ROLE OF PATHOGENESIS-RELATED PROTEINS IN PLANT DISEASE MANAGEMENT - A REVIEW

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ABSTRACT

The present paper reviews one of the most important defence strategy employed by plants against pathogens. Pathogenesis-related proteins (PR-proteins) are induced in plants in response to attack by microbial or insect pests and certain stress conditions. They have been classified into several groups (PR-1 through PR-17) based on their amino acid sequences and biochemical functions. In many cases, the expression of the PR-proteins either singly or in combination appears to improve resistance to multiple phytopathogens. Transgenics with PR protein encoding genes will have a major role in plant disease management.

Key words: â-1,3-glucanases, Chitinases, Defensins, Disease management, Pathogenesis-Related Proteins, Thaumatin-like proteins, Thionins,

Plants responding to infection by pathogens, or to various abiotic stresses, are induced to express a set of defence-related genes, such as the genes encoding pathogenesis-related proteins. Antoniw et al. (1980) coined the term “Pathogenesis-Related Proteins” and defined them as ‘proteins coded by the host plant but induced only in pathological or related situations’. PR proteins are a collective set of novel proteins associated with host defence mainly in incompatible interactions and thus are related primarily to a special type of pathogenesis, i.e., one culminating in the impediment of further pathogen progress.

PR proteins are a heterogeneous group of low molecular weight proteins with selective solubility at low pH, resistant to proteolytic degradation and predominantly accumulated in intercellular leaf spaces (Van-Loon, 1985; Bol et al., 1990; Schroder et al., 1992). They accumulate rapidly at the intra or extracellular level under various biotic and abiotic stimuli including fungal, elicitor and physical or chemical treatments (Heller and Gessler, 1986; Van-Kan et al., 1992; Van-Loon and Van-Strien, 1999; Graham et al., 2003). The importance of PR proteins to plant defence has been related to: (a) their rapid and early accumulation often associated with incompatibility, (b) their antimicrobial activity and (c) their ability to reduce symptoms development (Schroder et al., 1992; Wang et al., 2005).

Currently PR proteins are categorized into 17 families according to their properties and functions (Table 1), including â-1, 3-glucanases, chitinases, thaumatin-like proteins, peroxidases, ribosome-inactivating proteins, defensins, thionins, nonspecific lipid transfer proteins, oxalate oxidase, and oxalate-oxidase-like proteins.

PR-Proteins having vital role in plant defence mechanisms: The first PR-1 protein was discovered in 1970. Since then, a number of PR-1 proteins have been identified in Arabidopsis, barley, tobacco (Bormann et al., 1999), rice, pepper, tomato, wheat and maize (Liu and Xue, 2006). This PR-1 group has 14 to 17 kD molecular weight and mostly basic in nature. PR-1 proteins have antifungal activity at the micromolar level against a number of plant pathogenic fungi, including Uromyces fabae, Phytophthora infestans and Erysiphe graminis (Niderman et al., 1995).

Plant Glucanas es (PR-2): Plant ß-1,3-glucanases (ß-1,3-Gs) family comprises of large and highly complex gene families involved in pathogen defence as well as wide range of normal developmental processes. ß-1,3-Gs have molecular mass in the
range from 33 to 44 kDa (Hong and Meng, 2004; Saikia et al., 2005). These enzymes have wide range of isoelectric pH. Most of the basic â-1,3-Gs are localized in vacuoles of the plant cells while the acidic â-1,3-Gs are secreted outside the plant cell. Wounding, hormonal signals like methyl jasmonate and ethylene (Wu and Bradford, 2003), attack of fungal pathogen (Ji and Ku, 2002) and some fungal elicitors released from pathogen cell wall (Boller, 1995) can also induce â-1,3-Gs in the various parts of plant (Wu and Bradford, 2003; Saikia et al., 2005). â-1,3-glucanases are involved in hydrolytic cleavage of the 1,3-â-D-glucosidic linkages in â-1,3-glucans, a major component of fungal cell wall (Simmons, 1994; Hoj and Fincher, 1995). Cell lysis and cell death occur as a result of hydrolysis of glucans present in the cell wall of fungi.

