classes and they exhibit *in vitro* β-1,3-endoglucanase activity (Beffa and Meins, 1996; Nielsen et al. 1997). Class I is of basic proteins having molecular mass of ~33 kDa and are located in cell vacuole. On the other hand, classes II and III are acidic in nature and are of about 36 kDa size and are found extracellularly. Class I proteins differ from class II and III in a sense that class I proteins are produced as a precursor in the form of pre-pro-proteins that are modified by cleaving C-terminal post-translationally before they get activated enzymatically. Member of this family (PR-2) are identified in wide range of plant species including *A. thaliana,* fruits, grains, peas and tobacco as well (Rezzonico et al. 1998). These proteins functions against a wide range of pathogens viz, *Aspergillus fumigatus*, *C. albicans*, *Rhizoctonia solani* at micromolar levels (50 µg/ml). They showed antifungal activity by *in vitro* enzyme and whole-cell assays (Stintzi et al. 1993) along with *in vivo* experiments of transgenic plants in which genes for PR-2 protein overexpressed (Jach et al. 1995). β-glucan is the main constituent of cell wall in fungi and is hydrolyzed by these (1,3) β-glucanases especially at hyphal tips of filamentous fungi which is responsible for weak cell walls ultimately resulting in lysis and death of these fungal cells.

Family PR-3

Members of this class show *in vitro* chitinase activity and have molecular weights in between 26-43

kDa (18). On the basis of sequence homology and size, Chitinases (from all sources including PR-3 proteins) are categorized into 5 classes (I, II, III, IV and V). These proteins were isolated and identified from wide range of organisms including beans, cucumber, grains, peas, fungi and even bacteria (Huynh et al. 1992; Melchers et al. 1994; Chernin et al. 1997; Mathivanan et al. 1998; Kang et al. 1999; Lee et al.1999; Ye et al. 2000). They showed antifungal activity against broad range of plant and human pathogens viz, *Alternaria radicina, A. solani, Botrytis cinerea, Coprinus comatus*, *Fusarium oxysporum, Guignardia bidwellii, R. solani* and *Trichoderma reesei*. As compared to β-glucanases, PR-3 proteins act in a very simple manner. These proteins cleave glycosidic bond present in chitin of fungal cell wall, hence weakens the cell resulting in reduction of osmotic sensitivity. Members of PR-2 (β-glucanases) and PR-3 (chitinases) families work correspondingly by rapturing fungal cell wall both *in vitro* and in planta (Jach et al. 1995).

Family PR-4

Molecular weights of the members of this family are about 13-14.5 kDa and usually considered as chitin binding proteins. They are divided into two classes (I and II). Class I shows sequence resemblance to a chitin binding polypeptide i.e. hevein which is a lectin (chitin binding superfamily). On the other hand, class II does not have chitin-binding domain. Members of this family (PR-4) have been reported in barley, potato, tobacco, tomato and several other plants species (Van Damme et al. 1999). They showed antifungal properties against a range of plant and human pathogens including *B. cinerea*, *Fusarium graminearum*, *F. culmorum, Trichoderma harzianum.* Class I proteins targets β-chitin present in cell wall of fungi and thus disrupts the polarity of call wall and ultimately inhibits the fungi. On the contrary, class II lacks the chitin binding domain and their exact mode of action is still unknown. Antifungal activities also shown by chitin binding proteins but they are not PR-Proteins and have been isolated from several sources, comprising crustacean, insects, bacteria and plants (Bormann et al. 1999; Chae et al. 1999). Class II binding proteins include antifungal protein 1 (AFP1) has size of 9.8 kDa (Bormann et al. 1999), tachystatins (6.8 to 7.4 kDa) (Osaki et al. 1999), the penaeidins are of molecular weight of 5.5 to 6.6 kDa (Destoumieux et al. 1997) and many others. These proteins inhibit several pathogenic fungi including *Alternaria brassicola*, *Aspergillus* spp., *B. cinerea*, *F. oxysporum, N. crassa* and *Paecilomyces variotii*. They have same mechanism of action as that of members of class I proteins present in PR-4 family.

