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Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance

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ABSTRACT

Pathogenesis-related (PR) proteins and antimicrobial peptides (AMPs) are a group of diverse molecules that are induced by phytopathogens as well as defense related signaling molecules. They are the key components of plant innate immune system especially systemic acquired resistance (SAR), and are widely used as diagnostic molecular markers of defense signaling pathways. Although, PR proteins and peptides have been isolated much before but their biological function remains largely enigmatic despite the availability of new scientific tools. The earlier studies have demonstrated that PR genes provide enhanced resistance against both biotic and abiotic stresses, which make them one of the most promising candidates for developing multiple stress tolerant crop varieties. In this regard, plant genetic engineering technology is widely accepted as one of the most fascinating approach to develop the disease resistant transgenic crops using different antimicrobial genes like PR genes. Overexpression of PR genes (chitinase, glucanase, thaumatin, defensin and thionin) individually or in combination have greatly uplifted the level of defense response in plants against a wide range of pathogens. However, the detailed knowledge of signaling pathways that regulates the expression of these versatile proteins is critical for improving crop plants to multiple stresses, which is the future theme of plant stress biology. Hence, this review provides an overall overview on the PR proteins like their classification, role in multiple stresses (biotic and abiotic) as well as in various plant defense signaling cascades. We also highlight the success and snags of transgenic plants expressing PR proteins and peptides.

1. Introduction

Plants being sessile are constantly challenged by various pathogenic microorganisms (e.g., fungi, oomycetes, bacteria and viruses) that compromise plant survival and their fitness (Cramer et al., 2011). These pathogens lead to significant reduction in annual crop yield as well as pose serious threat to the future food security. Plants defend these enemies by using an array of defense mechanisms in order to survive or retain their fitness (Roux et al., 2014). There are two modes of plant immunity namely, pathogen-associated molecular pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Pathogen-associated molecular patterns (PAMPs) generally consists of microbial or pathogen structures like flagellins, lipopolysaccharides and fungal cell wall components (chitins and glucans), and these are recognised by special plant receptors called pattern recognition receptors (PRRs) that further activates PTI (Zipfel and Felix, 2005). On the other hand, microbial pathogens secrete effector proteins which are recognised by a special group of resistance (R) proteins that stimulates the activation of induced defense response so called ETI (Dangl and Jones, 2001). These effector proteins are key elements produced by fungal pathogen for its virulence against plants and are particularly important during the biotrophic phase of infection (Sonah et al., 2016). However, the significance of PR proteins during plant–fungal pathogen interactions has been widely recognised, and there is a growing list of identified pathogen effector proteins that directly interact with PR proteins during infection (Breen et al., 2017). The complexity and efficiency of plant defense system to combat pathogen attack varies within the plant species (Jones and Dangl, 2006).

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Plants use both preformed (structural and biochemical) as well as inducible defense responses to combat various biotic stresses (Slusarenko et al., 2000). Preformed defense system includes cutin, waxes, rigid lignin deposition on cell walls and production of antimicrobial molecules like phytoanticipins, and are generally considered as first line of defense to prevent further invasion of pathogens (VanEtten et al., 1994; Osbourn, 1996). However, many pathogens cross this first defense barrier and they must possess an alternative defense approach to counter these pathogens. One such defense mechanism is pathogen inducible defense response which includes hypersensitive response followed by generation of reactive oxygen species (ROS), cell wall cross-linking, synthesis of antimicrobial molecules like phytoalexins, and eventually the production of PR proteins (Van Loon et al., 1994; Van Loon and Van Strien, 1999; Van Baarlen et al., 2007). Among them are PR proteins which are the key ingredients of SAR, an inducible plant immune response that prevents further infection to noninfected parts of the host.

