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RESEARCH ARTICLE

Role of Pathogen related protein families in defence mechanism with potential role in applied biotechnology

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Abstract

Plants are threatened by various pathogen attacks from a wide range of pathogenic organisms like fungi, oomycetes, bacteria, viruses and insect which causes various devastating diseases resulting in reduced crop production. In this response plants have evolved various diverse mechanisms to defend themselves against various pathogenic speices attack. The plant response to pathogen attack activates various host plant signal transduction pathways and accumulates pathogenesis-related (PR) proteins. Therefore, PR proteins are usually induced upon attack of various pathogens and stress such as drought, osmotic, cold, salinity, metal stress, UV light. In plants, PR proteins plays a diverse multiple functions like plant defense, disease resistace, cell wall rigidification, development, antifungal activity, enzymatic functions (β-1,3-glucanase, chitinase, defensins) and adaption to stress. Currently PR proteins are categorized into seventeen families (PR 1-17) according to their biochemical functions and properties. The antimicrobial activity of pathogenesis-related proteins provides valuable tools for engineering resistance in plants with ecomomic improtance. Future exploitation of these proteins reflects their potential role in various agribiotechnological applications. This review discusses about the comprehensive function of PR proteins in plant development, classification, disease resistance and their utility in genetic engineering for enhancing disease resistance in crop plants with essential measures in disease controlling strategies.

Introduction

Plants constitute the largest and important group of autotrophic forms of life on the earth. Their abundant organic material serves as a nutritional source for all organisms including animals, insects, microbes and human. Plants, like animals, are continually exposed to pathogen attack from a wide range of pathogenic organisms which attack the plant with diverse and unique mode of pathogenicity causing a significant threat to the food biosecurity. Currently, worldwide crop losses caused by pathogens (viruses, fungi, bacteria, protozoa, mycoplasma and nematodes) are 20-40% of global agriculture productivity leading a major constraint to agricultural production (Oerke, 2006). A classical strategy for plants to fight against pathogens is to breed for resistance. Natural resistance found in wild relatives is introgressed in crop plants which provides specific resistance to particular races of a pathogen. This high specificity between a particular host (cultivar) and pathogen (race) genotype is the basis of gene-for-gene hypothesis confirmed by the molecular and functional identification of plant *R* genes (resistance genes) and matching avirulence (*Avr*) genes in the pathogen (Flor, 1971).

Plants have evolved an array of synergistic defense mechanisms in response to pathogen attack or other abiotic stresses which is crucial for the plant survival. The resistance or susceptibility to a particular pathogen depends on

pathogen recognition, activation of host plant signal transduction pathways and induction of active defense molecules. Plants activate the defense responses after recognition of race-specific or race nonspecific pathogen derived elicitors. Plant stimulates signalling pathways after the pathogen recognition which leads to the activation of various defence mechanisms such as production of reactive oxygen species (ROS), cell wall modification, accumulation of phytoalexin, induction of pathogenesis-related proteins (van Loon et al., 1994), as well as systemic acquired resistance (SAR) and hypersensitive response.

Antoniw et al. (1980) coined the term "pathogenesis-related proteins" (PR) as host-specific proteins induced *de novo* upon pathogen attack as well as after wounding and certain abiotic stress conditions (van Loon et al., 2006). Pathogenesis-related protein are widely distributed in various groups of the plant, animal and fungal kingdoms. PRs are multifunctional proteins having low molecular weight (10-40 kDa) located in the vacuole, cell wall and inter and intra cellular spaces. PR proteins are soluble, stable at low pH and usually have extreme isoelectric points. PR proteins can survive in extreme harsh environments due to their various biochemical properties and are resistant against proteolytic cleavage (van Loon et al., 2006). Furthermore, PR proteins induces a hypersensitive response characterized by localized and rapid death of cells (necrosis) surrounding the site of pathogen infection which inhibit multiplication and further spreading of infection (Heath, 2000). PR proteins were first described by van Loon and van Kammen (1970) as components of the hypersensitive response in leaves of tobacco plants induced by tobacco mosaic virus (TMV). The PR proteins plays a diverse functions like plant defense, disease resistance, cell wall rigidification, development, antifungal activity, enzymatic functions with increase adaption to environmental stress. These proteins are up-regulated in disease or stress conditions playing a crucial role in plant cellular defence. Therefore PR proteins belong to the family of "stress-inducible" proteins. Currently PR proteins are categorized into seventeen families (PR-1 - PR-17) according to their molecular weight, biochemical functions and properties (Table 1).

The PR proteins have anti-microbial activities which makes this family an attractive candidate for genetic engineering with enhanced resistance by constitutive expression of defense inducible genes. The antimicrobial activities many PR proteins are of great interest in agribiotechnological as well as in the pharmaceutical industry because of their potential use in generating transgenic crops with improved pathogen resistance and reducing pesticides used in the agricultural industry (Dempsey, 1998; Stotz et al., 2009b). Ten PR families (PR-1, PR-2, PR-3, PR-4, PR-5, PR-8, PR-11, PR-12, PR-13 and PR-14) have direct activities against fungal pathogens. Furthermore these proteins have the potential as the lead compounds for the development of novel antimicrobial therapeutics. The member of pathogenesis-related proteins PR-1, PR-2 and PR-5 are often used as molecular markers which elicits the SA-dependent SAR pathway where as the members of PR-3 and PR-4 are used as markers for JA-dependent SAR pathway. Some members of PR-protein families are known as 'plant food allergens' varying in allergenic potential in different plant species. PR-protein families PR-1, PR-2, PR-3, PR-4, PR-5, PR-8, PR-10 and PR-14 are plant-derived allergens which help in understanding various allergy syndromes.

This review is focused on recent advances in the study of PR proteins which shed some light to understand their functions in plant development, disease resistance and their utility in genetic engineering for enhancing disease resistance in crop plants to initiate a plethora of defense mechanism for all families is illustrated in table 2.