Chitinases (PR3): Most chitinases have molecular mass in the range of 15 kDa and 43 kDa. Chitinase can be isolated from chickpea (Saikia et al., 2005), cucumber, barley, tobacco, tomato (Wu and Bradford, 2003), black turtle bean (Chu and Ng, 2005), and grapes (Slyuter et al., 2005). They can be divided into two categories: exochitinases, demonstrating activity only for the non-reducing end of the chitin chain and endochitinases, which hydrolyse internal â-1,4-glycoside bonds.

Wounding and methyl jasmonate induces gene chi 9 for chitinase expression in the tomato seeds (Wu and Bradford, 2003). These chitinases have significant antifungal activities against plant pathogenic fungi like Alternaria sp., Bipolaris oryzae, Botrytis cinerea, Curvularia lunata, Pestalotia theae and Rhizoctonia solani (Chu and Ng 2005; Saikia et al., 2005; Kirubakaran and Sakthivel, 2006). The mode of action of PR-3 proteins is relatively simple i.e., chitinase cleaves the cell wall chitin polymers in situ, resulting in a weakened cell wall and rendering fungal cells osmotically sensitive (Jach et al., 1995).

Chitin Binding Protein (CBP, PR4): CBP can be isolated from plants like sugar beet, tobacco, pepper, tomato and potato (Nielsen et al., 1997; Bormann et al., 1999; Lee et al., 2001, Yang and Gong, 2002). Molecular weight of the CBP was found to be 9 kDa to 30 kDa with basic isoelectric pH (Nielsen et al., 1997; Bormann et al., 1999; Yang and Gong, 2002). Expression of the CACBP1 chitin-binding protein isolated from cDNA library of pepper (Capsicum annuum L.) was rapidly induced in the incompatible interactions upon pathogen infection, ethephon, methyl jasmonate or wounding. (Lee et al., 2001; Wan et al., 2008). CBP shows strong inhibitory effect against Aspergillus spp, Cercospora beticola and Xanthomonas campestris (Nielsen et al., 1997; Bormann et al., 1999; Lee et al., 2001; Yang and Gong, 2002). CBP binds to insoluble chitin and enhances hydrolysis of chitin by other enzyme like chitinase (Houston et al., 2005; Vaaje-Kolstad et al., 2005).

Thaumatin-Like Protein (TLP, PR5): Thaumatin-like proteins comprise of polypeptides that share homology with thaumatin, sweet protein from Thaumatococcus danielli (Bennett) Benth (Cornelissen et al., 1986). TLPs can be isolated from barley, maize, tobacco, tomato and wheat (Wurms et al., 1999; Fecht-Christoffers et al., 2003; Anand et al., 2004; Zamani et al., 2004). TLPs have a molecular weight in the range of 18 kDa to 25 kDa and have acidic pH (Fecht-Christoffers et al., 2003 and Zamani et al., 2004). They are involved in the Systemic Acquired Resistance (SAR) and in response to biotic stress, cause inhibition of hyphal growth and reduction of spore germination, probably by a membrane permeabilization mechanism and/or by interaction with pathogen receptors (Thompson et al., 2007). TLPs isolated from cherry, apple and banana showed antifungal nature against Verticillium albo-atrum having endo-â-1,3-glucanase activity (Menu-Bouaouiche et al., 2003). AP24 from tobacco and NP24 from tomato caused lysis of sporangia of Phytophthora infestans. Osmotin from tobacco caused fungal spore lysis, inhibition of spore germination, reduced germling viability and damage to membrane permeability (Narasimhan et al., 2001).

Proteinase-inhibitor Proteins (PR-6): Proteinase inhibitors (PIs) are highly stable defensive proteins of plant tissues that are developmentally regulated and also induced in response to insect and pathogen attacks. They were isolated from seeds of Leguminosae, Gramineae, and Solanaceae. Geoffroy et al. (1990) identified induction of strong inhibitory activity in tobacco during the hypersensitive reaction to tobacco mosaic virus with PIs. PIs were strongly induced along with other PR
genes during the incompatible interaction of barley seedlings with the fungus Stagonospora nodorum (Stevens et al., 1996). Lilley et al. (1996) investigated the presence of proteinases in cryostat sections of the soybean cyst-nematode, Heterodera glycines.