The word “PR proteins” indicates a group of diverse proteins that are induced by phytopathogens as well as defense-related signaling molecules. After pathogen challenge, activation of defense signaling pathways viz., salicylic acid (SA) and jasmonic acid (JA) take place which further leads to the accumulation of PR proteins that minimises pathogen load or disease onset in uninfected plant organs. In general, there are two types of pathogens viz., biotrophic and necrotrophic, the first one activates the SA pathway that stimulates the transcription of NPR1 (non-expressor of pathogen-related gene 1) which in turn leads to activation as well as accumulation SA signature gene (PR1, PR2 & PR5) products locally as well as systematically leading to systemic acquired resistance (SAR). The second i.e., necrotrophic pathogen stimulates JA pathway that induces the activation JA signature genes (PR3, PR4 & PR12) and leads to accumulation of their product locally, and hence provides only local acquired resistance (LAR) (Fig. 1) (Ali et al., 2017b). The SAR provides enhanced resistance to a wide range of pathogens (Sticher et al., 1997; Van Loon et al., 2006; Fu and Dong, 2013). Moreover, PR proteins are widely distributed in plant domain and are present in all plant organs being particularly rich in the leaves, and forms 5–10% of total leaf proteins (Van Loon et al., 1994). These proteins have been successfully isolated from diverse plant species belonging to different families (Takeda et al., 1991). Based on biochemical features PR proteins largely differ from each other. They are generally low-molecular weight proteins approximately 6–43 kDa, thermo stable, resistant to proteases and remain soluble at low pH (< 3) (Van Loon et al., 1994). The PR proteins have two subgroups namely acidic PR protein that is usually secreted to the extracellular space, and second subgroup is basic PR protein which is generally transported to the vacuole by a signal sequence located at the C-terminal end (Takeda et al., 1991). Pathogenesis-related proteins predominantly accumulate in the apoplastic region however they are also vacuolar (Van Loon et al., 1994). Transcriptomic studies have revealed that PR genes are significantly induced by both biotic and abiotic stresses, and makes them one of the most promising candidates for developing multiple stress tolerant crop varieties (Seo et al., 2008; Fountain et al., 2010; Archambault and Strömvik, 2011; Gupta et al., 2013; Jiang et al., 2015; Dai et al., 2016; Ali et al., 2017a,b, 2018).

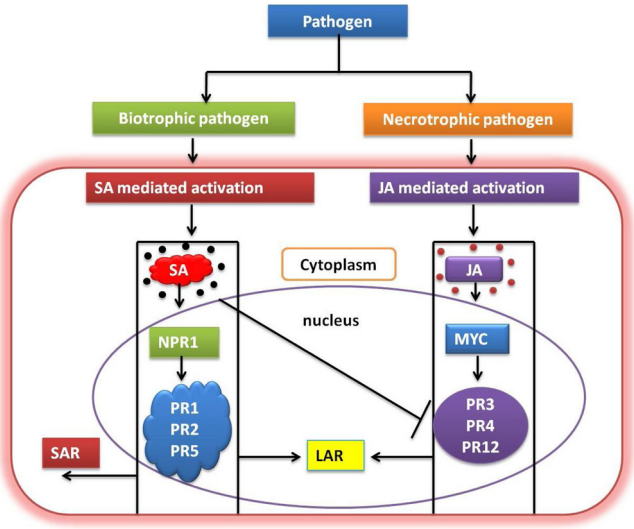

Fig. 1. An overview of activation of signaling cascades in plants after biotrophic and necrtrophic pathogenic infection. Accumulation of plant defense hormones like SA and JA further activates PR genes through selective transcription factor dependent pathways. SA accumulation also leads the activation SAR pathway. Increased expression of PR1 and PR2 genes have routinely been used as a molecular marker of SAR.

Some of the PR proteins are so called antimicrobial peptides (AMPs) which are usually cysteine rich molecules posses potential and broad range of antimicrobial activity. They include PR6 protein family (proteinase inhibitors), PR12 protein family (plant defensins), PR13 protein family (plant thionins) and PR 14 protein family (lipid transfer proteins) respectively. Generally, AMPs are ubiquitous in nature and forms an important part of host defense against a broad range of microbial pathogens and pests in different living forms ranging from microbes to plants (Egorov et al., 2005).

Therefore, the present review has been drafted to provide an overview on the PR proteins like their classification, role in multiple external stresses as well as in plant defense signaling cascades, and also highlights the success and snags of transgenic plants expressing PR proteins and peptides.

2. History and classification of PR proteins and peptides

Pathogenesis-related proteins were first discovered in tobacco plants infected by tobacco mosaic virus (TMV) (Van Loon and Van Kammen, 1970; Bol et al., 1990). Initially, only five major classes of PR proteins viz., PR1, PR2, PR3, PR4 and PR5 were reported in tobacco plants based on the biochemical and molecular approaches (Bol et al., 1990). However, in later studies many new PR proteins have been isolated and identified in various plants. In 1994, a proper nomenclature technique was employed to group PR proteins into different families based on different criteria like molecular, biochemical, serological and other biological or enzymatic activity. Later on, PR proteins were grouped into 11 families in tobacco and tomato plants which serve as a platform for isolating the homologs of PR proteins in other plant species including both monocots and dicots (Van Baarlen et al., 2007). There are two fundamental features for adding newly isolated protein in the PR protein family viz, first, it must show basal level expression in tissues but significantly increased expression upon pathogen exposure, and the second one is this increased expression should be confirmed in various plant pathological labs or must occur in similar fashion during different plant pathogen interactions. Presently, PR proteins are grouped into 17 families that are mainly based on their protein sequence similarities, enzymatic activities and other biological features which are shown in (Table 1) (Sels et al., 2008). Interestingly, PR proteins show diverse functions such as â-1, 3-glucanase (PR2), chitinases (PR3), thaumatin like (PR5), peroxidases (PR9), plant defensins (PR12) and thionins (PR13) (Van Loon and Van Strien, 1999).

