

Pathogenesis related-10 proteins are small, structurally similar but with diverse role in stress signaling

Parinita Agarwal · Pradeep K. Agarwal

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Abstract Pathogenesis related-10 proteins are small proteins with cytosolic localization, conserved three dimensional structures and single intron at 185 bp position. These proteins have a broad spectrum of roles significantly in biotic and abiotic stresses. The RNase activity, ligand binding activity, posttranslational modification (phosphorylation) and phytohormone signaling provide some information into the mechanism of the regulation of PR-10 proteins, however the presence of isoforms makes it difficult to decipher its exact mode of function. The involvement of phosphorylation/dephosphorylation events in its activation is interesting and provides unique and unbiased insights into the complexity of its regulation. Studies on upstream region of different PR-10 genes indicate the presence of *cis*-acting elements for WRKY, RAVI, bZIP, ERF, SEBF and Pti4 transcription factors indicating their role in regulating PR-10 promoter. In this review, we discuss in detail the structure and mechanism of regulation of PR-10 proteins.

Keywords Abiotic · Biotic · Ligand binding · Pathogenesis related proteins · Phosphorylation · Promoter · RNase activity

Abbreviations

ABA	Absciscic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
CSBP	Cytokinin-specific binding protein
ET	Ethylene

ETI	Effector triggered immunity
HR	Hypersensitive response
HyH	Hypericin
IPR	Intracellular pathogenesis-related proteins
JA	Jasmonic acid
LTPs	Lipid transfer proteins
MeJA	Methyl jasmonate
NCS	(S)-Norcoclaurine synthases
NLS	Nuclear localization signal
PAMP	Pathogen associated molecular patterns
PR-10	Pathogenesis related-10 proteins
PTI	PAMP triggered immunity
PTM	Post translational modification
ROS	Reactive oxygen species
SA	Salicylic acid
SAR	Systemic acquired resistance
SEBF	Silencer element binding factor
TF	Transcription factor
TLPs	Thaumatococcus-like proteins
TMV	Tobacco mosaic virus

Introduction

Plants in the fields are exposed to multiple stresses simultaneously and therefore, develop unique strategies for responding to environmental conditions through an intricate network of abiotic and biotic stress signal transduction pathways [1–3]. The plants responsiveness and adaptability to environmental stress result from the constant re-adjustment at physiological, biochemical and molecular level during their entire lifecycle. Biotic stress results due to infection by various living organisms such as bacteria, fungi, parasites, beneficial and harmful insects and weeds.

P. Agarwal (✉) · P. K. Agarwal
Discipline of Wasteland Research, CSIR-Central Salt & Marine
Chemicals Research Institute, Gijubhai Badheka Marg,
Bhavnagar 364 002, Gujarat, India
e-mail: Parinitaa@csmcricri.org

Drought, heat, cold and salinity are among the major abiotic stresses that adversely affect plant growth and productivity, worldwide. These stress factors are a menace for plants and prevent them from reaching their full genetic potential and limit the crop productivity worldwide. Abiotic stress in fact is the major cause of crop failure worldwide, dipping average yields for most of the major crops by more than 50 % [4]. The stress response of plants is a complex process and therefore it is difficult to decipher the molecular mechanism of stress responses. The response to stress generally involves induction of certain signaling pathways that allows production of secondary signaling molecules leading to induction of several defense related genes or proteins that help the plant cope against the stress. The adaptation to abiotic stresses is mainly regulated by the activity of interconnected ABA-dependent and ABA-independent signaling pathways [3], whereas, the plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play major roles in disease resistance. The role of ABA in disease resistance through ATAF1 transcription factor (TF) has been reported [5]. These hormones do not follow a linear signaling pathway but form a complex cross talking network of synergistic and antagonistic interactions [5]. Plants have protective systems like hardening of the cell wall, producing antibiotic compounds called “phytoalexins” and antibiotic proteins, and by triggering hypersensitive reaction to suppress the spread of infectious pathogens. The plant's innate immune system corresponds to two interconnected branches termed PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) [6]. PTI is elicited by pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) and ETI is triggered by plant disease resistance (R) proteins that activate highly efficient defense reactions upon specific recognition of pathogen effectors. PTI and ETI activate local immune responses and long-distance defense reactions including systemic acquired resistance (SAR) [7].

Pathogenesis related (PR) proteins have been defined as proteins encoded by the host plant but induced by various types of pathogens such as viruses, bacteria, and fungi and also by the application of chemicals that mimic the effect of pathogen infection or induce similar stresses. Accumulation of PR proteins is triggered by pathogen attack (biotic), abiotic stress, hypersensitive response (HR) and SAR [8]. PR proteins have been classified into 17 different families, ranging from PR-1 to PR-17 based on their primary structure, serological activity, and biological activity in mono and dicotyledonous plants [8]. PR-1 family is induced by SA and pathogen and is commonly used as a marker for SAR [9]. The functions of PR-1 family of proteins are not yet known. The PR-2 family consists of β -1,3-glucanases (glucan endo-1,3- β -glucosidases) and catalyzes the hydrolysis of β -1,3-glucans. They