PR-1 family

PR-1 is first discovered and highly expressed class of PR proteins contributing 10% of total protein found in infected leaves. PR-1 proteins are synthesized by plants in response to the recognition of pathogen by host plant cells which leads to the activation of transduction pathways. The PR-1 group has ~15 to 17 kDa molecular weight, present in both basic and acidic isoforms and have homology to the superfamily of cysteine-rich proteins (van Loon et al., 1999). The PR-1 proteins are found in yeasts, insects, vertebrates and angiosperms. The first report of an allergen in PR-1 protein family was detected in muskmelon (Cuc m 3). PR-1 proteins are useful molecular marker genes in plant disease/defense for the SAR response in many plants species. However to date, very little is known about the *in vivo* function and mode of action of PR-1 (van Loon et al., 2006). The *N. tabacum* PR-1a is the first characterized PR-1 protein. Since then, a number of PR-1 proteins have been identified in *Arabidopsis*, maize, wheat, tomato, tobacco, barley, rice and pepper. The defense function of PR-1 proteins was confirmed by constitutive expression of transgene expression in plants. Niderman et al. (1995) demonstrated that *in vitro* and *in vivo* antifungal activity in tomato (P14c) and tobacco (PR-1 g) PR-1 proteins against *P. infestans*. The *Nicotiana tabacum* encodes acidic PR-1 proteins such as PR-1a PR-1b and PR-1c present in the xylem, extracellular spaces

and vacuoles along with basic PR-1 proteins present in vacuoles which are biochemically and genetically considered as best characterized PR proteins. The PR-1 genes in tobacco are induced via signal transduction pathways involving salicylic acid, jasmonic acid and/or ethylene. A SA-dependent pathway induces the expression of acidic PR-1 genes whereas the JA-dependent pathway and/or ethylene-dependent pathway activate the expression of basic PR-1 genes (Niki et al., 1998). The PR protein occur as multigene families in various plant species for instance 22 PR-1 genes were identified in Arabidopsis, 39 PR gene in rice and 16 genes in tobacco respectively. Further it has been showed that in rice acidic pathogenesis-related class 1 cDNA (*OsPR1a*) from JA-treated rice (cv.Nipponbare) seedling leaf can be used as an important gene markers in rice with potential use in analyzing plant defense responses (Agrawal et al., 2000a). Further it was demonstrated that in transgenic tobacco plants, the basic PR-1 protein from pepper (CABPR1) overexpression confers enhanced disease resistance to the pathogens *Phytophthora nicotianae*, *Ralstonia solanacearum* and *Pseudomonas syringae* along with partial tolerance to heavy metal stress (Sarowar et al., 2005)

PR-2 family: β-1,3-glucanases

The PR-2 proteins are β-1,3-glucanases which possess 1,3-β-endoglucanase activity *in vitro*. The β-1,3-glucanase are highly abundant enzymes which catalyze endo-type hydrolytic cleavage of 1,3-β-D-glucosidic linkages in β-1,3-glucans resulting in the weaking of cell wall of pathogen to death. Plant β-1,3-glucanases have molecular 33-36 kDa having both acidic and basic isoforms. β-1,3-glucanases are widely distributed among bacteria, fungi and plants. β-1,3-glucanases play important roles in physiological and developmental processes such as pollen germination, flowering, cell differentiation, fertilization, embryogenesis, fruit ripening, mobilzation of storage reserves and bud dormancy. The β-1,3-glucanases are strongly induced in response to wounding, cold, ozone, UV and against array of fungal pathogens attack in plants. Therefore, β-1,3-glucanases is an attractive member because it has strong antifungal activity against a wide range of fungi (Walsh et al., 2000). The β-1,3-glucanase gene was first characterized in rice. Many plant species have multiple copies of β-1,3-glucanase genes like in tobacco plants 14 multiple β-1,3-glucanase genes copies have been reported. The PR-2 family is a plant allergen detected in the latex of *Hevea brasiliensis* (Hev b 2). Hevea latex allergy has been found to be associated with hypersensitivity fruit allergens especially avocado, banana, chestnut, fig, bell pepper, kiwi, pineapple, peach, and tomato causing latex-fruit syndrome. Another novel major allergen Ole e 9 has been isolated from olive pollen. β-1,3-glucanases are grouped into three classes on the basis of amino acid sequence analysis. Class I glucanases are 33 kDa basic proteins found in the plant vacuole. Classes II and III are 36 kDa acidic proteins found in extracellular space. Further it has been showed that the expression of classI rice chitinase gene *RCC2* in transgenic cucumber with showed increased disease resistance to *Botrytis cinerea* which causes gray mold diseases (Kishimoto et al., 2002). The expression of β-1,3-glucanase gene (PpGlu) from the *Pyrus pyrifolia* Nakai cv Huobali shows enhance resistance to three pathogenic fungi *Phomopsis* sp., *Alternaria* sp. and *Fusarium* sp. in transgenic tobacco (Liu et al., 2013). Further it has been demonstrated that transgenic *Linum usitatissimum* (flax) plant overexpressing β-1,3-glucanase showed significant and improved resistance to pathogen *Fusarium oxysporum* and *F. culmorum* infection with potential influence on composition of flax fibres (Wojtasik et al., 2013). Recently, β-1,3-glucanase (Bgn13.1) gene isolated from the biocontrol fungus *Trichoderma virens*-10 expressed in transgenic *Brassica napus* confers antifungal activity against phytopathogenic fungus *Sclerotinia sclerotiorum* (Kheiri et al., 2014).

PR-3, PR-4, PR-8, PR-11 family: Chitinases

Chitinases are hydrolytic enzymes catalyzing the hydrolysis of β-1,4-linkage of the *N*-acetylglucosamine polymer of chitin. Plant chitinases have molecular weight varying from 25 to 40 kDa with both acidic and basic isoforms constitutively present in stems, seeds, flowers and tubers. Chitinases have lysozymal activity, present in low level in plants but are strongly and coordinately up-regulated by various abiotic and biotic stress conditions. The transgenic plants overexpressing chitinases confers increase resistance to pathogen attack. Chitinases comprises four PR families namely PR-3, PR-4, PR-8, and PR-11. Chitinases play a role in plant defense by directly or indirectly inhibiting the hyphae growth which invades the intercellular space or release fungal elicitors. Chitinase are classified into two categories endochitinases (EC 3.2.1.1.4) and exochitinases (EC 3.2.1.14) with respect to their hydrolytic sites. Endochitinases cleaves chitin at internal bonds producing soluble, low molecular mass multimers of N-acetyl glucosamine such as chitotrose, chitotetraose and di-acetylchitobios. The endochitinases includes PR-3, PR-4, PR-8 and PR-11 families. Exochitinases cleave chitin by releasing diacetylchitobiose without producing N-acetyl glucosamine or oligomers. Chitinases are widely present in different tissues with multiple isoforms and gene clusters in both monocots and dicots plant species. Further on the basis of structure, specificity, catalatic mechanism and inhibitor sensitivity the plant chitinases are classified into seven classes I-VII. In tobacco Class I, V and class VI chitinases posses anti-fungal activity *in vitro* against *Fusarium solani*, *Alternaria radicina* and *Trichoderma viride*.