3. PR proteins as antifungal agents

Fungi are rated as one of the most detrimental phytopathogens causing significant yield losses in most agriculturally important crops across the globe (Dean et al., 2012). Based on their lifestyle, plant fungal pathogens are grouped into three categories viz, biotrophs, hemibiotrophs and necrotrophs. To gain access, fungal pathogens generally produce a blend of hydrolytic enzymes like cutinases,

Table 1
Shows classification, property and source of PR-proteins isolated from different plant systems.

PR-Proteins	Gene Accession No.	Property/function	Source	Reference
PR1	YOO707	Antifungal	Nicotiana. tabacum	Antoniw et al. (1980)
PR2	M59443.1	β-1,3-glucanases	N. tabacum	Antoniw et al. (1980)
PR3	X77111.1	Class I, II, IV, V, VI, VII Chitinases	N. tabacum	Van Loon (1982)
PR4	NW_015888419.1	Class I, II Chitinases	N. tabacum	Van Loon (1982)
PR5	NW_015793016	Thaumatin-like proteins	N. tabacum	Van Loon (1982)
PR6	NW_004196001.1	Proteinase inhibitor	Solanum lycopersicum	Green and Ryan(1972)
PR7	NC_015445.2	Endoproteinase	S. lycopersicum	Vera and Conejero (1988)
PR8	NC_026660.1	Class III Chitinase	Cucumis sativus	Metraux et al. (1988)
PR9	EC 1.11.1.7	Peroxidase	N. tabacum	Lagrimini et al. (1987)
PR10	NC_026940.1	Ribonuclease-like proteins	Petroselinum crispum	Somssich et al. (1986)
PR11	gi|899342	Class I Chitinase	N. tabacum	Melchers et al. (1994)
PR12	NC_025209.1	Defensin	Raphanus raphanistrum	Terras et al. (1995)
PR13	gi|1181531	Thionin	*Arabidopsis thaliana*	Epple et al. (1995)
PR14	gi|1045201	Lipid-transfer protein	Hordeum vulgare	Garcıa-Olmedo et al. (1995)
PR15	gi|2266668	Oxalate oxidase	Hordeum vulgare	Zhang et al. (1995)
PR16	gi|1070358	Oxidase-like	H. vulgare	Wei et al. (1998)
PR17	–	Antifungal and antiviral	N. tabacum	Okushima et al. (2000)

pectinases, cellulases and proteases that hydrolyzes plant cell walls. In order to defend fungal pathogens, plants use different immune strategies starting from pathogen recognition, activation of defense signal pathways and production of antifungal compounds like PR proteins which further restricts pathogen invasion and its replication (Bowles, 1990; Sels et al., 2008).The genetic transformation of key defense molecules offers an alternative strategy for preventing fungal diseases. Among them are PR proteins that are excellent targets for generating long lasting and broad spectrum disease resistant crop varieties against fungal pathogens (Stuiver, 2011; Ali et al., 2017a, 2018). Various transcriptional studies have shown the up-regulation of PR genes in many crops after fungal infections which further reveal their role in disease resistance. Under control conditions, PR genes show basal level expression but increases dramatically after fungal infection both at local infected site as well as in non infected parts of the host thereby activating systemic acquired resistance (SAR) pathway (Ahuja et al., 2012; Návarová et al., 2012; Ali et al., 2017b). Furthermore, many in vitro studies have revealed that PR proteins targets fungal cell wall or hydrolyse them, and lead to cell death. Among PR proteins PR2, PR3, PR4, PR5, PR12 have been rated as the potent antifungal proteins in plants. In addition, over-expression of PR genes alone or combination in various crops leads to enhanced disease resistance against biotrophic and necrotrophic fungal phytopathogens (Honee, 1999; Shi et al., 2006; Wally and Punja, 2010; Ceasar and Ignacimuthu, 2012; Jiang et al., 2015; Dai et al., 2016)

4. Role of PR proteins in bacterial resistance

Plant bacterial partnership is one of the most fascinating associations, and can be either beneficial or harmful to the plants. Bacterial pathogens of plants are known since a decade, however the first confirmed bacterial disease was fireblight of apples and pears in 1878 (Burrill, 1878). Thereafter, a number of bacterial pathogens were isolated and identified from different agriculturally important crops leading to massive yield losses. Bacterial pathogens get entry to the host through an array of routes such as stomata; lenticles, mechanical wounding or insect feeding on leaves as well chemo attraction. Plants defend the bacterial pathogens by different immune responses. The key feature of the first line of defense towards bacterial pathogen is its recognition by host PRRs. Later on, this plant bacterial battle activates two key immune responses in host namely PTI and ETI (Fig. 2). Interestingly, immune response triggered by PAMP is an early and effective way to arrest infection priors the disease establishment in the host (Zeidler, 2004; Ausubel, 2005). On the other hand, ETI leads to activation of various signaling cascades in like activation of SA pathways and activation of SAR as well as production of PR proteins (Maleck and Dietrich, 1999; Jones and Dangl, 2006).