act in fungal defense by hydrolyzing fungal cell walls and also by generating elicitors. The PR-3, PR-4, PR-8 and PR-11 proteins are plant chitinases. PR-3 family members hydrolyze the β -1,4 glycosidic bonds that link the *N*-acetylglucosamine residues of chitin, they impart disease resistance based on antimicrobial activity [10]. PR-4 proteins are termed endochitinases [11] and possess a C-terminal domain called Barwin and are inducible by pathogen attack. The PR-5 family of proteins includes permatins, osmotins, zeamatin, and thaumatin-like proteins (TLP). TLPs cause osmotic breakage of transmembrane pore on the fungal plasma membranes. PR-6 proteins are referred to as proteinase inhibitors. They reduce the pathogen's ability to use its lytic enzymes and complete its replication cycle [12]. They also respond to wounding by activating JA and ET signaling pathways finally providing systemic induced resistance. PR-7 acts as an endoproteinase and is characterized in tomato [13]. PR-9 family of proteins is referred to as peroxidases, and are key enzymes in cell wall building processes. They catalyze lignin deposition and strengthen the cell wall of plants during pathogen attack [14]. PR-12 proteins are referred to as plant defensins, their precise role remains unclear but it is regarded that they possess the ability to inhibit the growth of fungi and yeasts. PR-13 proteins are referred to as thionins, these are identified with antimicrobial effects causing permeabilization of cell membranes. Thionins are a source of sulphur, found in storage proteins in seeds [12]. PR-14 proteins are referred to as lipid transfer proteins (LTPs), LTPs are involved in cutin synthesis, β -oxidation, plant defense signaling and plant defense [12]. PR-15 and PR-16 are reported from barley and are induced by *Erysiphe graminis* and have oxalate oxidase property. The PR-17 has tobacco PRp27 as type member [15]. In this review, we would discuss the structure and evolution of PR-10 gene and its role in abiotic and biotic stresses, involving its protein activities and the mechanism of its regulation by post translational modifications (PTMs). PR-10 proteins including the tree pollen allergens are present as multigene family in seed plants and are developmentally and environmentally regulated. Based on similarities in their amino acid sequences, subcellular location and putative function, they are classified into two distinct groups: intracellular pathogenesis-related proteins (IPR) with homology to ribonucleases and (S)-norcoclaurine synthases (NCS). In dicotyledonous plants, PR-10 proteins were reported in parsley, pea, potato, bean, soybean, celery, alfalfa. In monocots, they are present in asparagus, rice, lily, sorghum. Among tree species, they were reported in *Pinus lambertiana*, *Pinus strobus*, *Pinus monticola*, *Pseudotsuga menziesii*, *Pinus pinaster*, *Picea glauca*, *Fagus sylvatica*, *Betula verrucosa*, *Jatropha curcas* (Table 1).

Table 1 PR-10 genes isolated from different plants and their transcript response to various abiotic and biotic factors

Gene source	Nomenclature	Accession number	Gene size (bp)	Transcript induction by		References
				Abiotic factors/signaling molecules	Biotic factors	
<i>Arachis hypogaea</i> L.	<i>AhSIPR10</i>	DQ813661	474	Salinity, heavy metal, cold, drought, SA, MeJA, H ₂ O ₂ , ABA	–	[48]
	<i>AhPR10</i>	AY726607	453	–	<i>Fusarium oxysporum</i> , <i>Rhizoctonia solani</i>	[54]
<i>Asparagus officinalis</i>	<i>AoPR1</i>	X64452	474	Wounding	–	[44, 99]
<i>Capsicum annuum</i>	<i>CaPR-10</i>	AF244121	477	SA, MeJA, NaCl, ABA, methyl viologen, H ₂ O ₂	TMV, <i>Xanthomonas campestris</i> , <i>Phytophthora capsici</i>	[17]
<i>Chrysanthemum cinerariaefolium</i>	<i>CcPR10</i>	JN211117	471	Wounding, JA	<i>Spodoptera litura</i>	[100]
<i>Crocus sativus</i>	<i>CsPR10</i>	GU220360	486	Wounding, sorbitol, JA, NPA, IAA, NaCl	<i>Fusarium oxysporum</i>	[18]
<i>Glycine max</i>	<i>SAM22</i>	X60043	474	Wounding, senescence, SA, H ₂ O ₂ , sodium phosphate, methyl viologen, chitosan	–	[101]
<i>Jatropha curcas</i>	<i>JcPR-10a</i>	JN566052	483	SA, MeJA, NaCl	<i>Macrophomina phaseolina</i>	[25]
<i>Lilium longiflorum</i>	<i>PR-10</i>	AF021853	473	ABA, MeJA, okadaic acid, staurosporine	–	[57]
<i>Lithospermum erythrorhizon</i>	<i>LePR1</i>	AF047178	468	Wounding, SA, H ₂ O ₂ , ABA, JA	<i>Erwinia stewartii</i> , <i>Pseudomonas syringae</i> , <i>Aspergillus nidulans</i> , <i>Penicillium funiculosum</i>	[102]
<i>Lycopersicon esculentum</i>	<i>TSI-1</i>	Y15846	534	SA	<i>Fusarium oxysporum</i>	[103]
<i>Malus domestica</i>	<i>Ypr10*Md.b</i>	AY026908	477	Wound, SA, ethephon	<i>Venturia inequalis</i>	[50]
	<i>Ypr10*Md.d</i>	AY026909	477			
<i>Medicago sativa</i>	<i>MsPR10.1A</i>	ADB44209	471	ABA, ethylene, wounding	<i>Xanthomonas campestris</i> pv. <i>alfalfae</i>	[64]
	<i>MsPR10.1B</i>	CAC86467	471			
<i>Oryza sativa</i>	<i>RPR10a</i>	AAF85972	474	SA, JA	<i>Magnaporthe grisea</i>	[104]
	<i>RPR10b</i>	AAF85973	480			
	<i>JIOsPR10</i>	AF395880	480	Cantharidin, endothall, okadaic acid, light, SA, JA, ET, ABA, H ₂ O ₂	<i>Magnaporthe grisea</i>	[29]
	<i>RSOsPR10</i>	AB127580	480	Drought, NaCl, JA, probenazole	<i>Piricularia grisea</i>	[30]
	<i>RSOsPR10</i>	AB127580	480	NaCl, drought, Wounding, JA, ACC, NaCl + SA ⁺ , wounding + SA ⁺ , JA + SA ⁺ , ACC + SA ⁺	–	[31]
<i>Panax ginseng</i>	<i>PgPR10-1</i>	P80889	465	Coldness, NaCl, JA, H ₂ O ₂ , ABA	–	[105]
	<i>PgPR10-2</i>	GU086324	462			
	<i>PgPR10-3</i>	HQ171906	462			
<i>Pinus monticola</i>	<i>PmPr10-2</i>	AY596271	486	Low temperature, wounding	–	[19]
	<i>PmPr10-3</i>	AY596272	486			
<i>Prunus domestica</i>	<i>PdPR10</i>	EU117124	483	–	<i>Monilinia fructicola</i>	[106]
		EU117126	483			
<i>Retama raetam</i>	<i>RPR-10</i>	AF439272	474	Dormancy, cold	–	[47]
<i>Solanum surattense</i>	<i>SsPR10</i>	AY660753	483	MeJA, SA, ABA, GA ₃ , H ₂ O ₂ , Cu ²⁺	<i>Piricularia oryzae</i>	[34]
<i>Solanum tuberosum</i>	<i>pSTH-2</i>	M25155	465	Arachidonic acid, eicosapentanoic acid, wounding	<i>Phytophthora infestans</i>	[107]
	<i>pSTH-21</i>	M25156	465			
<i>Tamarix hispida</i>	<i>ThPR10</i>	FJ463256	486	NaCl, PEG, cold, CdCl ₂ , ABA		[65]
<i>Theobroma cacao</i>	<i>TcPR-10</i>		480	–	<i>Moniliophthora perniciosa</i> , <i>Saccharomyces cerevisiae</i>	[55]
<i>Trifolium subterraneum</i>	<i>TsPR10-a</i>	–	471	Ethephon	<i>Halotydeus destructor</i>	[108]
<i>Vitis pseudoreticulata</i>	<i>VpPR10.2</i>	HQ634186	477	–	<i>Plasmopara viticola</i> , <i>Alternaria alternata</i>	[35]
<i>Vitis vinifera</i>	<i>VvPR10.1–10.17</i>	AJ291705 (10.1), AJ291704 (10.2), EU379313 (10.3)	204–486	–	–	[16]