The chitinases belong to glycosyl hydrolase families 18 and 19 according to the classification made by Henrissat and Bairoch (Henrissat et al., 1993). The class of chitinases III and V belong to glycosyl hydrolase families 18 and class I, II, IV, VI and VII belong to glycosyl hydrolase families 19. Further chitinases of classes I, II, IV, VI and VII belong to PR-3 family, class III chitinases belongs to PR-8 family and class V to PR-11 family respectively. Salzer et al. (2000) demonstrated that class III chitinase genes from *M. truncatula Mtchit3-1* and *Mtchit3-4* are induced in response to infection by pathogenic fungi *Phytophthora megasperma*, *Fusarium solani* in the roots. PR-3 (class I) chitinases also act as major allergens which are detected in chestnut (Cas s 5) and avocado (Pers a 1), banana (Mus a 2). Further in PR-4 (class II) family prohevein (Hev b 6.0) is a major allergen detected in *Hevea brasiliensi* latex. PR-8 class III chitinases hevamine (Hev b 14) is another allergen present in *Hevea brasiliensis* latex possing both lysozyme and chitinase activity. Other PR-8 chitinase as major allergens have been identified in *Zizyphus mauritiana* (Indian jujube) (Ziz m 1) and coffee (Cof a 1). Transgenic plants expressing chitinase gene have been transformed to a number of plant species showing enhanced disease resistance against various fungal pathogens. The overexpression of basic PR-3-class chitinase from bean in transgenic tobacco and canola showed resistance to *R. solani* which causes rice sheath blight disease (Grover et al., 2003). The constitutive expression of both chitinases and β-1,3-glucanases from *Citrus limon* seedlings shows the defense response against *Alternaria alternata* which causes early blight disease (Fanta et al., 2003). Recently Prasad et al. (2013) demonstrated that overexpression of a chitinase gene from rice (Rchit) in transgenic peanut plants confers enhanced resistance against soil borne pathogens *Phaeoisariopsis personata* and *Puccinia arachidis* respectively.

PR-5 family: Thaumatins

Members of the PR-5 proteins are called thaumatin-like proteins (TLPs) which have high sequence identity with thaumatins, a sweet-tasting protein isolated from the West African shrub *Thaumatococcus daniellii*. PR-5 family have diverse functions in plant disease resistance such as antifungal activity, antifreezing activity and osmotic stress tolerence. About 24 PR-5 genes have been identified in arabidopsis. PR5 family consists of TLP, osmotin, osmotin-like proteins (OLP) and zeamatin. TLPs posses molecular mass 20 to 26 kDa, with ~200 residues possessing 16 conserved cysteine residues forming eight disulphide bonds. According to their isoelectric point, PR5 family are grouped as acidic members characterized by the presence of an N-terminal signal peptide and the basic member proteins have an additional C-terminal extension targeting the vacuole. The PR5 proteins are induced and up-regulation in response to pathogens attack, osmotic stress, response to wounding, insect feeding and freezing tolerance. The PR-5 family represents pollen or food derived allergens that are present in various plant species. Several pollen allergens have been detected in Eastern red cedar (Jun v 3), Japanese cedar (Cry j 1), mountain cedar (Jun a 3), Arizona cypress (Cup a 3) and food allergens include apple (Mal d 2), sweet cherry (Pru av 2), bell pepper (Cap a 1), kiwi (Act d 2), peach (Pru p 2) and banana (Mus a 4). The thaumatin like proteins posseses antifungal activity by inhibiting hyphal growth, spore lysis and/or reduction in spore germination or viability of germinated spores. It is demonstrated that *in vitro* overexpression of PR-5 proteins in potato delayed development of disease symptoms of *P. infestans* which is the cause of late blight disease of potato (Liu et al., 1993). Osmotin is a 24-kDa, basic pathogenesis-related playing a defensive role during fungal infection against a variety of pathogenic fungi. Osmotin inhibit *in vitro* spore germination and hyphal growth against a variety the phytopathogenic fungi *Phytophthora infestans, Fusaritun oxysporum, Altermaria solani, Botrytis cinerea Fwarium oxysponun, Verticillium akhliae, P. nicotianae* and *Cercospora beticola* with delayed disease symptoms. Linusitin is a 25-kDa TLP isolated from flax seeds posses antifungal activity against *Alternaria alternata*. Further, Zeamatin a 22 kDa, antifungal protein isolated from maize play an important role in a plant defense. The antifungal activity of osmotin and zeamatin, a PR-5 protein from maize is due to fungal membrane permeabilization. Wang et al. (2011) showed that thaumatin-like proteins isolated from dessert plant *Cynanchum komarovii* seeds (CkTLP) confers strong antifungal activity against *Verticillium dahliae*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Botrytis cinerea* and *Valsa mali*. Futher in transgenic *Arabidopsis thaliana* the over-expression of an antifungal protein-CkChn134 and CkTLP from *C. komarovii* seeds confers enhanced the resistance against *V. dahliae* (Wang et al., 2011). Further, Zamani et al. (2012) demonstrated that the thaumatin like *tlp* gene isolated from cereal rye (*Secale cereal* L.) was expressed in Canola (*Brassica napus* L.) confers antifungal activity with enhance resistance to stem rot disease causing pathogen *S. Sclerotiorum*. Recently, it has been demonstrated that overexpression of TLP gene of *Camellia sinensis* (*CsTLP*) in transgenic potato plants shows tolerance to fungal pathogens, *Macrophomina phaseolina* and *Phytophthora infestans* (Acharya et al., 2013). Transgenic tobacco plants expressing TLP gene from *Arachis diogoi* (wild peanut) (AdTLP) showed enhanced resistance to fungal pathogen *Rhizoctonia solani* along with enhance stress resistance in crop plants (Singh et al., 2013).