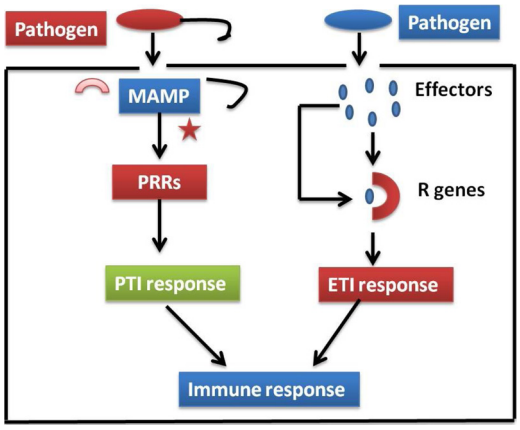

Fig. 2. An overview of plant immune response against bacterial pathogens. After pathogen attack, two types of immune responses are activated namely pathogen-associated molecular patterns-triggered immunity (PTI) and effector-triggered immunity (ETI).

Pathogenesis-related proteins are well known weapons against bacterial pathogens which have been used to develop bacterial resistant plants. Many in-vitro studies have shown the antibacterial properties of the PR proteins like PR10 (Ribonuclease-like proteins), PR12 (defensins), PR13 (thionins) and PR14 (Lipid-transfer protein) (Park et al., 2004; Patkar and Chattoo, 2006; Jiang et al., 2015). Among them PR10 shows broad spectrum of antibacterial activity against Pseudomonas syringae, Agrobacterium tumefaciens, A. radiobacter, P. aureofaciens and Serratia marcescens (Xie et al., 2010; Jiang et al., 2015). Over-expression of lipid transfer protein (PR14) in rice plants showed increased resistance to bacterial as well as fungal pathogens (Patkar and Chattoo, 2006). Future studies are required to validate antibacterial activity of other PR proteins as well as AMPs against wide range of bacterial pathogens in economically important crops.

5. Antiviral activity of PR proteins

Plants are infected by viruses that are obligate biotrophic pathogens hijacking the host machinery and cause detrimental effects on plant health as well as suppress the immune response. In response to viral attack, plants produce a variety of antiviral agents or proteins such as RNA-binding proteins (RBPs), ribosome-inactivating proteins (RIPs) and PR proteins which can function as virus suppressors (Okushima et al., 2000; Park et al., 2004; Musidlak et al., 2017). In this review, we

are highlighting the role of PR proteins as well as AMPs against viral pathogens. After viral infection, PR proteins or AMPs get accumulated in non-infected organs thereby blocks further virus propagation. Interestingly, PR2a and PR3 universally known as antifungal proteins from Nicotiana tabacum showed strong antiviral activity against TMV (Sindelarova and Sindelar, 2005). In addition, PR9 (peroxidase) protein which is a novel member of PR family also possesses antiviral activity (Nawrot et al., 2014). Interestingly, Capsicum annuum PR10 protein (CaPR10) showed ribonucleolytic activity against TMV. Previous studies have revealed that phosphorylation of CaPR10 significantly enhanced its antiviral activity against TMV (Park et al., 2004). Antimicrobial peptides like PR-12, PR-13 and PR-14, knottin and hevein type peptides are also known to possess antiviral activity. These peptides not only inhibit cell-viral fusion but also targets virus envelope thereby causing pores in the envelope and finally the lysis of the viral pathogen (Yount and Yeaman, 2013; Nawrot et al., 2014). Earlier studies have shown that overexpression of PR1b protein in tobacco plants leads to enhanced resistance to TMV (Cutt et al., 2005). Based on these finding PR proteins as well AMPs seems to be the possible candidate genes for developing viral resistant transgenic crops besides their antifungal or antibacterial properties. However, the antiviral activity of most of the PR proteins and AMPs has not been fully studied at molecular level therefore future studies are needed.