Table 1 continued

Gene source	Nomenclature	Accession number	Gene size (bp)	Transcript induction by		References
				Abiotic factors/signaling molecules	Biotic factors	
<i>Zea mays</i>	<i>ZmPR10</i>	AY953127	480	H ₂ O ₂ , CuCl ₂ , darkness, coldness, wounding, SA, ABA*	<i>Erwinia stewartii</i> , <i>Clavibacter michiganensis</i> , <i>Aspergillus flavus</i>	[32]
	<i>ZmPR10.1</i>	FJ897503	480	H ₂ O ₂ , CuCl ₂ , darkness, coldness, wounding, SA, MeJA, NAA, ABA*	<i>Erwinia stewartii</i> , <i>Erwinia chrysanthemi</i> , <i>Clavibacter michiganensis</i> , <i>Aspergillus flavus</i>	

* Down regulation of transcript

Structure and evolution of PR-10 proteins

The PR-10 proteins are referred to as multi-gene family. There are at least five PR-10 genes in *Pisum sativum*, eighteen *Mal d 1* genes in *Malus domestica*, ten *Bet v 1* genes in birch, eight *Fra a 1* genes in strawberry, six PR-10 genes in *Solanum surattense*, eight in yellow lupine, five in rice and seventeen PR-10 genes in *Vitis vinifera* [16]. The PR-10 IPR group encodes polypeptides of 151–162 amino acids with molecular weight of 15–18 kDa, show cytosolic localisation and conserved three dimensional structures. However, the PR-10 gene from *Physcomitrella patens* (34 kDa) and some *Arabidopsis* homologs have high molecular weight. Gene duplication event occurs within the genome making multiple copies of PR-10 which are present in the form of chromosomes clusters [8]. This is contemplated as a consequence of evolution.

The cDNA sequences of IPR proteins reveal that they have an open reading frame (ORF) of around 456–489 bp encoding a polypeptide of 151–162 amino acids. However, the members of the PR-10 NCS group are found to be having longer ORFs from 633 to 696 bp with approximate molecular mass of 26 kDa, and they share 28–38 % identity as well as putative structural homology with IPR proteins. The ORF codes for an acidic small protein with conserved sequence features, and is possibly involved in ribonuclease activity. The 3D structures of the birch Bet v1 and the yellow lupine PR-10 protein show that glutamic acid (E) at 96, 148 and tyrosine (Y) at 150 amino acid position are close together and thus form an activity site for the catalytic reaction of ribonuclease. The second important domain comprise the motif GxGGxGxxK (amino acid 47–55) that forms a P-loop supposed to have NTPase activity as they have binding site for ATP or GTP, critical for the RNase activity [8, 16]. In *Solanum tuberosum*, the P-loop is reduced to GXG (aa positions 45–47) with conserved lysine residues 13 aa upstream and 19 aa downstream [17]. No P-loop structure has been found in *Crocus sativus*, CsPR10 [18]. Another important feature of PR-10 proteins is a large Y-shaped hydrophobic cavity, as shown by the determination of three-dimensional structure. This