Family PR-5

PR-5 Proteins reveal substantial amino acid similarity to Thaumatin (a protein present in berry bush of South Africa having sweet taste) and thus called Thaumatin Like (TL) proteins. These proteins have been identified in *A. thaliana*, barley, beans, corn, flax, pumpkin, rice, soybeans, tobacco, tomato, wheat and several other plants (Singh et al. 1987; Hejgaard et al. 1991; Borgmeyer et al. 1992; Huynh et al. 1992; Cheong et al. 1997; Hu and Reddy, 1997; Koiwa et al. 1997; Moralejo et al. 1999; Ye et al. 1999). Mostly, molecular weight of TL proteins is 22 kDa having eight disulfide bonds due to which they are very stable and show strong endurance against proteases. In majority, PR-5 proteins having molecular masses of ~22 kDa and are stabilized via eight disulfide bonds. Due to this highly stabilized structure, PR-5 proteins become hugely resistant to protease degredation (Roberts and Selitrennikoff, 1990). The X-ray structure of two PR-5 proteins and thaumatin have been established thus confirming the above statement (Koiwa et al. 1998). Nevertheless, exact way of action of PR-5 proteins is still to be discovered but Coca et al. (2000) had several observations during their studies that these proteins inhibit fungi. As these proteins are responsible for the change in cell permeability and durability of fungal cells but on the other hand they do not effect or have very little effect on protoplasts of these cells (Roberts and Selitrennikoff, 1990). Another, assumption is that these proteins bind to β-glucan present in cell wall of fungus thus have β-glucanase activity *in vitro* (Trudel et al. 1998). Thirdly, another protein named zeamatin shows *in vitro* mammalian trypsin and insect α-amylase activities (Schimoler-O'Rourke et al. 2001). Fourthly, TL protein known as osmotin which has been identified in tobacco is responsible for agitations in cell wall of fungi (Yun et al. 1998). Regardless of the fungicidal properties, all of the above described observations are complementary due to which it is very hard to conform their one mode of action against wide variety of human and plant pathogenic fungi *in vitro.*

Family PR-6

Proteins belonging to this family are known and classified as a serine proteinase inhibitors (PIs) related to the tomato/potato inhibitor I (Glazebrook, 2005). They are having molecular weight of ±8 kDa typically (Van Loon et al. 1994). Serine proteinase inhibitors (PIs) are best investigated and are further divided into Bowman-Birk PIs, Kunitz-type PIs and tomato/potato class II PIs. This classification of PIs is questionable as it is only restricted to serine PIs while all other classes of PIs are also reported for their role in plant defense against many attacking organisms by working together with proteinases (Datta and Muthukrishnan, 1999; Haq et al. 2004; Christeller and Laing, 2005).

As far as their role in plant defense is concerned, PIs lowers the capability of pathogen by inhibiting their enzymatic machinery required for their pathogenicity, replications and to digest proteins of host ultimately resulting in less quantity of amino acids available for these pathogens (Urwin et al.1997; Gutierrez-Campos et al. 1999; Dunaevskii et al. 2005; Vila et al. 2005). Terras et al. (1993) reported their *in vitro* antifungal activity against *Ascochyta pisi, Alternaria brassicicola, Verticillium dahliae* and *Fusarium culmorum* in barley. Moreover, Lorito et al. (1994) put forward that PIs inhibit the endogenous trypsin which is compulsory for chitin synthase to produce chitin in call walls of fungi, resulting in decrease in fungal growth and development (Machida and Saito, 1993). Several other reports confirm that PIs have anti-insect activity *in vitro* experiments in which PIs reduce the insects growth rate that fed on artificial diets having PIs as compare to the insects growth rate that fed on diet not having PIs (Markwick et al. 1995; Tamhane et al. 2005; Harsulkar et al. 1999).