PR-6 family: Protease inhibitors

The PR-6 family proteinase inhibitors (PIs) are small proteins which act as natural antagonists of proteases. In plants the proteinase inhibitors are present mainly in storage tissues (tubers and seeds) and aerial parts inhibiting the function of proteases. Plant PI proteins ranges from 4 to 85 kDa with high cysteine residues composition which forms disulfide bridges. PIs play a role in plant defense and are induced in response to various stress including pathogens and insects attack, wounding and various environmental stresses (Koiwa et al., 1997). The defensive role of plant PIs depends on inhibition of proteases secreted or presents in insect guts which supresses various vital and biochemical processes of insects and other pathogens. Protease inhibitors exhibit broad spectrum activity against wide range of nematodes like *Globodera tabaccum, G. Pallida* and *Meloidogyne incognita* by cowpea trypsin inhibitor (CpTi). PIs have been isolated from many plants playing important roles in defense e.g. cowpea trypsin inhibitor, potato and tomato proteinase inhibitor II (PIN2), rice cysteine PI, barley trypsin inhibitor, soybean Kunitz trypsin inhibitor and maize PI. As many plant PIs potently inhibit the growth of pathogens therefore used as excellent candidates for the development of novel antimicrobial compounds. Protease inhibitors also inhibit the growth of microorganism by antifeedent mechanism. The PIs families are specific for the four classes of protinases they inhibit and based on the active amino acid in their "reaction center" are classified as serine, cysteine, aspartic and metallo-proteases (Koiwa et al., 1997). Among different types of plant PIs, serine PIs is a widespread throughout the plant kingdom. However, serine PIs are used to engineer insect resistance in transgenic plants as insects such as *Helicoverpa armigera* and *Spodoptera litura* use serine proteinases as major digestive enzymes. Cysteine proteinase inhibitors (cysteine PIs or cystatins) are widely distributed in plants, animals and microorganisms which inhibit the activity of cysteine proteases. The first isolated and well characterized cysteine PIs is oryzacystatin (OC-1). Aspartic inhibitors are rare and have been been isolated from sunflower, barley, cardoon flowers and potato tubers. In plants two families of metalloproteinase inhibitors have been identified the metallocarboxypeptidase inhibitor family in potato and tomato plants and cathepsin D inhibitor family in potato. The metallocarboxypeptidase inhibitors are small peptide inhibitors consisting of 38-39 amino acid residues with molecular mass ~4.2 kDa. This inhibitor accumulates in various tissues like potato tuber and leaf during the development. Further the potato inhibitor I and II families of serine PIs accumulate as a response to wounding in potato which strongly inhibits a broad spectrum of carboxypeptidases from various pathogens. The cathepsin D inhibitor, an aspartyl PI is a 27 kDa protein which inhibits serine proteases, trypsin, chymotrypsin, and aspartyl protease cathepsin D. Many plants species over-expressioning the various protinases inhibitor families have been used to develop insect-resistant transgenic plants. It has been demonstrated that the overexpression of serine proteinase inhibitor gene (SaPIN2a) from *Solanum americanum* in transgenic tobacco shows enhanced resistance to lepidopteran insects *Helicoverpa armigera* and *Spodoptera litura* which causes considerable economic loss in cotton, tobacco, sunflower, corn, pepper and tomato plants (Luo et al., 2009).

PR-7 family: Proteinase

Proteases (proteinase or peptidase) play important role in the regulation of various biological processes like growth, development, photosynthesis and induction of defence response against insects, herbivores and nematodes. Proteases directly degrade proteins from the pathogen and release peptide-based toxins or activate enzymes from their precursor proteins. *Arabidopsis* genome encodes over 800 proteases. The activity of proteases is inhibited by protease inhibitors. Proteases are of two types: endopeptidases and exopeptidases. Endopeptidases hydrolyse internal peptide bonds in polypeptide chains which include trypsin, chymotrypsin, pepsin, papain, elastase whereas exopeptidases hydrolyse terminal residues which include aminopeptidases, carboxypeptidase. Many plant proteases play role in defence which are secreted into the apoplast and are poorly understood due to uncharacterised substrates in plants. Proteases are further grouped according to their active sites and mechanisms of action into five major classes: (i) serine proteases (ii) aspartic proteases (iii) cysteine proteases (iv) threonine and (v) metalloproteases. Serine proteases are a largest class of proteolytic enzymes which cleave peptide bond in proteins where the active site Ser acts as a nucleophile. Serine proteases play role in various essential physiological pathways to regulate plant development and defense response. Cysteine proteases use a catalytic Cys as a nucleophile during proteolysis. Cysteine proteases regulate epidermal cell fate, flowering time, inflorescence architecture and pollen or embryo development. Cysteine proteases play an important role in localized programmed cell death (PCD) with response to pathogens infection. Cysteine proteases posses insecticidal property. Recently, demonstrated that the plant cysteine proteases isolated from *Gossypium hirsutum* (GhCP1) and *Arabidopsis* (AtCP2) shows resistance against insect cotton bollworm larvae (Mao et al., 2013). Aspartic proteases (AP) are the second largest protease class containing two aspartic residues which acts as the nucleophile during proteolysis. APs have two aspartic acid residues located within the conserved (Asp-Thr/Ser-Gly) motif responsible for catalytic activity. Aspartic protease regulates various

biological processes in plants like such as development, senescence, stress responses, programmed cell death and reproduction. Metalloproteases are protease enzyme where the catalytic mechanism involves catalytic metal ions which activate water molecule for nucleophilic attack while and stabilize the oxyanion hole. Metalloproteases play diverse roles in many physiological processes like growth, nodulation, thermotolerance, seed germination and development. Liu et al. (2001) demonstrated that in *Glycine max GmMMP2* gene is up-regulated in response to the infection from fungal pathogen *Phytophthora sojae* and bacterial pathogen *Pseudomonas syringae*