internal cavity could be responsible for the intracellular transport of apolar ligands, as diverse as fatty acids, flavonoids, cytokinins or brassinosteroids. Slight modifications of the structure and shape of this cavity would allow binding of different ligands, that would cause PR-10 proteins to perform diverse roles in plant defense and development [16]. Liu and Ekramoddoullah [19], identified that substitutions occur in the putative ligand-binding site of PmPR10-3 proteins as an adaptation during evolution, allowing a homologous PR-10 protein to obtain a new function. Most of the PR-10 proteins lack nuclear localization signal (NLS) as well as any other localization signal peptide indicating its cytoplasmic localization, interestingly, VvPR10.2 have dual distribution to both cell wall and nucleus, further suggesting that PR-10 proteins cooperate with other proteins for movement and distribution to subcellular compartments for different functions [16]. The binding of PR-10 proteins with MdAP [20] and with different physiological ligands including cytokinin, flavonoids and fattyacids has been reported [21]. Some PR-10 proteins including Bet v1, Arah8, AhSIPR10 contain the polyketide cyclase domain involved in binding/transport of lipids and in the synthesis of compounds like pigments, antibiotics and antitumour drugs. The polyketide cyclase/dehydrase-like domain of Bet v1 is ubiquitous domain involved in binding of large hydrophobic ligands [22]. The yellow lupine PR-10 protein (LIPR-10.2B) is capable of binding to hormone trans-zeatin, similar ligand binding has also been reported for plant cytokinin-specific binding proteins (CSBP) [23].

Using X-ray diffraction and NMR spectroscopy, crystal structures of a few PR-10 proteins have been identified, and shown to be similar. Their 3D structures consists of a 25-amino acid long C-terminal α -helix surrounded by a seven-stranded antiparallel β -sheet and their two N-terminal short α -helices lie between β 1 and β 2 strands, short loop structures join the α -helix or/and β -strand [8]. The conserved P-loop motif (GxGGxGxxK) is present between β 2 and β 3. A Y-shaped, hydrophobic pocket is also identified which play a role in ligand binding [8]. The crystal structure of PR10 proteins from yellow lupine consists of a

seven-stranded β -sheet wrapped around a long C-terminal helix with a large cavity between the two structural elements [24].

The genomic organizations of PR-10 proteins are also conserved, with the presence of two exons and one intron. The size of the intron varies from 76 to 359 bp but the position of intron is highly conserved in some plant families, viz. at amino acid codon 62 in Fagaceae, Rosaceae and Apiaceae families. Similarly in *JcPR-10a*, the intron is present at 185 bp, similar to PR-10 genes in *V. vinifera* [25]. The highly variable length and composition of the intron was important in classifying the birch isoforms to proper subfamily [26]. Certain modifications such as phosphorylation, glycosylation and myristoylation play an important role in regulating gene expression. Phosphorylation sites are found in number of PR-10 proteins from capsicum, cacao, potato, peanut and *Jatropha* [25]. In LaPR-10 (*Lupinus albus*) protein, several PTM sites are observed, including N-glycosylation site NYSI, several phosphorylation sites and N-myristoylation sites, and the cell-attachment determinant tripeptide RGD [27].

The varied number of functions attributed to PR-10 proteins is supposed to have originated due to exposure to the changing environmental conditions during millions of years. Exploring the evolutionary relationships in the PR-10 family provides insight into the origin and functional adoption of this universal family. PR-10 genes show low intraspecific variation and higher interspecific variation making them viable for phylogenetic markers [28]. A phylogenetic tree of 95 PR-10 proteins from a variety of seed plants including both angiosperm and gymnosperm species, was constructed by Liu and Ekramoddoullah [8], dividing PR-10 into two major clades. The large clade comprises of IPR proteins homologous to ribonucleases and small clade consist of NCS proteins. The large clade is further divided into four subclades—Dicots (subclade-I), monocots (subclades II and IV), and conifers (subclade-III). Most members of PR-10 family are probably due to multiple and independent gene duplication events in the common ancestors, or due to strong concerted evolution of species specific PR-10 loci [16]. Schenk et al. [26] reported different isoforms from *Betula* species with varied copy number among different species, due to evolutionary events like duplication, extinction and recombination. Slight modifications occur in the copies which contribute to functional diversification of the gene copies. For example, in birch or yellow lupine, some PR10 genes are constitutively expressed while others are induced under pathogen attack, abiotic stress or during plant development. However, numerous copies created during evolution are also lost rapidly because of pseudogenisation. For instance, in *Vitis vinifera*, the pseudogenes s7 and s9 share the highest nucleotide similarity with s4, suggesting its duplication.

Moreover, s6 and s5 shows similarity at the intron level [16].

There is a reduced sequence divergence observed i.e. low intraspecific variability is seen in PR-10 genes. The close proximity of repetitive duplicates facilitates gene conversion or unequal crossing over events leading to concerted evolution. In *V. vinifera*, PR-10 sequences were located on the chromosome 5, suggesting loss of some of the triplicate ancestral copies and/or translocation on a unique chromosome [16]. Evolution in the form of perpetual occurrence of repetitive small duplications could have lead to seventeen VvPR-10 sequences. Subsequent mutations and possible positive selection are responsible for the observed divergence within the sequences. However, low intra-specific variability is because a part of *V. vinifera* PR-10 sequences had been subjected to concerted evolution [16].

Role in biotic and abiotic stress

The induction of pathogenesis-related (PR) genes in response to pathogens and parasites ranging from microscopic viruses to insect herbivores and to different environmental cues qualifies their deployment as important gene for multiple stress tolerance. Pathogens such as viruses, bacteria, fungi, as well as abiotic factors like salinity, cold, dehydration are involved in inducible expression of PR-10 proteins (Table 1). Most PR proteins are induced through the action of the signaling compounds such as SA, JA, ABA or ET, and possess antimicrobial activities in vitro through hydrolytic activities on cell walls, contact toxicity, and hence are involved in multiple stress signaling.