6. PR proteins as defense signaling indicators

Plants produce a blend of phytohormones, like salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) in response to pathogen attack. The composition, quantity, and timing of these small versatile molecules significantly differ within plant species and rely on the pathogen lifestyle and their mode of infection (De Vos et al., 2005). Classically, SA pathway provides resistance to biotrophic pathogens, whereas jasmonic acid/ethylene (JA/ET) pathways mediate resistance to necrotrophic pathogens as well as to herbivorous pests (Fig. 1) (Glazebrook, 2005; Bari and Jones, 2009). Pathogenesis-related proteins are considered as the signature genes of SA and JA pathways in model and many crop plants. Increased expression of the PR1, PR2, and PR5 genes represents the activation of SA signaling pathway (Fig. 1) (Kunkel et al., 2005; Delaure et al., 2008; Ali et al., 2017b). Furthermore, SA mutants such as nim1, npr1 and sai1 as well as nahG transgenic Arabidopsis plants were impaired or failed to activate PR1, PR2 and PR5 gene expression which further provides the evidence that these PR genes are SA dependent genes (Cao et al., 1994; Delaney and Friedrich Ryals, 1995; Shah et al., 1997). In contrast, increased expression of PR3, PR4 and PR12 represent the activation of JA pathway in Arabidopsis. Interestingly, JA mutant lines like fad3/7/8, coi1, and jar1 failed to induce the expression of PR3, PR4 and PR12, and were found to be susceptible to a large number of pathogens (Staswick et al., 1998; Vijayan et al., 1998; Norman-Setterblad et al., 2000; Seo et al., 2008; Ali et al., 2017b). Hence, transcripts of PR proteins and other inducible molecules increased significantly after SA or JA treatments which possess broad range of antimicrobial activities (Fig. 1). Many reports have shown that plants expressing SA and JA signature genes or PR genes lead increased resistance to a broad spectrum of pathogens (Alexander et al., 1993; Datta et al., 2001; Mackintosh et al., 2007; He et al., 2008; Wally and Punja, 2010; Kaur et al., 2016). In addition to classical defense hormones (SA and JA) other phytohormones viz., abscisic acid (ABA), auxins, cytokinins, gibberellins and brassinosteroids have been shown to modulate the plant immune system however the molecular mechanism remains largely enigmatic (Pieterse et al., 2012).The transcription studies of PR genes in response to these hormones is limited therefore future studies are required to study the expression of SA and JA marker genes after growth hormonal treatments which will provide novel insights in the hormonal crosstalk during plant pathogen interaction.

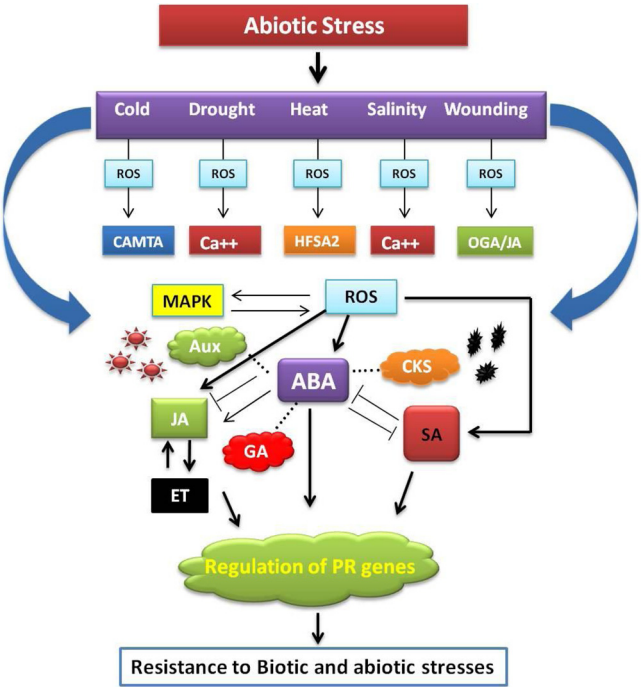

Fig. 3. An outline of regulation of PR genes after abiotic stresses. Abiotic stress results in changes in different hormones and their cross talk involved in PR gene regulation. Solid bold arrows shows proven pathways, dashed lines indicates postulated pathways, line with bar shows negative interactions and arrow showed positive interactions.

7. PR proteins and abiotic stress

Abiotic stress is one of the major threats to the modern agriculture that causes not only enormous yield losses but also provides the entry points to various microbial pathogens. Subsequently, the global climate change is another threat to crop system because of increased emergence of more virulent and broad host range pathogenic strains. Therefore, studying the molecular mechanisms of plant resistance or tolerance to either biotic and abiotic stresses or multiple stresses will provide novel opportunities to develop multiple stress tolerance crops. Pathogenesis-related proteins are induced by multiple stresses and seem to be important candidates for generating multiple stress tolerant crop varieties (Ali et al., 2017b). Abiotic stress mediated expression of PR genes is not fully understood at molecular level. However, we have shown the involvement of various signaling pathways that modulates the expression of PR genes after abiotic stress (Fig. 3). Previous reports have shown that salt and drought stress significantly increases the expression of PR genes in Arabidopsis plants (Seo et al., 2008; Singh et al., 2013). On the other hand, mRNA levels of PR1 (SAR marker gene) in pepper plants increased notably during abiotic stresses (Hong and Hwang, 2005). Classical antifungal PR proteins like PR2 and PR3 protect cell damage due to cold stress and also possess antifreeze activity (Janska et al., 2010). In addition, cold stress significantly induces the expression of AMps namely PR12 and PR13 in Oxytropis and wheat plants (Gaudet et al., 2003; Archambault and Strömvik, 2011). Recent studies have revealed that the mRNA levels of PR4 gene increases dramatically after cold, salinity and wound stress (Kim et al., 2014). Moreover, the up regulation of PR10 gene has been reported under multiple abiotic stresses in maize (Fountain et al., 2010). In our previous study, PR genes in Brassica juncea were significantly induced by both biotic and abiotic stresses (Ali et al., 2017b). The activation of transcription factors like dehydration-responsive element binding proteins (DREB), drought-induced protein 19 (Di19) and cup-shaped cotyledon (CUC) are also known to induce PR genes (Tsutsui et al., 2009). Plants may be challenged by different stresses under field conditions that may likely