PR-9 family: Peroxidases

Peroxidases are ubiquitous class of oxidoreductases which catalyze reduction of hydrogen peroxide and oxidation of a variety of organic and inorganic compounds. Peroxidase are the key enzymes involved in various physiological processes plant differentiation, development, auxin catabolism, hormonal signaling, wound healing, lignification, suberization, response to stresses. Plant peroxidases have molecular weight ~32-42 kDa, heme-containing enzymes which catalyses the oxidation of substrates like phenol and its derivates by hydrogen peroxide. Peroxidases are involved in the poymerization of the precussors of lignin playing different role in host plant defences against various necrotrophic or biotrophic pathogens. The peroxidases are often used as important resistance gene markers for disease resistance. Peroxidases have been characterized and purified in many plant species. It has been reported that *Arabidopsis thaliana* genome has 130 peroxidases. Peroxidase expression is induced by pathogens and various stress such as drought, light intensity, salinity, cold, metal stress and UV light. Peroxidases are broadly categorised into three classes based on the structural properties. Class I peroxidases are the intracellular peroxidases are located in chloroplasts, mitochondria, peroxysomes and cytosol. Class I peroxidases includes: yeast cytochrome c peroxidase, ascorbate peroxidase and cytosol of higher plants. Class II peroxidases are monomeric glycoproteins involved in the degradation of lignin which consists of secretory fungal peroxidases. Class II includes ligninases and manganese-dependent peroxidases. Class II peroxidases contain four conserved disulphide bridges with two conserved calcium-binding sites in the structure. Class III are glycoprotein secretory plant peroxidases located in vacuoles and cell walls which play role in various functions like defence responses towards wounding, indole-3-acetic acid, oxidation of toxic compounds, ethylene biosynthesis (Passardi et al., 2005). Class III peroxidases contain two conserved calcium ions, four conserved disulphide bridges and one N-terminal signal peptide. The over-expression of a Populus peroxisomal ascorbate peroxidase (PpAPX) gene in tobacco plants enhances stress tolerance. Sarowar et al. (2005) demonstrated that *Capsicum annuum* ascorbate peroxidase-like 1 gene (CAPOA1) overexpressed tobacco plants exhibited enhanced resistance to the oomycete pathogen, *Phytophthora nicotianae* and shows increased tolerance to oxidative stress. However transgenic tobacco, tomato and sweet gum overproducing anionic peroxidase enzyme exhibited greater resistance to insect attack. Recently, demonstrated that tobacco transgenic plants over-expressing ascorbate peroxidase *SbpAPX* gene from *Salicornia brachiata* shows salt and drought stress tolerance (Singh et al., 2013).

PR-10 family: Ribonuclease-like

PR-10 family are small acidic proteins with molecular masses of 15-18 kDa. PR-10 family are homologous to tree pollen allergens and major food allergens of celery or apple. PR-10 proteins are classified as ribonuclease-like PR proteins due to the structural similarity to ginseng ribonuclease. PR-10 proteins have RNase and ligand-binding activities which protects plants during programmed cell death around infection sites or act directly on the pathogens. The PR-10 proteins play important roles in plant growth, development and plant defense responses against various biotic and environmental stresses, such as drought, high salinity, low and high temperatures, wounding, heavy metals and UV exposure. PR-10 protein family members also act as pollen allergens, extensively studied in birch (Bet v 1) homologues. Several Bet v 1 homologus have been characterized and isolated in apple (Mal d1), sweet cherry (Pru av 1), apricot (Pru ar 1), celery (Api g 1), peach (Pru p 1), pear (Pyr c 1), carrot (Dau c 1), parsley (pcPR-1 and pcPR-2), potato (pSTH-2 and pSTH-21), hazelnuts (Cor a 1) and chestnuts (Cas s 1) (Hoffmann-Sommergruber 2002). PR-10 family have been isolated from dicots as well as monocot including bean, soybean, asparagus, sorghum, barley, rice, potato, apple and *Medicago sativa*. As pollen or food allergens high levels of PR-10 proteins are detected in birch pollen, hornbean, apple, celery, pear and soybean. The recombinant CaPR-10 protein from *Capsicum annuum* inhibits the growth of the *P. capsici* pathogen and exhibits ribonucleolytic activity against tobacco mosaic virus RNA. A novel tuber storage PR-10 protein Ocatin isolated from *the Oxalis tuberosa* confers antibacterial and antifungal activities. Fung et al. (2007) demonstrated that in *V. vinifera* these proteins are induced under salt or herbicide stress and also exhibit wide spectrum resistance to the powdery mildew fungus Erysiphe necator (Schw.) Burr. The constitutive expression of a pea PR10 gene in *Brassica napus* enhances their tolerance to salinity specifically during germination and seedling development. In *Panax ginseng*, (PgPR10) PR10

proteins have ribonuclease activity which confers various defense-related resistances against various stress (Lee et al., 2012). Recently, Xie et al. (2013) demonstrated that overexpression of PR10 family gene *ARAhPR10* play an important role in *Arachis hypogaea* seed resistance to *Aspergillus flavus*.

PR-12 family: Defensin

Plant defensins have low-molecular mass (~3 to 5 kda) with 45 to 54 amino acids long, positively charged with basic cysteine-rich residues that form disulfide bridges. The protein structures of defensins have βαββ architecture composed of a cysteine-stabilized αβ motif consisting of α-helix and a triple-stranded β-sheet. Defensin exhibit broad spectrum of potent antifungal activity, antibacterial activity, proteinase inhibitory activity and insect amylase inhibitory activity. The first defensins were isolated form wheat and barley endosperm. Later it was found that the genome of *A. thaliana* encodes more than 300 defensin-like peptides. Furthermore, the expression of plant defensin genes is highly expressed or upregulated in plants in response to biotic and abiotic stress. Defensins are classified into four groups. Group I defensins are known as morphogenic defensins as they cause morphological changes in susceptible fungi. Group II proteins are non-morphogenic group which inhibit fungal growth but do not cause morphological changes. Group III have no antifungal activity but inhibit α-amylases *in vitro*. Group IV have unique antifungal specificity. The antifungal activities of plant defension have been identified in various plants like pea, tobacco, radish and arabidopis. One of the best-characterized antifungal plant defensins was isolated from *Raphanus sativus* (*RsAFP1* and *RsAFP2)* which confers both *in vivo* and *in vitro* antifungal activity. Transgenic plants overexpressing defensins are strongly resistant to fungal pathogen providing broad spectrum resistance against pathogens. The plant defensin *AlfAFP*, isolated from the seeds of *Medicago sativa*, was transformed into potato provides increased resistance against *Verticillium dahliae*. Further it was demonstrated that the combination of β-1,3-glucanase and defensin gene provides enhance resistance to bacterial pathogens. In transgenic potato plants, the overexpression of *NmDef02* isolated form *Nicotiana megalosiphon* showed enhanced resistance against *Phytophthora infestans* (Portieles et al., 2010).