The expression of *JIOsPR10* gene in rice was induced by signaling molecules in presence of light, on the other hand in dark expression was not observed, indicating that light signal might be involved in regulating its transcript expression induced by signaling molecules [29]. The *JIOsPR10* gene showed response with blast pathogen (*Magnaporthe grisea*) in both a compatible and incompatible host-pathogen interaction, in compatible interaction the transcript accumulation was observed at 24 h with late visible symptom at 72 h, however, with incompatible interaction an early transcript response and dark brown necrotic symptoms were visible within 36 h leading to HR [29]. The expression of PR-10 genes is observed in almost all plant parts, be it vegetative or reproductive, and accordingly, they, perform their function in the plant. The PR-10 genes from *OsPR10a/PBZ1* to *RSOsPR10* showed organ specific induction in response to different stresses. The *RSOsPR10* was expressed in roots in response to salt, drought, blast fungus (*Pyricularia grisea*), JA and pro-benzole (chemical effective against rice blast fungal

disease). Similarly, *OsPR10a* was also expressed in roots by salt, drought and rice blast fungus (*Pyricularia grisea*), however, in response to probenazole it showed induction in shoots [30]. On simultaneous application of SA with NaCl, wounding, JA and ACC (1-aminocyclopropane-1-carboxylic acid) treatments, the expression of *RSOsPR10* were reduced [31]. Also *ZmPR10* and *ZmPR10.1* from maize were expressed mainly in roots [32]. The transcripts of two *PR-10* genes in four wheat genotypes also show differential patterns of expression in roots [33]. The *SsPR10* showed highest expression in roots under control conditions, however, in seedlings incubated with TMV (tobacco mosaic virus) sap, highest expression was observed in old yellow leaves. In control conditions also, the senescent leaves showed higher expression as compared to young leaves, indicating the role of *SsPR10* in development and senescence, apart from stress response [34]. The saffron *CsPR10* gene showed high expression in anther and tapetal tissues, low expression in stigmas and roots and no expression in leaves. The higher expression in flower parts indicate its role in protecting the short lived flower from environmental as well as infection stress. It showed induction by sorbitol, NaCl, wounding IAA, NPA and *Fusarium oxysporum* infection and faintly by temperature variations of heat and cold treatment [18]. The two *PR-10* genes from fungal resistant (*VpPR10.2*) and susceptible (*VvPR10.2*) grapevine show similarity at nucleotide level, however, *VpPR10.2* showed stronger induction in leaves in response to *Plasmopara viticola* infection and is localized in host cells and haustorium of *Plasmopara viticola*. Recombinant *VpPR10.2* showed antifungal activity against *Alternaria alternata* and its over expression in susceptible grapevine increased the resistance against *P. viticola* [35]. The *PR-10* gene from maize gets upregulated during kernel development, and was also induced upon *Aspergillus flavus* infection only in the resistant genotype, Chen et al. [36] report that a negative correlation exist between *PR-10* expression and kernel aflatoxin production in maize. Furthermore, the leaf extracts of transgenic tobacco plants expressing maize *pr-10* also inhibited the growth of *Aspergillus flavus* [37]. Similarly, *PR-10* gene from barley and cow pea were induced in epidermis of resistant cultivars on infection with *Rhynchosporium secalis* and *Uromyces vignae* [38, 39]. The *SsPR10* and *ZmPR10* also showed induction with signaling molecules and cold stress [32, 34]. The over expression of potato *PR-10* gene significantly enhances salt and osmotic stress tolerance [40]. Phytohormones like gibberellic acid, kinetin and ABA treatment also significantly induce the expression of *PR-10* genes of maize, potato [32, 34]. Kinetin upregulates *OsPR10* expression in rice leaves [41], however, kinetin does not affect the accumulation of *PR-10* genes in birch [42] and alfalfa [43]. Expression analysis revealed the

accumulation of an asparagus *PR-10* protein on wounding [44]. Constitutive expression of pea *PR-10.1* enhances the germination of *Brassica napus* under saline conditions, suggesting a crucial role for *PR-10* protein in its tolerance [45]. In vitro studies of WAP18, a *PR-10* from mulberry (*Morus bombycis* Koidz.), reveal cryoprotective activity for a freeze-labile model enzyme (L-lactate dehydrogenase, LDH), suggesting that some *PR-10* proteins might function in the freezing-tolerance mechanism during winter [46]. *PR-10* proteins are induced by dormancy and possess high numbers of polar residues (40 %), comparable to dehydrin (50 %) suggesting the role of *PR-10* protein in dormancy [47]. In desert legume *Retama raetam* the *PR-10* transcript accumulates with dormancy and decreases as the dormancy breaks [47]. Recently, Jain et al. [48] showed that over-expression of *AhSIPR10* gene from peanut enhance abiotic stress tolerance by reducing ionic and oxidative stress in transgenic plants exposed to salinity, heavy metal or drought stress. The *PR-10* protein in Douglas-fir was found localized in the cell walls and cytoplasm of roots infected with the fungal pathogen, *Phellinus sulphurascens* [49]. Also, *Malus domestica* is found to show antifungal activity against *Venturia inaequalis* [50]. *CaPR-10* showed antifungal activity against *Phytophthora capsici*, *TcPR-10* showed antifungal activity against *Moniliophthora perniciosa* and *Saccharomyces cerevisiae* (Table 1). Northern blot analysis by Park et al. [17] revealed that the transcripts corresponding to *CaPR-10* gene started to accumulate in the leaves and peaked at 6 days after initiation of infection with tobacco mosaic virus—P_{1,2} (TMV). There was about several dozens-fold reduction of TMV production in the leaves. This shows that some *PR-10* genes also aid in defense against viruses. However, limited success is observed in controlling diseases, employing *PR-10* genes with transgenic plants, as these genes reduce only a limited number of diseases in vivo, depending on the nature of the protein, plant species, and pathogen involved.