Table 2
Shows list of selected transgenic plants expressing PR proteins and AMPs for improving biotic and abiotic stress tolerance.

Family	Gene	Source	Host	Response	Reference
PR1	Antifungal	pepper	N. tabacum	Heavy metal and pathogen stress	Sarowar et al. (2005)
PR1	Antifungal	*Oryza sativa* cv. Nipponbare	*O. sativa*	*Magneporthe grisea* race 003	Mitsuhara et al. (2008)
PR1	Antifungal	*O. sativa*	*O. sativa*	Resistance to Alternaria alternata	Mitsuhara et al. (2008)
PR2	β-1,3-glucanase	*Flax*	*Potato*	Fusarium culmorum and Fusarium oxysporum	Wrobel-Kwiatkowska et al. (2004)
PR2	β-1,3-glucanase	*Barley*	*Wheat*	Fusarium graminearum	Mackintosh et al. (2007)
PR2	β-1,3-glucanase	*Tobacco*	*Groudnut*	Cercospora arachidicola and Aspergillus flavus	Sundaresha et al. (2010)
PR2	β-1,3-glucanase	Pichia pastoris	*Arabidopsis*	Leptosphaeria maculans	Oide et al. (2013)
PR2	β-1,3-glucanase	Hevea brasiliensis	H. brasiliensis	Phytophthora palmivora	Sunpao and Pornsuriya (2016)
PR2	endo-β-1,3(4)-glucanase	Humicola insolens Y1	P. pastoris GS115	Barley β-glucan and CMC-Na, birchwood xylan	Jinyang et al. (2017)
PR3	CHIT33, CHIT42	Trichoderma harzianum	T. harzianum	Biotic and abiotic stress	Cruz et al. (1992)
PR3	ChiA	Pseudomonas sp. BK1	Escherichia coli	Pheidole dentata and Pyropia yezoensis	Jang et al. (2005)
PR3	Chitinase	Hordeum vulgare	Daucus carota	Alternaria radicola, Botrytis cinerea	Jayaraj and Punja (2007)
PR3	Chitinase	Momordica charantia	*Oryza sativa*	Magnaporthe grisea	Li et al. (2009)
PR3	Chitinase	N. tabacum	N. tabacum	Ralstonia solanacearum	Tang et al. (2017)
PR4	Chitinase II	O. sativa L.	O. sativa L.	Drought stress and pathogen response	Wang et al. (2011)
PR4	Chitinase II	Vitis pseudoreticulata	V. pseudoreticulata	Powdery mildew	Dai et al. (2016)
PR4	Chitinase II EuCHIT2	Eucommia ulmoides	N. tabacum cv. Xanthi	Erysiphe cichoracearum DC	Dong et al. (2017)
PR4	Chitinase classII Zjchi2	zoysiagrass.	Zoysiagrass	Rhizoctonia solani AG2-2	Kang et al. (2017)
PR5	Thaumatin-like	Prunus domestica	P. domestica	Enhance resistance to fungal infection	El-kereamy et al. (2011)
PR5	Thaumatin-likeTaLr19TLP1	wheat	Wheat	Puccinia triticina	Yanjun et al. (2017)
PR5	Thaumatin-like protein (VaTLP)	V. amurensis	V. vinifera	Downy mildew-resistant grapevine "Zuoshan-1	Rongrong et al. (2017)
PR5	Thaumatin-like protein (TLP29)	V. vinifera L.	V. vinifera L.	Elsinoe ampelina, Erysiphe necator	Xiaoxiao et al. (2017)
PR6	proteinase inhibitor	Panax ginseng Meyer	P. ginseng Meyer	Hormonal, heavy metals and abiotic stress	Myagmarjav et al. (2017)
PR10	Ribonuclease-like'	Capsicum annuum	C. annuum	Ribonucleolytic activity against TMV	Park et al. (2004)
PR10	Ribonuclease-like'	JIOaPR10	Rice	Biotic and Abiotic stress	Wu et al. (2016)
PR12	Defensin	Wasabia japonica	Rice	Magnaporthe grisea	Kanzaki et al. (2002)
PR12	Defensin	Mungbean	Pichia pastoris	Fusarium oxysporum	Chen et al. (2004)
PR12	Defensin	Brassica nigra	Peanut	Pheaoisariopsis personata and Cercospora arachidicola	Anuradha et al. (2008)
PR12	BoDFN Defensin gene	Brassica oleracea var. italica	B. oleracea var. italica	Downy Mildew	Jiang et al. (2012)
PR12	JcDef	Jatropha curcas	N. tabacum	Sheath Blight disease resistance	Wang et al. (2017)
PR12	AtPDF1.1	1. thaliana	A. thaliana	Pectobacterium carotovorum	Hsiao et al. (2017)
PR12	VrPDF1	Vigna radiata	V. radiata	Weevils	Thao et al. (2017)
PR13	Thionin	Brassicaceae species	Solanum tuberosum	B. cinerea	Hoshikawa et al. (2012)
PR13	Thionin	Carrizo plant	Carrizo plant	Citrus Canker	Hao and Stover, 2016
PR13	Thionin	A. thaliana	S. tuberosum	Fusarium Spp.	Hammad et al. (2017)