PR-13 family: Thionin

Thionins is a group of small, low-molecular weight (approximately 5kDa) cysteine rich, basic polypeptides consisting of 44-48 amino acid residues stabilized by conserved six to eight cysteine residues which form 3-4 disulufide-linked bonds. Thionin have antibacterial, antifungal activities with ability to inhibit insect α-amylases and proteinases playing role as plant defense proteins. Till date large number of thionins have been identified and isolated in different plant species. In the cell membranes of phytopathogens, thionins induces formation of open pores on resulting in release of potassium and calcium ions from the cell. Thionins can be classified into five types (I-V) Type I thionins (purothionins) are isolated from endosperm of wheat and barley (hordothionins) which comprises 45 highly basic amino acid residues containing eight cysteine residues present in the central disulfide loop. Type II thionins are 46-47 amino acids long containing eight cysteine residues which form four disulfide bridges at the same positions as those of type I. The type III thionins comprises of 46 residues having three conserved disulfide bridges in the central disulfide loop. The type III thionins includes the viscotoxins and phoratoxins from mistletoes isolated from leaves and stems of *Dendrophthora clavata, Phoradendron tomentosum, Phoradendron liga* and *Viscum album*. The type IV comprises of 46 amino acids with neutral charge molecules and possesses three disulphide bonds. The type IV thionins corresponds to crambins isolated from the Abyssinian cabbage. Like type IV type V thionins are also neutral which have been identified in wheat endosperm and Aegilops species. Thionins are also classified into two groups on the basis of their 3D structure: α/β-thionins and γ-thionins. The α/β-thionins have two α-helixes, double-stranded β-sheets with a C-terminal coil region. The γ-thionins have one α-helix and three anti-parallel β-sheets and an α-helix and three anti-parallel β-sheets help to form amphipathic two-layer α/β sandwich. It has been shown that in various transgenic plants like tobacco, arabidopsis, rice and tomato expressing thionin exhibit resistance to bacterial and fungal pathogens thus playing a role in the plant defense against pathogens. Further, in transgenic *Ipomoea batatas* expressing barley thionin *αHT* showed enhanced resistance to black rot disease caused by *Ceratocystis fimbriata* which severely deteriorates plants growth and storage roots (Muramoto et al., 2012).

PR-14 family: Lipid transfer proteins

Lipid-transfer proteins (LTP) are small (9-10 kDa), basic, soluble, ubiquitous protein which have ability to bind lipids and other hydrophobic molecules. LTP have an alpha helical structure stablized by four disulphide bonds involving eight cystine residues which form tunnel-like hydrophobic cavity for ligand binding. LTP plays diverse role in plant development and defence like cutin synthesis, β-oxidation, somatic embryogenesis alergenics, pollen

adherence, signalling and plant defense against phytopathogens. LTPs expression is induced in response to environmental changes such as drought, cold, high salinity. Further various signalling molecules such as abscisic acid, salicylic acid, ethylene and methyl jasmonate of signaling pathway are responsible for the LTP expression. LTPs are present in high concentation in aerial and vascular tissues of the plant exposed to pathogens at a high concentration. In plants LTPs, are encoded by a small multigene family from various plant species having vital role in various processes including cuticle biosynthesis and embryogenesis. LTPs comprises of two families: LTP_1 and LTP_2. LTP_1 are basic with molecular masses of ~10 kDa. LTP_1 consists of 90-95 amino acid residues with eight conserved cysteines residues which form four disulfide bridges to stabilize the protein structure. The LTP_2 family have molecular masses of ~7 kDa, having average 70 amino acids. PR-14 family members are most important allergens detected in many plant species like peach (Pru p 3), apple (Mal d 3), apricot (Pru ar 3), cherry (Pru av 3), plum (Pru d 3), hazelnut (Cor a 8), chestnut (Cas s 8), maize (Zea m 14), barley (Hor v 14), *Parietaris judaica* (Par j 1), grape (Vit v l), asparagus (Aspa o 1), *H. brasiliensis* latex (Hev b 12), mugwort (Art v 3), ambrosia (Amb a 6) (Hoffmann-Sommergruber 2002). It have been demonstrated that LTPs in radish, maize and grape exhibit *in vitro* antimicrobial activities against various fungi and bacteria. The constitutive expression of CALTPI and CALTPII genes in tobacco plants showed enhanced resistance to oomycete pathogen, *Phytophthora nicotianae* and bacterial pathogen *Pseudomonas syringae pv. tabaci* (Sarowar et al., 2009). Recently Zhu et al. 2012 demonstrated that transgenic *Triticum aestivum* plants overexpressing lipid transfer protein gene *TaLTP5* shows increase resistance to *Cochliobolus sativus* and *Fusarium graminearum* which causes common root rot and Fusarium head blight disease in wheat repectively.

PR-15, 16 family: Oxalate oxidase

In plants oxalate oxidases (OXO) are present in low concentration playing crucial role in the defense against biotic and abiotic stress. The enzyme catalyzes the aerobic oxidation of oxalic acid and oxygen into CO_2 and hydrogen peroxide H_2O_2. Further H_2O_2 triggers signal transduction cascade which activates plant defence mechanisms leading to the synthesis of pathogenesis-related proteins and phytoalexins (Mittler, 2002). 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. Oxalate oxidase was first isolated and characterized in *Hordeum vulgare* and *Triticum aestivum*. 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. In barley six different oxalate oxidase and oxalate oxidase-like proteins have been characterized. 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. Recently, Xiaoling He et al. (2013) showed that transgenic *Colocasia esculenta* (Taro) transformed with oxalate oxidase gene gf2.8 from wheat confer increased resistance to Taro pathogen *Phytophthora colocasiae*.