Mechanism of regulation of *PR-10* genes

The molecular mechanism through which *PR-10* proteins perform various functions in different plant parts is still not well understood. The fact that *PR-10* proteins play a crucial role in plant adaptation to biotic and abiotic stress cannot be ruled out, however the presence of isoforms makes it difficult to predict the localization or the function of *PR-10* genes. The *PR-10* protein interacts with stress signaling molecules and cross talk with different stress signal transduction pathways. The RNase activity, ligand binding activity, posttranslational modification (phosphorylation) and phytohormone signaling of *PR-10* proteins provide an insight into the mechanism by which these proteins perform their function in the plant system. Some information

is also learnt from the promoter identified for some PR-10 genes. The regulation of PR-10 protein also bears relationship in activating other PR-10 proteins, silencing of MtPR10-1 from *Medicago truncatula* induced other PR proteins and increased tolerance against infection with *Aphanomyces euteiches* [51]. Some specific function are also observed in PR-10 proteins, Hyp-1 encoding an enzyme for hypericin (HyH) biosynthesis shows 45 % homology to Betv1 class allergens [52]. The *AoPR1* gene is involved in phenylpropanoid pathway [53].

Nuclease activity

RNase activity has been reported for some PR-10 proteins and is regarded to be involved with its antimicrobial activity, being released by infected host cells and acting directly on pathogens or during programmed cell death (PCD) at and around the plant infection sites. The ribonucleases from plants are classified into four main families depending on the size of the protein, pH needed for activity and sensitivity to EDTA. It has been possible to classify only some PR-10 proteins according to this biochemical classification like PR-10 from saffron, peach. For some PR-10 proteins like CsPR-10, TcPR-10, AhPR-10, JcPR-10 and SsPR-10 both antifungal and ribonuclease activity has been studied while for others like PR-10 protein from birch, lupine, gossypium only ribonuclease activity has been examined. Park et al. [17] reports the relationship between ribonucleolytic activity and antiviral activity of CaPR-10. In internalization experiments using AhPR-10 mutant protein, Chadha and Das [54], show that RNase activity domain interacts with fungal membrane, denaturation of TcPR-10 led to loss of both RNase activity and antifungal activity and further inability to be taken by fungal cells [55]. Similarly boiled JcPR-10a protein lacked both RNase and antifungal activity [25]. The RNase activity shows variation with the presence of metal ions and also with hormone zeatin. The RNase activity of one isoform of peach PR-10 protein is increased significantly in the presence of cytokinin zeatin whereas for another isoform the activity shows no variation [56]. The activity of ZmPR-10 protein was significantly reduced by Ag^+ and Cu^{+2} metal ions [32]. Interestingly, the antifungal activity of TcPR-10 is less in mutants with impaired copper transport although the internalization or uptake of protein is similar in both mutants and wild type, therefore either Cu^{+2} promotes toxicity or RNase activity of TcPR-10 or lack of copper causes metal homeostasis affecting toxicity of PR-10 protein [55]. Most of the PR-10 proteins lack DNase activity, however, recently, VvPR10.2 and JcPR-10a are reported to show DNase activity [25, 35].

Phytohormone signaling

Phytohormones play a pivotal role in regulating the signal transduction pathways in response to biotic and abiotic stress. These include SA, JA, ET, abscisic acid (ABA). ABA and ET are involved in abiotic stress response signaling whereas SA and JA correspond to biotic stress signaling. The phytohormones play an important role in stress regulated responses. The differential expression of PR-10 genes in response to phytohormones indicates the involvement of different stress signal transduction pathways in its regulation. Wang et al. [57], reports that in lily anthers, there exist two pathways for induction of PR-10 genes through ABA and MeJA. The *OsPR10a/PBZ* and *RSOsPR10* genes are induced by biotic and abiotic stresses possibly through JA signaling pathway as these genes are not upregulated by ABA or SA [30, 58]. The regulation of *RSOsPR10* gene shows strong inhibition when SA treatment is given along with NaCl, wounding, JA and ACC indicating that there is an antagonistic effect of the SA signal transduction pathway on the JA/ET pathway [31]. Another rice gene (*JIOsPR10*) also showed stronger induction with JA [29]. Similarly, *CsPR10* was induced by JA and not by ABA and thus is also activated by JA pathway [18].

The expression of *AhSIPR10* gene is elevated by signaling molecules like ABA, SA and MeJA and this expression is further enhanced by salt and drought treatment. Interestingly, the MeJA or ABA induced expression of this gene gets antagonized by SA treatment both in the presence or absence of salt stress. Both SA and JA are important defense signaling molecules. SA inhibits JA-dependent responses and vice versa [59]. A type 2C protein phosphatase known to negatively regulate ABA responses gets upregulated by combined SA and MeJA treatments [60]. Also recently an RCAR1 protein (component of ABA receptor complex in *Arabidopsis*) showing structural similarity to Betv1 mediates ABA independent inactivation of type 2C phosphatase [61]. The transgenics overexpressing *ABR17* gene, show reduced level of ABA as compared to wild type plants [62, 63]. The alfalfa *MsPR10.1A* and *MsPR10.1B* expression was induced by ABA [64]. The *ThPR10* gene was also regulated via ABA dependent signaling pathway [65].