occur simultaneously; a greater effort must be made to study these types of stresses both individually or combined under lab conditions which will provide novel insights in the field of plant stress biology (Mittler and Blumwald, 2010). Based on these studies PR proteins as well as AMPs can be utilised for developing both biotic and abiotic stress tolerant crop varieties in modern agriculture.

8. Antimicrobial peptides (AMPs) and disease resistance

For improving disease resistance, AMPs are gaining more attention due to their all-rounder performance against multiple stresses such as antifungal, antibacterial, antiviral and also their role in abiotic stress tolerance. For instance, the transcript levels of AMPs in tomato plants are increasing significantly after bacterial and fungal infections, thus implying their role in diseases resistance (Pautot and Holzer, 1991). Based on in-vitro studies PR6 peptides have shown effective antimicrobial activity against an array of fungal pathogens (Terras et al., 1993). Among antimicrobial peptides, PR12 or plant defensins are known to be the most important antifungal peptides in plants. The in vitro studies revealed that plant defensins shows antifungal activity against many fungal pathogens (Terras et al., 1995; Jha and Chattoo, 2009). In addition, overexpression of plant defensin peptides both in model and crop plants have shown enhanced and long lasting disease resistance (Kanzaki et al., 2002; Chen et al., 2004; Anuradha et al., 2008; Ntui et al., 2010; Ghag et al., 2012; Kaur et al., 2016). Interestingly, Lacerda Lacerda et al. (2016) reported first time that transgenic Pichia pastoris plants expressing defensin gene leads to enhanced resistance against obligate biotrophic fungi Fusarium tucumaniae and Colletotrichum gossypii var. Besides their role in biotic stress, they are also induced by diverse abiotic stresses including cold (Koike et al., 2002), drought (Do et al., 2004), heavy metals (Mirouze et al., 2006) and wounding (Rawat et al., 2017). Another important group of AMPs are PR13 and PR14 peptides which also play important defensive role against a wide range of pathogens. For instance, overexpression of PR13 peptides in tomato and potato plants has shown enhanced disease resistance against fungal pathogens (Chan et al., 2005; Chandrashekhara et al., 2010; Hoshikawa et al., 2012; Muramoto et al., 2012). On the other hand, in vitro studies have revealed that various PR14 peptides or lipid transfer proteins show antifungal and antibacterial activity (Cammue et al., 1995; Wang et al., 2004). Hence, these results further highlight the multifaceted role of AMPs which will serve as future candidate genes for developing multi-trait resistance crop plants. More studies are required to focus on these small antimicrobial peptides with respect to their gene regulation and possible functions during multiple stresses.

9. Transgenic PR plants success and snags

After the beginning of plant genetic engineering era, PR proteins have always been the first choice of researchers to develop transgenic plants for improving disease resistance against wide range of pathogens. For example, overexpression of PR1 or like proteins in various

plants lead enhanced disease resistance to many pathogens (Alexander et al., 1993; Sarowar et al., 2005). Nevertheless, overexpression of PR2 alone or in conjunction with PR3 conferred increased resistance to wide range of fungal pathogens (Yamamoto et al., 2000; Anand et al., 2003; Chye et al., 2005; Li et al., 2009; Kovacs et al., 2013; Fujimori et al., 2016). Furthermore, their combined expression in transgenic plants can also provide broader and effective disease resistance against an array of pathogenic strains or races of the same pathogen (Joshi and Nayak, 2010). Recently, overexpression of PR4 gene in Vitis vinifera leads enhanced resistance to powdery mildew infection (Dai et al., 2016). Another important member of PR protein family is a PR5 protein or thaumatin like proteins which are considered as important antimicrobial weapons and when overexpressed in tobacco or wheat plants showed improved resistance to wide range of pathogens (Liu et al., 2012; Yanjun et al., 2017). Consistent with this notion, many reports have shown that overexpression of AMPs like PR12 and PR13 also increased disease resistance against broad range of pathogens. The results obtained from these studies were promising and showed several advancements in plant pathogen interactions. We have summarised the list of transgenic plants overexpressing PR proteins leading to improved resistance against microbial pathogens (Table 2).