**Ribosome Inactivating Protein (RIP, PR10):** RIP has an inherent antifungal activity. It has been isolated from peanut, (Vivanco et al., 1999), tobacco (Kim et al., 2001), pea (Ye et al., 2000) etc., having molecular mass of 30 kDa. RIP isolated from tobacco, termed as TRIP, releases adenine residues from the ribosomal and non-ribosomal substrata that inhibits translation in Fusarium oxysporum, Pestalotia sp., Erwinia amylovora and Pseudomonas solanearum (Kim et al., 2001).

**Plasma membrane-permeabilizing Proteins (PR-12, PR-13 and PR-14):** Plasma membrane-permeabilizing ability proper to PR-12, PR-13 and PR-14 contributes to plasmolysis and damage of fungal and bacterial pathogens, inhibiting their growth and development (Vigers et al., 1992; Abad et al., 1996; Van Loon and Van Strien, 1999, and Van Loon, 2001; Selitrennikoff, 2001). This effect may be due to electrostatic interactions of PRs with membrane components, leading to conformational changes, dissipation of membrane gradient, and formation of pores in membranes (Abad et al., 1996; Cheong et al., 1997; Anzlovar et al., 1998).

**OVER-EXPRESSION OF PR PROTEINS IN TRANSGENIC PLANTS AS A DEFENCE STRATEGY:** The induction of PR proteins has often been interpreted as an attempt by the plant to prevent or limit the spread of the pathogen. In most cases, an assortment of PR proteins belonging to diverse subclasses, rather than a single member of a single family of PR proteins has resulted in increased disease resistance. There is a good correlation between a rapid and high-level expression of one or more PR proteins and the resistance reaction of the host plant. These findings have led to the hope that a genetic engineering strategy involving constitutive, high-level expression of combinations of PR proteins with different modes of action against target organisms may provide broad-spectrum, durable resistance to a variety of diseases.

The most attractive candidates for combination are genes encoding chitinases or â-1, 3-glucanases because these two enzymes hydrolyze chitin and â-1-3-glucans which are structural components of the cell walls of several fungi. In the first report of success with this approach, the expression of a bean chitinase gene in tobacco and Brassica napus resulted in decreased symptoms by Rhizoctonia solani (Broglie et al., 1991). A rice chitinase gene (RC7) isolated from R. solani-infected rice plants was introduced into indica rice cultivars IR72, IR64, IR68899B, MH63 and Chinsurah Boro II and the transformants showed increased tolerance to R. solani (Datta et al., 2001). Tohifdar et al. (2009) showed that transgenic cotton expressing a bean chitinase exhibited enhanced resistance against Verticilium dahlia in greenhouse and in-vitro assay as compared to the non-transgenic plants. Nirala et