Ligand binding activity

The PR-10 proteins show general ligand binding, where both PR-10 and ligand show mutual conformational changes allowing transport of ligands from cytosol to their receptors [66, 67]. Ligand binding activity of PR-10 supports its stress response signaling. According to Mongensen et al. [21], Betv1 has two ligand binding sites, lys54

adjacent to P-loop in AhPR-10 may interact with RNA and hydrophobic ligand binding site with the receptor on the fungal membrane [54]. Cytokinin is an important plant hormone that promotes cell division facilitating plant growth and development. Cytokinins also affect plant resistance to biotic [68, 69] and abiotic stresses [70]. Increased cytokinin levels maintain high cellular redox potential during drought and hence reduce damage by reactive oxygen species (ROS) [71]. Recently, it has been shown that plant cytokinin systemically induce resistance against infection by modulating SA signaling [70, 72]. The cytokinin-specific binding protein from mung bean (VrCSBP) and intracellular PR protein from moss have shown cytokinin binding activity [73, 74]. Btvl and PR-10c from birch shows affinity for a broad range of physiological ligands including fatty acids, flavonoids including cytokinin [21, 75]. Constitutive expression of pea ABR17 in transgenic *Brassica* and *Arabidopsis* enhanced germination under saline conditions. These transgenics also showed increased cytokinin levels [62, 63], and further, microarray revealed upregulation of a number of CK-responsive genes [76]. The increased cytokinin levels enhance resistance against salinity by functioning as antioxidants, cytokinins also function as antioxidants in germinating soybean seedlings [77]. The *AhSIPR10* gene confers abiotic stress tolerance in transgenic tobacco and also display adequate photosynthetic CO₂ assimilation rate under stress conditions [48]. It is regarded that cytokinin binding ability of PR-10 protein promotes photosynthesis during stress, as cytokinins are known to promote photosynthesis [78] and also protect photosynthetic machinery [79].

Post translational modification: phosphorylation

Post translational modification especially phosphorylation must be involved in regulating the function of PR-10 gene as most of the PR-10 proteins show the presence of phosphorylation sites. Park et al. [17] report that phosphorylation of CaPR10 protein increased its ribonucleolytic activity to cleave invading viral RNAs. The phosphorylated CaPR-10 has shown a 12.4-fold higher RNase activity than the non-phosphorylated form in a yeast tRNA-degradation. The RNase activity of SsPR10 is also attributed to phosphorylation at particular sites and phosphorylation is believed to enhance the specificity of its RNase activity [34]. The specificity is necessary as non-specificity might be injurious to plant cells. The activation of potato PR-10a protein requires phosphorylation of PBF-1 (PR-10a binding factor 1). The PBF-1 is phosphorylated in tissues where *PR-10a* transcript accumulates, in vivo experiments employing protein phosphatase and kinase inhibitors have provided evidence that wounding and elicitor treatment

induce the phosphorylation of PBF-1 leading to gene activation [80]. The differential expression of *JIOsPR10* gene on treatment with protein phosphatase inhibitors, okadaic acid, cantharidin and endothall strongly suggest the involvement of phosphorylation/dephosphorylation events in its activation. Furthermore, this expression pattern is influenced by light signal [29]. The RNase activity of PR-10 proteins regarded to be directly or indirectly involved with antifungal role also gets modulated by phosphorylation. ABA and MeJA follow distinct pathways in the lily anthers. Applying okadaic acid a phosphatase inhibitor inhibited the MeJA induced PR-10 protein meaning that dephosphorylated protein is required for this pathway, whereas it increased the ABA induced expression of PR-10 protein implying that dephosphorylation is not needed in ABA signaling. The staurosporine blocked both the ABA and MeJA induced pathways showing that staurosporine sensitive kinases leading/causing phosphorylation are essential for induction of PR-10 proteins [57]. On the contrary, staurosporine enhanced the JA induced OsPR10 expression and interestingly, protein phosphatase inhibitors cantharidin and endothall also induced its expression [41]. Another *JIOsPR-10* gene also showed enhanced expression in response to phosphatase inhibitors [29]. Thus protein phosphorylation/dephosphorylation is an important component in activation of PR-10 protein during defence signaling. It is likely that a majority of PR-10 proteins can be phosphorylated by multiple kinases, adding layers of complexity to how the cell integrates information.

Activity of PR-10a promoter

Transcriptional regulation is an important part of plants response to both abiotic and biotic stresses. The transcription factors bind to *cis* elements of promoter, of the genes they regulate and thereby repress or activate gene activity. The promoter for PR-10 genes has been isolated from angiosperms and gymnosperms (Table 2). The parsley PR-10 (*PcPRI-1*) promoter was the first to be characterized [81]. The three PR-10 family members of parsley (*PcPRI-1*, *PcPRI-2*, *PcPRI-3*) are regulated by WRKY1, 2, 3, 4 and 5 transcription factors [82, 83]. Turck et al. [84] has shown that WRKY1 binds to parsley *PcPRI-1* (PR-10 gene family) promoter in a stimulus-dependent manner from 1 to 4 h after elicitor stimulation. The *PR-10a* promoter from potato shows the presence of several *cis*-acting elements (Table 2). The sequence PyTGTCNC in silencer elements served as the binding site for silencer element binding factor (SEBF), furthermore, the binding activity of *PR-10a* promoter is inversely related with its binding [85]. The activation of *PR-10a* from potato requires recruitment of StWhy 1 to the ERE [86]. PR-10a is negatively regulated by SEBF and Pti4 (Pto interacting protein 4). Both are

