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Pathogenesis-Related Proteins for the Plant Protection

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Abstract : Fungi are far more complex organisms than viruses or bacteria and can developed numerous diseases in plants that cause loss of big portion of the crop every year. Plants have developed various mechanisms to defend themselves against these fungi which include the production of low molecular weight secondary metabolites, proteins and peptides having antifungal activity. In this review, brief information like biochemistry, source, regulation of gene expression, mode of action of defense mechanism of various pathogenesis-related proteins is given. Proteins include pathogenesis-related protein 1, â-glucanases, chitinases, chitin binding protein, thaumatine like protein, glycine-histidine rich proteins, ribosome inactivating protein, and some newly discovered antifungal proteins.

Key words : Pathogenesis-related Proteins, â-Glucanase, Chitinases, Thaumatine like protein, Glycine-histidine rich proteins and Ribosome inactivating protein.

Introduction

Fungi are an extremely diverse group of organisms, with about 250,000 species widely distributed in essentially every ecosystem. They can use almost any surface e.g., bathroom tile, skin, or leaves for their growth. They are proficient at colonizing and using plants, humans, and animals as substrates.

During the past two decades, invasive fungal infections have emerged as a major threat to immunocompromised hosts. Fungal infections are a frequent cause of death among immunocompromised patients, and the increasing number of immunosuppressed patients has spurred development of new antifungals (Shoham and Levitz, 2005). Patients with primary immunodeficiencies exhibit immune deficits that confer increased susceptibility to fungal infections. Numerous fungi, have been invariably implicated in causing disease in patients with chronic granulomatous disease, severe combined immunodeficiency, chronic mucocutaneous candidacies, hyper-IgE syndrome, myeloperoxidase deficiency, leukocyte adhesion deficiency, defects in the interferon-ã/interleukin-12 axis, DiGeorge syndrome, X-linked hyper-IgM syndrome, Wiskott-Aldrich syndrome and common variable immunodeficiency (Antachopoulos *et al*., 2007). Unfortunately there are few species of fungi that infect the human and animals. But among the all microbes fungi are the most causative agent of disease in plant.

When a pathogen attacks a plant, it either successfully infects the plant or plant prevents the infection. Plants do not have circulating or phagocytic cells. Instead their cells have a thick, complex wall that acts as a barrier to invasion. Plants display an innate pathogen-specific resistance by producing responses like oxidative burst of cell, change of cell wall composition that prevent infection and *de-novo*

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synthesis of compounds like phytoalexin and pathogenesis-related proteins. All this responses can be triggered by exposing the plant to virulent, avirulent, and nonpathogenic microbes, or artificially with low molecular weight and sometimes volatile molecules like such as salicylic acid, jasmonate (Delaney *et al*., 1994; Xu *et al*., 1994; Wu and Bradford, 2003), 2,6-dichloro-isonicotinic acid or benzo(1,2,3) thiadiazole-7-carbothioic acid S-methyl ester(BTH) (Vallad and Goodman, 2004). These types of resistance are called as Systemic Acquired Resistance (SAR) or Induced Systemic Resistance (IAR). Among all induced responses, production of "Pathogenesis Related (PR) proteins" is most important because they can lead to the increased resistance of the whole plant against a pathogenic attack (Adrienne and Barbara, 2006). Large numbers of small, basic, cysteine-rich antimicrobial proteins are produced by many organisms throughout all kingdoms. They display a great variety in their primary structure, in species specificity, and in the mechanism of action (Leiter *et al*., 2005). There are more than 13 different pathogenesis-related proteins are known to us.

Antifungal PR proteins are of great biotechnological interest because of their potential use as food and seed preservative agents and for engineering plants for resistance to phytopathogenic fungi (Dempsey *et al*., 1998). Various studies have revealed that transgenic plants over expressing genes of the PR-1, PR-2, PR-3, and PR-5 families mediate host plant resistance to phytopathogenic fungi. Co-expression of multiple antifungal protein genes in transgenic plants seems to be more effective than expression of single genes (Bormann *et al*., 1999).