Family PR-7

A pathogenesis-related protein named Proteinase (P69), were produced in *Lycopersicon esculentum* as a result of pathogen invasion. Its molecular weight is 69-kDa hence known Proteinase (P69) (Tornero et al. 1996). In response to viral infections in plants, series of PR-proteins have been produced, among these proteins, an exclusive protein which showed endoproteolytic activity have been identified in tomato and called as PR-P69. The activity of P69 is usually triggered by Ca^{+2}. This protein is based in the intercellular spaces of plants under viral infection (Vera and Conejero, 1989; 1988). Induction of P69 has been reported by pathogens like fungi and nematodes (Fischer et al. 1989), as well as exogenous application of chemical elicitors like salicylic acid and ethylene which act as a signal molecules to enhance the defense mechanism in plants (Vera and Conejero 1989, 1990). The analysis of nucleotide sequence showed that P69 is produced as a pre-proenzyme, which is a polypeptide consists of 745 amino acids with a signal peptide of 22 amino acids, along with 92 amino acid polypeptide and a 631 amino acid polypeptide. P69 is secreted as a calcium-activated endopeptidase and it is like subtilisin (a PR proteinase) that work together with other proteins of plant's defense mechanism against pathogens (Tornero and Conejero, 1996).

Family PR-8

The family comprises of Class III chitinases which shows lysozyme activity against pathogens. They do not show any sequence homology to other classes of chitinases (I or II) but all the members of this family (PR-8) have extremely analogous sequences and the main difference between them is of isoelectric points (Zhang, 2006). Chitinases functions by releasing cell wall components of fungi by targeting the chitin present in fungal cell wall and after cleavage, these pieces of cell wall serves as a signal to activate the defense mechanism against forth coming pathogens. Moreover, these proteins targets cell wall of pathogens by disruption or deposition and eventually restrict the pestilence (Graham and Graham, 1991). Members of this family have showed *in vitro* antifungal activity and in transgenic plants as well (Yamamoto et al. 2000). Pathogenesis related proteins class III chitinases also show lysozyme function and have been identified Arabidopsis, chickpea, cucumber and tobacco against wide range of pathogens. Well known chitinase present in this PR-8 family is Hevamine which was first recognized in *Nicotiana tabacum* (Van Loon, 1999; Neuhaus, 1999).

Family PR-9

Plants responds with variety of defense mechanisms, among them production of peroxidase (POX) holds its importance because it is one of the earliest response against pathogen attacks (Lin and Kao, 2001). The member of this family (peroxidases) plays a vital role in lignin formation (Van Loon, 1997) and the process of lignification helps to strengthen cell walls by accumulation of lignin upon the onset of pathogen attack (Bateman et al. 2002). Many reporters have categorized PR-9 as a family of peroxidases on many occasions and described their role in plant disease resistance including in potatoes (Young et al. 1995; Collinge and Boller, 2001; Yoshida et al. 2003). They play their role in different ways including (i) lignification of plant cell walls which provide strength against wide range of pathogens (Vance et al. 1980; Hammerschmidt and Kuc, 1982) and (ii) by producing toxic radicals like H_2O_2 (Peng and Kuc, 1992; Way et al. 2000). Members of PR-9 family were convincingly produced in leaflets from KB!US-1 (Wang et al. 2005). Moreover, they may also take part in the activation of R-gene mediated resistance (Van Pelt-Heerschap and Smit-Bakker, 1999).