PR-17 family: Unknown

The PR17 proteins play important role in plant defence against pathogens but the 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). In tobacco, NtPRp27 is induced in response to tobacco mosaic virus infection and mechanical wounding (Okushima et al., 2000). Further it was demonstrated that two encoded proteins from barley (HvPR-17a and HvPR-17b) belong to this family. These proteins are monomeric polypeptides with molecular weights of 26 and 24 kDa respectively (Christensen et al., 2002). These proteins are apoplastic which accumulates in the barley mesophyll apoplast and in leaf epidermis when attack with powdery mildew fungi *Blumeria graminis* f.sp. *hordei*. The HvPR-17a and HvPR-17b proteins are present at diferent level in the plant

Conclusion

Pathogenesis-related proteins provide effective defense mechanisms against many pathogenic and phytopathogenic microorganisms. The increase amount of data of PR proteins gives better idea regarding the plant development and disease resistance. The PR genes are considered as "stress-inducible" proteins having various applications in genetic

engineering for crop improvement. The antimicrobial property of PR proteins is used in agribusiness to create genetically modified plants with increased feild resistance. Further a combination of various PR proteins opens new ways for genetic engineering and provides insights into pathogen defense mechanism with improved disease resistance for developing new and improved crop varieties. Thus pathogenesis-related proteins potentially help to increase in crop production with an increase plant disease resistance to various pathogens and reduction in the extensive use of the chemical fungicides.

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Table: 1. The families of pathogenesis-related proteins (Van Loon and Van Strien, 1999).

Protein Family	**Type member**	**Molecular Size (kDa)**	**Properties**	**Gene Symbols**	**Proposed microbial targets**
PR-1	Tobacco PR-1a	15-17	Antifungal	*Ypr1*	Unknown
PR-2	Tobacco PR-2	33-36	β-1,3glucanase	*Ypr2, Gns2 'Glb'*	β-1,3-Glucan
PR-3	Tobacco P,Q	25-30	Chitinase type I, II, IV, V, VI, VII	*Ypr3, Chia*	Chitin
PR-4	Tobacco 'R'	15-20	Chitinase type I, II	*Ypr4, Chid*	Chitin
PR-5	Tobacco S	25	Thaumatin-like	*Ypr5*	Membrane
PR-6	Tomato Inhibitor I	8	Proteinase-inhibitor	*Ypr6, Pls 'Pin'*	
PR-7	Tomato P69	75	Endoproteinase	*Ypr7*	
PR-8	Cucumber chitinase	28	Chitinase type III	*Ypr8, Chib*	Chitin
PR-9	Tobacco "lignin-forming peroxidase"	35	Peroxidase	*Ypr9, Prx*	
PR-10	Parsley "PR1"	17	Ribonuclease-like	*Ypr10*	
PR-11	Tobacco "class V" chitinase	40	Chitinase, type I	*Ypr11, Chic*	Chitin
PR-12	Radish Rs-AFP3	5	Defensin	*Ypr12*	Membrane
PR-13	Arabidopsis THI2.1	5-7	Thionin	*Ypr13, Thi*	Membrane
PR-14	Barley LTP4	9	lipid transfer proteins (LTPs)	*Ypr14, Ltp*	Membrane
PR-15	Barley OxOa (germin)	20-25	Oxalate oxidase	*Ypr15*	
PR-16	Barley OxOLP	20	Oxalate-oxidase-like	*Ypr16*	
PR-17	Tobacco PRp27	27	Unknown	*Ypr17*	

Table 2: Role of PR proteins in genetic engineering to enhance disease resistance in crop plants.

PR class	**Transgenic plant**	**Donor plant**	**Genes and sources**	**Diseases**	**References**
PR-1	Tobacco	Tobacco	PR1a	*Pernospora tabacina, Phytophthora parasitica*	Alexander et al., 1993
	Tobacco	Pepper	CABPR- 1	*Phytophthora nicotianae, Ralstonia solanacearum* and *Pseudomonas syringae pv. tabaci*	Sarowar et al., 2005
PR-2	Indian mustard	Tomato	Class I glucanase	*Alternaria brassicae*	Mondal et al., 2007
	Flax	Potato	β-1,3-glucanase	*Fusarium oxysporum* and *F. culmorum*	Wrobel-Kwiatkowska et al 2004
	Banana	Soybean	β-1,3-glucanase	*Fusarium oxysporum*	Maziah et al., 2007
	Groundnut	Tobacco	β-1,3-glucanase	*Cercospora arachidicola and Aspergillus flavus*	Sundaresha et al., 2010
	Brassica napus	*Trichoderma virens*	β-1,3-glucanase (Bgn13.1)	*Sclerotinia sclerotiorum*	Kheiri et al., 2014
	Wheat (spring)	Wheat (Sumai-3 cultivar)	Glucanase,	*F. graminearurn*	Anand et al., 2003

			Chitinase and PR-5		
	Wheat	Barley (glucanase), wheat (thionin) and thaumatin-like protein 1 (tlp-1)	class-II β-1,3-glucanase, thaumatin-like protein 1, a-1-purothionin	*F. graminearum*	Mackintosh et al., 2007
	Indica rice	Tobacco	chitinase and β-1,3-glucanase	*Rhizoctonia solani*	Sridevi et al., 2008
	Carrot	Tobacco	chitinase PR-3d and and class I glucanase PR-2e	*Alternaria dauci, A. radicina, Cercospora carotae, and Erysiphe heraclei*	Melchers et al., 2000
	Tall fescue	Alfalfa (glucanase)	β-1,3-glucanase, AGLU1, frog dermaseptin SI gene	*Magnaporthe grisea, Rhizoctonia solani*	Dong et al., 2007
	Peanut	Rice and Alfalfa	rice chitinase and an alfalfa glucanase	*S. sclerotioriurn*	Chenault et al., 2005
	Tobacco	*Pyrus pyrifolia*	β-1,3-glucanase gene, PpGlu	*Phomopsis sp., Alternaria sp. and Fusarium sp*	Liu et al., 2013
	Psium satium	Barley	β-1,3 glucanase gene (gluc) and *S. olivaceoviridis* chitinase gene (Chit30)	*T. harzianum, C. acutatum, B. cinerea and Ascochyta pisi*	Amian et al., 2011
PR-3, PR-4, PR-8, PR-11	Carrot	Wheat and rice	Wheat class IV chitinase (Chi83), acidic wheat β-1,3-glucanase (Glu638), and rice cationic peroxidase (POC1)	*B. cinerea and Sclerotinia sclerotiorum*	Wally et al., 2009
	Peanut	Rice	Chitinase (Rchit)	fungal pathogens	Prasad et al., 2013
	Tobacco	Sugarbeet	class III Chitinase	*C. nicotianae*	Pasonen et al., 2004
	Grapevine	Rice	class I Chitinase	*Elisinoe ampelina*	Yamamoto et al., 2000
	Rice	Rice, Alfalfa, Barley	RCH10, RAC22, β-glucanase, β-RIP	*Magnaporthe grisea*	Zhu et al., 2007
	Canola and Tobacco	Bean	class III Chitinase	*Rhizoctonia solani*	Grovers et al., 2003
	Solanum tuberosum cv. *Savalan*	chitinase and glucanase *Trichoderma*	chit42 + bgn13.1	*Rhizoctonia solani*	Esfahani et al., 2010