Pathogenesis-related (PR) protein 1

The first PR- 1 protein was discovered in 1970. Since then, a number of PR-1 proteins have been identified in *Arabidopsis, Hordeum vulgare* (barley), *Nicotiana tabacum* (tobacco), *Oryza sativa* (rice), *Piper longum* (pepper), *Solanum lycopersicum* (tomato), *Triticum* sp. (wheat) and *Zea mays* (maize) (Liu and Xue, 2006). These PR-1 having 14 to 17 kD molecular weight and mostly of basic nature. Non-expressors of Pathogenesis-Related Genes1 (NPR1) regulate systemic acquired resistance via regulation pathogenesis-related 1 (PR-1) in *Arabidopsis thaliana*. The interaction of nucleus-localized NPR1 with TGA transcription factors, after reduction of cysteine residues of NPR 1 by salicylic acid (SA) results in the activation of defense genes of PR-1. In the absence of TAG 2 and/or SA expression of PR-1 not occur in *Arabidopsis thaliana* (Després *et al*., 2000; Rochon *et al*., 2006). 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). The exact mode of action of the antifungal activities of these proteins are yet to be identified but a PR-1-like protein, helothermine, from the Mexican banded lizard have been found to be interacting with the membrane-channel proteins of target cells, inhibiting the release of Ca^{2+} (Monzingo *et al*, 1996).

â-Glucanase (PR2):

Plant ß-1,3-glucanases (ß-1,3-Gs) comprises of large and highly complex gene families involved in pathogen defense as well as a 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 ß-1,3-Gs are secreted outside the plant cell. Wounding, hormonal signals like methyl jasmonate and ethylene (Wu and Bradford, 2003), pathogen attack like fungous *Colletotrichum lagenarium* (Ji and Ku, 2002) and some fungal elicitors releases from pathogen cell wall (Boller, 1995) can also induced â-1,3-Gs in the various parts of plant

(Wu and Bradford, 2003; Saikia *et al*., 2005). The enzyme â-1,3-Gs was found to be strongly induced by ultraviolet (UV-B; 280–320 nm) radiation in primary leaves of French bean (*Phaseolus vulgaris*), so that UV-induced DNA damage is a primary step for the induction of â-1,3-Gs.(Kucera *et al*., 2003). â-1,3-glucanases and chitinases are down regulated by combination of auxin and cytokinin while Abscisic acid (ABA) at a concentration of 10 µM markedly inhibited the induction of â-1,3-glucanases but not of chitinases (Rezzonico, 1998; Wu *et al*., 2001). These enzymes are found in wide variety of plants like *Arachis hypogaea* (peanut), *Cicer arietinum* (chickpea), *Nicotiana tabacum* (tobacco), etc. and having resistivity against various fungi like *Aspergillus parasiticus, A. flavs, Blumeria graminis, Colletotrichum lagenarium, Fusarium culmorum, Fusarium oxysporum, fusarium udum, Macrophomina phaseolina* and *Treptomyces sioyaensis* (Rezzonico, 1998; Wu and Bradford, 2003; Hong and Meng, 2004; Wróbel-Kwiatkowska *et al*., 2004, Liang *et al*., 2005; Roy-Barman *et al*., 2006). â-1,3-glucanases are involves in hydrolytic cleavage of the 1,3-â-D-glucosidic linkages in â-1,3-glucans, a major componant of fungi cell wall (Simmons, 1994; Høj and Fincher, 1995). So that cell lysis and cell death occur as a result of hydrolysis of glucans present in the cell wall of fungi.