Family PR-10

PR-Proteins grouped together in this family are ribonuclease-like proteins (Van Loon et al. 1994). These proteins are having low molecular masses of 16-19 kDa and are acidic in nature. They have been characterized in wide range of plants involving dicots such as beans, parsley, pea, potatoes, soybean (Fristensky et al. 1988; Somssich et al. 1988; Walter et al. 1990; Crowell et al. 1992) and monocots like rice, asparagus, and lily (Warner et al. 1992; Huang et al. 1997; Jwa et al. 2001). Many workers have described that these proteins are localized nearby the sites of microbial entrance, wounds and under ecological stresses (Crowell et al. 1992; Warner et al. 1993; Pinto and Ricardo, 1995; Breda et al. 1996)

which signifies their role in plant defense system. Their exact mode of action in plant defense mechanism is still mysterious. On the other hand, expression pattern as well structural similarities such as amino acid sequences homology resembles with ginseng ribonuclease which indicates that these proteins possess RNase activity that may activate as a defense response (Moiseyey et al. 1997). PR-10 proteins were identified in dry seeds and constitutive expression was observed in roots as well. It was proposed that they have very vital role development of plant due to their constitutive expression in stems or flower parts (Barratt and Clark, 1993; Warner et al. 1994; Legocki et al. 1997).

Family PR-11

This family comprises of endochitinases which targets the chitin present in cell walls hydrolyzing it during the fungal attack (Garcia-Olmedo et al. 1998). The exhibits their resemblance to zinc and do not show any sequence similarities or homology with other classes of chitinases. As far as their induction and accumulation is concerned, they are even produce in viral attacks and exposure to UV radiations. They were first discovered in tobacco under fungal infection and their homologue is exists in pepper as well. The molecular weight of these proteins is 18 kDa and they do not possess typical chitin binding domains (CBD) (Bravo et al. 2003).

Family PR-12

Proteins belonging to this family are known as plant defensins and were first isolated and identified during the year 1990 from barley and wheat (Colilla et al. 1990; Mendez et al. 1990). They were characterized as a unique thionin, because of their molecular mass which is 5 kDa and cysteines present in them. As far as their structure is concerned, especially the location of disulfide bonds, they are totally different from a- and b-thionins (Bruix et al. 1993), and hence classified as g-thionins. Afterwards, these g-thionins were called as ‘‘plant defensins’’ due to their structure resemblance with defensins presents in insects and mammals. Terras et al.(1995) identified and classified these PDFs under family PR-12 during their experiments conducted on *Raphanus sativus*. In their study, two defensins named Rs-AFP3, Rs-AFP4 were observed in infected and uninfected leaves of radish with fungi but infected leaves possessed more PDFs (Terras et al. 1995). In the past, several PDFs have been purified from different parts of plants including floral organs, leaves, roots, seeds and stems as well. These proteins show several biological activities such as a-amylase activity, antimicrobial activity and ion-channel blocking *in vitro* (Pellegrini et al. 2005). Terras et al. (1995) reported their broad spectrum antifungal activity against *A. brassicicola*, *B. cinerea* and *F. culmorum* in the members of Brassicaceae family. The exact mode of action of PDFs is still mystery among scientists as they plays their roles in development, abiotic and biotic stresses such as drought, heavy metal stress, cold and microbes (Vanoosthuyse et al. 2001; Koike et al. 2002; Armengaud et al. 2004; Do et al. 2004; Zimmerli et al. 2004; Mirouze et al. 2006). Terras et al. (1995) and GAO et al.(2000) reported the overexpression of PDF gene (Rs-AFP2) resulting in enhanced resistance against *Alternaria longipes* and *Verticillium dahliae* in tobacco and alfalfa respectively.