		species			
	Tobacco	*Metarhizium anisopliae*	chitinase gene CHIT 42	*Rhizoctonia solani*	Kern et al., 2010
	Banana	Rice	chitinase gene	*Micosphaerella fijiensis*	Kovács et al., 2013
PR-5	Tobacco	Rice	PR-5	*Alternaria alternata*	Velazhahan et al., 2003
	Carrot	Rice	PR-5	*A. dauci, Alternaria petroselini, A. radicina, B. cinerea, R. solani* and *S. sclerotiorum*	Punja et al., 2005
	Tomato	Rice	tlpD34	*Alternaria alternata*	Radhajeyalakshmi et al., 2005
	Potato	*Camellia sinensis*	CsTLP	*Macrophomina phaseolina* and *Phytophthora infestans*	Acharya et al., 2013
	Canola	cereal rye	tlp	*S. Sclerotiorum*	Zamani et al., 2012
	Tobacco	*Arachis diogoi*	AdTLP	*Rhizoctonia solani*	Singh et al., 2013
	Arabidopsis thaliana	*C. komarovii*	CkChn134 and CkTLP	*V. dahliae*	Wang et al., 2012
PR-6	Pisum sativum	Bean	α-amylase inhibitor 1	*Bruchus pisorum* (pea weevil)	Morton et al., 2000
	Sugarcane	Soybean	BBI and Kunitz inhibitors	*Diatraea saccharalis* (sugar-cane borer)	Falco and Silva, 2003
	Apple	*Nicotiana alata*	proteinase inhibitor	*Epiphyas postvittana*	Maheswaran et al., 2007
	Oilseed rape	Rice	cysteine protease inhibitor oryzacystatin	*Myzus persicae*	Rahbé et al., 2003
	Tobacco	*Solanum americanum*	serine proteinase inhibitor gene SaPIN2a	*Helicoverpa armigera* and *Spodoptera litura*	Luo et al., 2009
	Rice	Maize	maize proteinase inhibitor (mpi)	*Chilo suppressalis* (striped stem borer)	Vila et al., 2005
	Arabidopsis	Papain-like Cys proteases	RD19	*Pseudomonas syringae*	Xia et al., 2004
PR-9	Tobacco	*Stylosanthes humilis*	Shpx2	*Phytophthora parasitica var nicotianae* and *Cercospora nicotianae*	Way et al., 2000
	Tobacco	*Capsicum annuum*	CAPOA1	*Phytophthora nicotianae*	Sarowar et al., 2005
PR-10	Tobacco	Maize	ZmPR-10	*Aspergillus flavus*	Chen et al., 2006
	Arabidopsis	*Parax ginseng*	Pg PR-10	Fungal and bacterial resistance	Lee et al., 2012
PR-12	Potato	*Nicotiana megalosiphon*	NmDef02	*Phytophthora infestans*	Portieles et al., 2010
	Brassica napus	Pea	defensin gene	*Leptosphaeria maculans* and *R. solani*	Wang et al., 2001
	Tomato	*Capsicuum annuum*	cdef1	*Phytophthora infestans* and *Fusarium sp*	Zainal et al., 2009
	Rice	*Dahlia merckii*	Dm-AMP1	*Magnaporthe oryzae* and *Rhizoctonia solani.*	Jha et al., 2009
	Papaya	*Dahlia merckii*	Dm-AMP1	*Phytophthora palmivora*	Zhu et al., 2007

	Tobacco	Mustard	defensin	*Fusarium moniliforme* and *Phytophthora parasitica*	Swathi et al., 2008
	Peanut	Mustard	defensin	*Pheaoisariopsis personata, Cercospora arachidicola*	Swathi et al., 2008
	Tomato	tobacco & alfalfa	tobacco β-1,3-glucanase (GLU) and defensin gene alfAFP	*Ralstonia solanacearum*	Chen et al., 2006
	Tomato	Radish	Rs-AFP1 and Rs-AFP2	*Alternaria solani*	Parashina et al., 2000
	Pichia pastoris	Pea and Peach	pea (Psd1) and peach (PpDfn1)	*Penicillium expansum* and *Botrytis cinerea*	Wisniewski et al., 2003
PR-13	*Ipomoea batatas*	Barley	thionin αHT	*Ceratocystis fimbriata*	Muramoto et al., 2012
	Tomato	*Arabidopsis thaliana*	thionin Thi2.1	*Fusarium oxysporum* and *Ralstonia solanacearum*	Loeza-Angeles et al., 2008
	Rice	Barley	Asthi1	*Burkholderia plantarii* and *B. glumae*	Iwai et al., 2002
PR-14	wheat	*Allium cepa*	Ace-AMP1	*Blumeria graminis* and *Neovossia indica*	Roy-Barman et al., 2006
	Arabidopsis	Pepper	LTP	*P. syringae pv. tomato DC3000* and *Botrytis cinerea*	Jung et al., 2005
	Carrot	Wheat, Barley	LTP and chitinase	Foliar fungal pathogen	Jayaraj and Punja, 2007
PR-15, 16	Sunflower	Wheat	OXO	*Sclerotinia sclerotiorum*	Hu et al., 2003
	Soybean	Wheat	gf-2.8	*Sclerotinia sclerotiorum*	Donaldson et al., 2001
	Poplar	Wheat	OXO	*Septoria musiva*	Liang et al., 2001
	Maize	Wheat	OXO	*Ostrinia nubilalis*	Ramputh et al., 2002
	Tobacco	Rice	OsLOL2	*Pseudomonas syringae pv. tabaci*	Bhatti et al., 2008
	Tomato	Wheat	OXO	*Botrytis cinerea and Sclerotinia sclerotiorum*	Walz et al., 2008
	Potato	Wheat	OXO	*Phytophthora infestans, Streptomyces reticuliscabiei*	Schneider et al., 2002
	Peanut	Barley	OXO	*Sclerotinia sclerotiorum*	Livingstone et al., 2005
	Colocasia esculenta	Wheat	gf-2.8	*Phytophthora colocasiae*	He et al., 2013