Chitinases (PR3)

Most of Chitinase having molecular mass in the range of 15 kDa and 43 kDa. Chitinase can be isolated from *Cicer arietinum* (chickpea) (Saikia *et al*., 2005), *Cucumis sativus* (cucumber), *Hordeum vulgare* (barley) (Kirubakaran and Sakthivel, 2006), *Nicotiana tabacum* (tobacco) (Pu *et al*., 1996), *Phaseolus vulgaris* (black turtle bean) (Chu and Ng, 2005), *Solanum lycopersicum* (tomato) (Wu and Bradford, 2003) and *Vitis vinifera* (grapes) (Sluyter *et al*., 2005). Chitinases 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. Many plant endochitinases, especially those with a high isoelectric point, exhibit an additional lysozyme or lysozyme like activity (Collinge *et al*., 1993; Brunner *et al*., 1998; Schultze *et al*., 1998; Subroto *et al*., 1999). Chitinase and â-1,3-Glucanase are differentially regulated by Wounding, Methyl Jasmonate, Ethylene, and Gibberellin. Wounding and methyl jasmonate induces gene *chi 9* for Chitinases expression in the tomato seeds (Wu and Bradford, 2003). In some study, it is also found that Chitinase gene are also expressed in response to stress like cold up to -2 to -5ºC (Yeh *et al*., 2000). These Chitinases have significant antifungal activities against plant pathogenic fungi like *Alternaria* sp. For grain discoloration of rice, *Bipolaris oryzae* for brown spot of rice, *Botrytis cinerea* for blight of Tobacco, *Curvularia lunata* for leaf spot of clover, *Fusarium oxysporum, F. udum, Mycosphaerella arachidicola, Pestalotia theae* for leaf spot of tea and *Rhizoctonia solani* for sheath blight of rice (Chu and Ng 2005; Saikia *et al*., 2005; Kirubakaran and Sakthivel, 2006). The main substrate of Chitinases is chitin - a natural homopolymer of â-1,4- inked N-acetylglucosamine residues (Kasprzewska, 2003). The mode of action of PR-3 proteins is relatively simple i.e. Chitinases 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):

All chitin binding proteins do not possess antifungal activities. CBP can be isolate from plant *Beta vulgaris* (suger beat), *Hydrangea macrophylla* (hortensia), *Nicotiana tabacum* (tobacco), *Piper longum* (pepper), *Solanum lycopersicum* (tomato) and *Solanum tuberosum* (potato) and bacteria like *Streptomyces tendae* (Nielsen *et al*., 1997; Bormann *et al*., 1999; Lee *et al*., 2001, Yang and Gong, 2002,). Moleculer weight of the

CBP was found to be in the range of 9 kDa to 30 kDa and having 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.) (CACBP1) gene was rapidly induced in the incompatible interactions upon pathogen infection, ethephon, methyl jasmonate or wounding (experimental model plant pepper). The CACBP1 gene was organ-specifically regulated in plants. High level of expression occurs in phloem of vascular bundles in leaves of pepper (Lee *et al*., 2001; Wan *et al*., 2008). CBP shows strong inhibitory effect against fungi *Aspergillus* species, *Cercospora beticola*, *Xanthomonas campestris* and many more and several crop fungal pathogen (Nielsen *et al*., 1997; Bormann *et al*., 1999; Lee *et al*., 2001; Yang and Gong, 2002). Enzymeticaly CBP has not any function but it 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 classes that share homology with thaumatin, sweet protein from *Thaumatococcus danielli* (Bennett) Benth (Cornelissen *et al*., 1986). Thaumatin-like proteins can be isolated from *Hordeum vulgare* (barley), *Actinidia deliciosa* (kiwifruit), *Zea mays* (maize), *Pseudotsuga menziesii* (douglas-firs), *Nicotiana tabacum* (tobacco), *Solanum lycopersicum* (tomato) and *Triticum* sp. (wheat) (Wurms *et al*., 1999; Fecht-Christoffers *et al*., 2003; Anand *et al*., 2004; Zamani *et al*., 2004). Most of the TLPs have a molecular weight in the range of 18 kDa to 25 kDa and have a pH in the range from 4.5 to 5.5 (Fecht-Christoffers *et al*., 2003; Zamani *et al*., 2004). Constitutive levels of Thumatin-Like Protein is typically absent in healthy plants, with the proteins being induced exclusively in response to wounding or to pathogen attack like *Uncinula necator*, *Phomopsis viticola* (Monteiro *et al*., 2003). Although the specific function of many PR5 in plants is unknown, they are involved in the Acquired Systemic Resistance and in response to biotic stress, causing the 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). Linusitin is a 25-kDa Thaumatin-Llike Protein isolated from flax seeds. Linustin shows antifungal activity against *Alternaria alternata* by the mechanism of membrane permeabilization. Concentration of protein and lipid and composition of cell wall of fungi play a major role in these mechanisms (Anzlovar *et al*., 1998). In one study by Menu-Bouaouiche *et al*., (2003), Thaumatin-like proteins were isolated from cherry, apple and banana shows antifungal activity against *Verticillium albo-atrum* and having endo- â1,3-glucanase activity.