Family PR-13

Proteins which are classified in PR-13 family are thionins which are of small molecular mass (5 kDa), having cysteine rich peptides and are of basic in nature. Thionins were formerly identified in cereals (Bohlmann et al. 1988). Up till now, almost 100 DNA sequences in 15 plant species which are responsible for the production of thionins have been characterized. The main functions of thionins are of broad spectrum antimicrobial activities in different plants such as *A. thaliana* and barley. And their antimicrobial activity is due to the permeabilization of cell membranes (Cammue et al. 1992; Epple et al. 1995; Stec, 2006) and has been reported against bacteria (Castro and Fontes, 2005), fungi (Terras et al. 1993), mammalian cell lines (Carrasco et al. 1981) and yeast as well (Castro and Fontes, 2005). Thionins along with lipid-transfer proteins (LTP) show synergism and enhanced antifungal activity, proposing that these two proteins work together in permeability and membrane binding (Molina et al. 1993). Several workers have reported that incorporation of gene (Thi2.1) responsible for thionin production in several plants species including *A. thaliana* and tomato have enhanced their antimicrobial resistance against wide range of pathogens including bacteria and fungi (Epple et al. 1997; Chan et al. 2005). RNAi in transgenic plants or knockout of thionin genes and their role on plant defense mechanism have not been studied yet. However, it is assumed that they have defensive and regulatory role like thionins having thioredoxin activity and by this means in the redox regulation on enzymes it could act as secondary messengers (Johnson et al. 1987). Thionine act as storage proteins in seeds particularly as sulfur source (Castro and Fontes, 2005).

Family PR-14

Lipid transfer proteins (LTPs) are small peptides having molecular masses of 7-9 kDa. These peptides are cysteine rich and have been discovered in large number of plant species including *A. thaliana*, barley, grapevine, onion, spinach and wheat (Molina et al. 1993; Segura et al. 1993; Cammue et al. 1995; Girault et al. 2008; Sun et al. 2008). LTP comprises of a single peptide which target them to the secretory

pathway are classified into two classes LTP1s (7 kDa) and LTP2s (7 kDa) (Carvalho and Gomes, 2007). Hence, their name indicates that they are responsible for the *in vitro* transfer of phospholipids among membranes (Kader, 1975) such as galactolipids, phosphatidylcholine and phosphatidylinositol (Castro and Fontes, 2005). As far as their specificity is concerned, they are very little specific towards their substrate and hence named non-specific lipid transfer proteins (Kader, 1996). LTPs are confined to cell walls (Thoma et al. 1993), glyoxosomes where they are associated with catabolism of lipid (Tsuboi et al. 1992) and in protein storage vacuoles of seeds (Carvalho et al. 2001). Genes for the production of LTP usually show their expression flowers and leaves but seldom in roots of plants (Arondel et al. 2000). These genes show high levels of expression in newly formed leaves as compared to old leaves which suggest their role in transportation of cutin monomers and association with the assemblage of wax and cutin (Chassot et al. 2007). However, Park et al.(2000) observed during their studies on *Lilium longiflorum* that most of the LTP genes are expressed in floral parts of plants because LTP are required for the adherence of pollens to stigma during pollen elongation. Moreover, LTP genes are expressed in biotic and abiotic stresses such as cold, drought and salt (Jung et al. 2003). There are several reports of their role in β-oxidation (Tsuboi et al. 1992), cutin synthesis (Pyee et al. 1994) and plant defense signaling (Buhot et al. 2001). As far as their part in plant defense is concerned, LTPs exhibits *in vitro* antimicrobial properties (Wang et al. 2005) by interacting with biological membranes, possibly causing membrane permeabilization (Kader, 1996).

Family PR-15

In plants, oxalate oxidases (OXO) are present in low concentration playing crucial role in the defense against biotic and abiotic stress (Mittler, 2002). Oxalate oxidase is an enzyme that degrades oxalate to CO, and H_2O_2, has been characterized in some plant species including barley *(Hordeum vulgare)* (Sugiura et al. 1979). Further H_2O_2 triggers signal transduction cascade which activates plant defense mechanisms leading to the synthesis of pathogenesis-related proteins and phytoalexins (Mittler, 2002). Oxalate oxidase was first isolated and characterized in *Hordeum vulgare* and *Triticum aestivum*. The oxalate oxidase play various physiological and defense roles like germination, fruit ripening, floral induction, seed development, embryogenesis, nodulation production of H_2O_2 and Nitrogen fixation. In nature, OXO has two forms soluble and membrane bound. Wheat oxalate oxidases (germin) are the best-characterized enzyme having extreme resistance to heat and protease. Therefore, germin are used as a marker for early plant development and have been isolated from both dicots and monocots species. Six different types of OXO and OXO type proteins have been identified in barley (Singh et al. 2014). Zhou et al.(1998) characterized oxalate oxidase (PR-15) in leaves of barley infected with fungus powdery mildew *Blumeria graminis* f. sp. *hordei* and suggested that these OXO plays their role in signal transduction pathway for regulation of the hypersensitive response.