In addition to antimicrobial property, PR proteins are known to play important role in plant development and abiotic stress tolerance. For example, potato transgenic plants expressing PR2 and PR3 genes not only showed resistance to fungal pathogens but also improved root growth when compared to non-transgenic potato plants (Chye et al., 2005). One of the major goals in crop biotechnology is to improve the crop yield which is mainly affected by biotic and abiotic stresses. Previous studies have revealed that plants expressing stress responsive genes showed better progress in stress adaption as well as increased yield (Li et al., 2014; Shi et al., 2014). Recently, overexpression of PR10 leads increasing yield in rice which may be due to its improved adaption to various stresses (Wu et al., 2016). Various studies have shown the multiple roles of PR proteins when expressed in different crop systems like overexpression of PR1 and PR3 in tobacco plants leads enhanced tolerance to salt and heavy metals (Sarowar et al., 2005; Dana et al., 2006). Similarly, overexpression of PR-5 also showed enhanced tolerance to abiotic stress (Frendo et al., 1992; Singh et al., 2013). The recent study by Wu et al. (2016) demonstrated the role of PR10 in abiotic stress (drought, salt) tolerance when overexpressed in rice. Hence, PR genes can serve as potential candidate genes for improving multi trait factors in crops through genetic engineering. However, future studies are required to evaluate the PR transgenic plants in response to many traits like biotic, abiotic as well as plant development and yield.

Besides above promising results, many reports have revealed pitfalls of PR transgenic technology. For example overexpression of tobacco basic chitinase gene in carrot showed improved resistance to three out of five tested pathogens, but fails to provide resistance when the chitinase was obtained from petunia. In addition, when these chitinases were overexpressed in cucumber no resistance was seen against pathogens (Punja and Raharjo, 1996). To solve this problem, researchers should introduce more than one PR gene into host plants for improving disease resistance because the function of a lone PR gene might be altered due to some mutation in the respective pathogen Avr gene (Moosa et al., 2017). Therefore, PR proteins or AMPs should be characterised in a number of crops using different pathogens. The overall success rate of transgenic technology using PR proteins or AMPs to improve disease resistance is highly dependent upon the recipient host as well as the source of the PR gene. Another problem is that most of the researchers have used constitutive promoters to drive the expression of PR genes in crop plants for uplifting resistance which causes a number of hitches like homology dependent gene silencing, leading to fitness consequence in plant growth and development. Hence, untimely and uncontrolled activation of PR genes or AMPs is harmful for plant growth and development. Second problem is the uses of CaMV 35 s promoter from viral origin has lots of ethical issues. To solve this problem, spatially and temporally inducible promoters that are less exhaustive are needed to develop transgenic plants resistant to pathogens. Therefore, we must focus on plant based pathogen inducible promoters to drive the expression of PR genes or AMPs to develop disease resistant transgenic crops.

10. Conclusion

In modern agriculture biotic stress has become a great challenge, and many research organizations are actively working to develop resistant varieties using different approaches including the PR proteins. The best feature of PR proteins are that they can be effective against multiple biotic agents like fungi, bacteria or even insects, which really attracts the attention of most of the active researchers for using them against multiple stresses. In this regard, genetic engineering is the best option to use PR proteins for the development of transgenic resistant plants. However, more innovations or novel approaches are required in PR protein transgenic technology to further improve the agronomic traits across the globe. So far, plant transgenic technology has shown remarkable success in plant disease resistant program and will continue to improve plant health. However, plant pathologists have more focused on commonly known PR proteins therefore, future studies are required to characterize or overexpress other PR proteins as well in different model and crop plants against different traits which may be the breakthrough in disease development. Another future challenge is that increasing rate of global climatic change will possibly increase the emergence of virulent strains of phytopathogens with broad host range. Hence, there is need to functionally characterize as well as identify novel PR genes/alleles to cope such drastic challenges. In this regard, advances in “omics” approaches viz., genomics, transcriptomics, phenomics, proteomics, metabolomics, and ionomics will greatly help us to understand the detailed network of PR genes as well as the interaction of PR proteins with other proteins belonging to both plants and pathogen. These studies will definitely provide us new genetic stocks of PR genes that can be effectively used to counter the disease epidemic. Therefore, PR proteins can serve as the potential candidates for engineering crop plants to improve resistance to multiple stresses.

Conflict of interest

The authors declare that there is no conflict of interest.

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