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

<table>
<thead>
<tr>
<th>Family</th>
<th>Type member</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-1</td>
<td>Tobacco PR-1a</td>
<td>Antifungal</td>
</tr>
<tr>
<td>PR-2</td>
<td>Tobacco PR-2</td>
<td>â-1, 3-Glucanase</td>
</tr>
<tr>
<td>PR-3</td>
<td>Tobacco P, Q</td>
<td>Chitinase type I, II, IV, V, VI, VII</td>
</tr>
<tr>
<td>PR-4</td>
<td>Tobacco “R”</td>
<td>Chitinase type I, II</td>
</tr>
<tr>
<td>PR-5</td>
<td>Tobacco S</td>
<td>Thaumatin-like</td>
</tr>
<tr>
<td>PR-6</td>
<td>Tomato Inhibitor I</td>
<td>Proteinase-inhibitor</td>
</tr>
<tr>
<td>PR-7</td>
<td>Tomato P6g</td>
<td>Endoproteinase</td>
</tr>
<tr>
<td>PR-8</td>
<td>Cucumber chitinase</td>
<td>Chitinase type III</td>
</tr>
<tr>
<td>PR-9</td>
<td>Tobacco “lignin-forming peroxidase”</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>PR-10</td>
<td>Parsley “PR1”</td>
<td>Ribonuclease-like/Ribosome Inactivating Protein</td>
</tr>
<tr>
<td>PR-11</td>
<td>Tobacco class V chitinase</td>
<td>Chitinase type I</td>
</tr>
<tr>
<td>PR-12</td>
<td>Radish Rs-AFP3</td>
<td>Defensin</td>
</tr>
<tr>
<td>PR-13</td>
<td>Arabidopsis TH12.1</td>
<td>Thionin</td>
</tr>
<tr>
<td>PR-14</td>
<td>Barley LTP4</td>
<td>Lipid-Transfer Protein</td>
</tr>
<tr>
<td>PR-15</td>
<td>Barley OXa (germin)</td>
<td>Oxalate oxidase</td>
</tr>
<tr>
<td>PR-16</td>
<td>Barley OXaL</td>
<td>Oxalate oxidase-like</td>
</tr>
<tr>
<td>PR-17</td>
<td>Tobacco PRp27</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
al. (2010) showed that expression of a rice chitinase gene enhances antifungal potential in transgenic grapevine exhibited higher chitinase activity than non-transgenic plants that showed increased tolerance to Uncinula necator through delayed onset of the disease and smaller lesions. Girhepuje and Shinde (2011) reported that transgenic tomato plants expressing a wheat endochitinase gene resulted in enhanced resistance to Fusarium oxysporum f. sp. lycopersici. Furthermore, â-1,3-glucanases and chitinases have been shown to act synergistically against fungi under in vitro condition (Mauch et al., 1988). Transgenic tomato plants expressing both chitinase and â-1,3-glucanase transgenes had significantly higher resistance to F. oxysporum than the plants expressing only chitinase or â-1,3-glucanase alone (Jongedijk et al., 1995).

Expression of the barley RIP cDNA under the control of a wound-inducible promoter in transgenic tobacco plants conferred protection against the soil-borne pathogen R. solani as judged by height differences between control and transgenic plants grown in infected soil (Logemann et al., 1992). Jach et al. (1995) demonstrated high-level expression of the transferred genes was detected in transgenic plants when cDNAs encoding three proteins from barley, a class II chitinase (CHI), a class II â-1,3-glucanase (GLU), and a Type-I ribosome inactivating protein (RIP) were expressed in tobacco plants. The performance of tobacco plants co-expressing the barley transgene GLU/CHI or CHI/RIP in a R. solani infection assay showed significantly enhanced protection against fungal attack. These data indicated synergistic protective interaction of the co-expressed antifungal proteins as antifungal defenses.

Transgenic potato expressing the tobacco osmotin (TLP) gene showed a delay in the development of disease symptoms against the potato pathogen (Liu et al., 1994). Zhu et al. (1996) reported transgenic potato expressing osmotin-like protein from potato plants showed delayed development of disease symptoms when inoculated with P. infestans. Transgenic tomato plants with a TLP gene showed resistance to B. cinerea, Oidium lycopersicum, Leveillula taurica and P. infestans and the resistance was confirmed up to T_3 generation (Veronese et al., 1999).

The T_2 tobacco plants transformed for radish Rs-AFP2 plant defensin showed a sevenfold reduction in lesion size upon infection with the fungal pathogen Alternaria longipes compared to untransformed plants (Terras et al., 1995). Transgenic tomato plants expressing an acidic endochitinase (pcht28) isolated from Lycopersicon chilense showed resistance to Verticillium dahliae (Tabaeizadeh et al., 1999).

**CONCLUSIONS**

PR proteins play an important role in disease management. The increasing knowledge about the PR proteins gives better idea regarding the defense system of plants. Genetic engineering strategy involving constitutive, high-level expression of combinations of PR proteins with different modes of action against target organisms may provide broad-spectrum, durable resistance to a variety of diseases. Hence transgenic plants capable of over-expressing one or combinations of PR proteins can be used for the effective and successful management of phytopathogens. Primary aspects of the gene regulation of the PR proteins are understood but the study of exact mechanism of gene regulation and receptor cascade will pave new ways for the plant genetic engineering technology for crop protection against diseases.

**REFERENCES**