Family PR-16

Multiple structural, receptor and enzymatic functions are detected in do-all germins and germin-like proteins are referred to as PR-15 and PR-16, respectively (Park et al. 2004a; Park et al. 2004b). The remaining PR-like proteins, classified as PR-15 and PR-16 protein families, are pathogen-induced germin-like oxalate oxidases and germin-like oxalate oxidase-like with superoxidase dismutase activity. These are thought to be involved in signal transduction pathway for the regulation of Hypersensitive Response (Van Loon et al. 2006). The PR-15 and PR-16 have been proposed to release H_2O_2 which is necessary for cross-linking of components of cell wall during papillae (Carter et al. 1998; Wei et al. 1998). The constitutive expression of OXO confers enhanced resistance to OA-generating pathogens like *Sclerotinia sclerotiorum, Cristulariella pyramidalis* and *Septoria musiva*. The pathogen synthesizes and secretes millimolar concentrations of oxalic acid into infected host tissues. The OXO significantly reduces the Sclerotinia disease by degrading the OA produced by *Sclerotinia* toxin which reduces the damage in the plant tissues caused by the pathogen and produces the defense inducing molecule H_2O_2 (Singh et al. 2014).

Family PR-17

Members of PR-17 family are known for their significant role in disease defense mechanism against pathogens but their exact molecular functions have not been defined. Further it has been demonstrated that over-expression of PR17 protein in wheat (WCI-5) confers resistance in wheat against with powdery mildew fungi *B. graminis* f.sp. *tritici* (Schweizer et al. 1999). PR-17 proteins are yet to be characterized and have been discovered in barley, wheat and tobacco. It has already been demonstrated for two barley (*Hordeum vulgare* L.) proteins (HvPR-17a and HvPR-17b) belonging to the PR-17 family that they accumulated in the mesophyll apoplast along with leaf epidermis when barley plants were inoculated with *Blumeria graminis* f.sp. *hordei*.

These proteins are monomeric polypeptides with having molecular masses of 26 and 24 kDa respectively. It has only one representative in barley and characterized mainly by its cDNA. The central C terminal part of the deduced amino acid sequence has five domains that are highly conserved. They share

sequence similarities with aminopeptidase's form the eukaryotes and thermolysins from bacteria suggesting a proteolytic like activity. The PR-17 family has affinity towards zinc and therefore is similar to Zinc metal, loproteinases. The C-terminal A to E domains are highly conserved and are similar to thermolysins. Domain A has protein kinase C phosphorylation site and the domain B has conserved similarities with aminopeptidases (Christensen et al. 2002).

Conclusion

Pathogenesis-Related Proteins are important for plants because they play a significant role in seed germination, plant development and disease resistance and to cope with unfavourable environmental conditions. This review encourages the incorporation of PR genes through genetic engineering for crop protection and improvement. Though, plant molecular biology lacks the genetic studies of PRs genes responsible for the production of PR-Proteins especially the process of gene regulations which includes signal transduction pathways and molecular targets required to induce these PRs genes which is a crucial step for studies involved in crop protection. Preliminary studies have been carried out and the primary attributes of gene regulation of the PR-Proteins are very well known but the exact mode of action of these proteins, mechanism of their gene regulation and expression pattern of these genes will open new horizons in field of plant genetic engineering for the betterment of crop protection related research.

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