Table 2 The *cis*-elements and activity of *PR-10* promoters

Gene source	Size	Elements	Localization	Activity	References
<i>Asparagus officinalis</i>	982	ACCTGACC, ACCTAGCC, CCTACCN(7)CT, two set of direct repeats	Wound sites, sites of pathogen invasion, pollen, sites of active lignifications in stem and roots, developing seeds	HR response, lignification	[53, 89, 99]
<i>Betula pendula</i>	1,000	Box II, GT-1, LAT52, GTGA, ABRE, RY, C-repeat DRE, HSE, WUN, AE box, GAG motif, LAMP	Pollen	Induction by temperature and ABA, pollen development	[97]
<i>Medicago sativa</i>	657	Six ABA responsive elements, ten dehydration responsive elements, one each for wounding and heat shock and three WRKY elements	Leaves and stem	Induction through ABA, wounding, heat shock, dehydration	[64]
<i>Medicago truncatula</i>	1,548	–	Stem, flower and leaf	–	[109]
<i>Oryza sativa</i>	1,000	W box, three W box like element 1 (WLE1), three RAVIAAT elements, ASF1 motif element, several ABREs	Leaves	Induction on SA treatment	[110]
<i>Petroselinum crispum</i>	840	Elicitor responsive	–	Elicitor	[82]
<i>Pinus monticola</i>	1,316	Three CAAT box, W box, Elicitor responsive element, two CGTCA motifs, DRE element, G boxes, two WUN like motifs, MRE sequence, six G box or G box like motifs, two WUN like motifs	Hypocotyls, cotyledons, stems and leaves	Induction upon wounding and pathogen infection	[111]
<i>Pinus monticola</i>	1,675	AAAG-boxes, gibberellins responsive elements, silencer consensus, elements recognized by bZIP910	Root, stem and leaves	–	[112]
<i>Pinus strobus</i>	1,750	Six Dof elements, two TAACAAA like box, one E box, one GATA motif	Roots	NaCl, mannitol, SA, JA, ABA	[95]
<i>Solanum tuberosum</i>	1,015	Silencer element, wound responsive element and TAATNN motif	Tubers	Wounding, elicitation	[80]

required for their recruitment to silencer element (SE). It is interesting to note that both StWhy1 and SEBF are single strand DNA binding protein [85, 86] indicating that *PR-10a* promoter exists in single stranded or melted state [87]. Lamothe et al. [88] show that Pti4 is recruited to *PR-10a* promoter, despite the fact that there is no Pti4 consensus binding site in *PR-10a*, however, this interaction is SEBF-dependent. The W-box like element of *OsPR-10a* promoter play an important role in SA mediated PR gene expression, for mutation in WLE-1 completely abolished the expression of *OsPR-10a* promoter by SA. *OsPR-10a* promoter also contain W- box, RAVIAAT and ASF1 elements which serve as binding site for WRKY, RAVI and bZIP proteins, suggesting the role of these TF in regulating *OsPR-10a* promoter. Takeuchi et al. [31] report the involvement of OsERF TF in JA/ET signaling cascade in inducing *RSOsPR10* expression. The upstream region of *RSOsPR10* contains two GCC box which are binding sites for ERF TFs, and also in case of NaCl and JA treatment OsERF showed earlier induction as compared to *RSOsPR10*. The wound responsive *AoPR-10* promoter was

induced during different developmental stages at the sites of phenylpropanoid accumulation and by wounding, pathogen infection, H₂O₂ treatment, but not expressed by SA, MeJA, ET and NO [89]. The *AoPR-10* promoter shows similar expression by oxidation stress in tobacco and *Arabidopsis* [89–91]. In *Arabidopsis* the promoter expression was observed with respect to wounding and HR, however it was not observed in tobacco plants [92], indicating that different signaling components regulate the promoter activity in tobacco and *Arabidopsis* [89]. The WRKYb TF, was demonstrated to bind to the *CaPR10* promoter and activate the defense signaling pathway in pepper [93]. Recently, it has been shown that *CaPR10* binds to leucine-rich repeat protein 1 (LRR1) and positively regulates phosphorylation and RNase activity of PR-10 protein. Furthermore, the PR10/LRR1 overexpression in *Arabidopsis thaliana* confers enhanced resistance to *Pseudomonas syringae* pv *tomato* and *Hyaloperonospora* [94].

PR-10 promoter is also studied for few gymnosperms. The *Pinus monticola* *PmPR-10-1.13* promoter showed expression with wounding and pathogen infection in

transgenic *Arabidopsis*. The presence of W-box, DRE elements and MRE sequence indicate the involvement of WRKY, DREB or MYB TFs in its regulation. Another *PsPR-10* promoter from *Pinus strobus* show root specific expression which increases with biotic and abiotic signal molecules. The presence of Dof (TAACAAA) and GATA *cis* elements, which help in tissue specific expression, might be involved in directing its root specific expression [95]. The *MsPR-10A* promoter from alfalfa showed expression in leaves vein and phloem tissues of stem [64]. The bean *Ypr10c* promoter showed dark dependent and SA inducible expression [96]. This promoter has its elements responsible for tissue specific expression.

The expression, allergenicity and structure of Bet v1 have been extensively explored, yet limited studies are being carried out on its regulation. *Bet v1a* promoter shows pollen specific expression [97]. The transformation of grapevine with a chimeric gene containing alfalfa *PR-10* promoter and *Vst1* (V1 *cis* synthase 1) gene resulted 5–100 fold accumulation of resveratrol, a major phytoalexin, and enhanced tolerance against *Botrytis* [98], indicating the application of PR-10 promoters towards enhancing disease tolerance.

Conclusion

The PR-10 proteins are interesting multifunctional proteins playing an important role in plants response to intrinsic and extrinsic factors, however the signaling pathway involved in its activation remains unclear. The major role of PR-10 proteins is reported in response to biotic and abiotic stresses. The organ/tissue specific and differential expression patterns in same plant species suggest that PR-10 proteins have diverse roles in plant growth and development. The RNase activity of PR-10 proteins supports its antimicrobial activity either directly or indirectly. Studies on the ligand binding property are gaining momentum with special emphasis on cytokinins. Although the mechanism of regulation of PR-10 protein is still not very clear. The presence of W-box, ERE and other *cis* elements in promoter region suggests its interaction with different transcription factors and provide a model for its regulation. It is important to study the transcriptional machinery as well as the involvement of post translational modification of PR-10 proteins, to unravel its signaling pathway. Also gene knock out studies will help in understanding its potential role towards stress tolerance.

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