Glycine-Histidine Rich Protein

Many insects like holotrichin and flesh fly synthesized some Glycine-Histidine Rich Antifungal Proteins. The mode of action of this protein is not understood completely. Phenoloxidase Interacting Protein (POIP) isolated from *Tenebrio molitor* (Tenecin) interacts with phenoloxidase (Yoo *et al*., 2001) and inhibits some fungi like *Candida albicans* and *Saccharomyces cerevisiae* (Kim *et al*., 2001) and bacteria like *Bacillus subtilis*, *Proteus vulgaris* and *Streptococcus aureus* (Kim *et al*., 2001).

Ribosome Inactivating Protein (RIP, PR10)

RIP has an inherent antifungal activity. It has been isolated from *Arachis hypogaea* L. (peanut), *Mirabilis expansa* (mauka) (Vivanco *et al*., 1999), *Nicotiana tabacum* (tobacco) (Kim *et al*., 2001), *Pisum sativum* (pea) (Ye *et al*., 2000), *Solanum surattense* (nightshade) and *Volvariella volvacea*

(mushroom) (Lam and Ng, 2001) having molecular mass of around 30 kDa. Numerous RIPs have been identified but among them some have antifungal activity. RIP isolated from tobacco, termed as TRIP, releases adenine residues from the ribosomal and non-ribosomal substrata. this is the probable mode of action of inhibition of translation in many fungi like *Cytospora cankar*, *Fusarium oxysporum*, Pestalotia sp. and *Trichoderma reesei* and bacteria like *Ervinia amylovora*, *Pseudomonas solancearum*, *Rhizobium leguminosarum*, *Salmonella typhimurium*, *Shigella asonei* (Kim *et al*., 2001). Some of these RIP also inhibit the reverse transcriptase of human immunedeficiency virus (HIV) -1 with an IC_{50} of about 5.2 nm (Lam and Ng, 2001).

Other Proteins

Theis *et al*, 2003 investigated the inhibitory effects of the antifungal protein (AFP) from *Aspergillus giganteus*. AFP is a highly basic (pI 8.8) polypeptide of 51 amino acids with a high content of cysteine, tyrosine, and lysine residues. MICs of AFP were determined and ranged from 0.1 µg/mL against *Fusarium oxysporum* to 200 µg/mL against *Aspergillus nidulans*. They also showed that the growth inhibitory effect of the AFP is caused by permebealization of the fungal membranes by using an assay based on the uptake of the fluorescent dye SYTOX Green. Pozo *et al*., 2002 also found the same AFP protein from the *Aspergillus giganteus*, it promotes charge neutralization and condensation of DNA as demonstrated by electrophoretic mobility shift and ethidium bromide displacement assays. Hagen *et al*., 2007 found AFP can inhibit the chitin synthesis by the *In situ* chitin synthase activity assays. These three results indicate that AFP causes cell wall stress and disturbs cell integrity by inactivating chitin synthase that results in membrane permeability.

Conclusion

PR proteins play important role in disease resistance, seed germination and also help the plant to adapt to the environmental stress. The increasing knowledge about the PR proteins gives better idea regarding the development and defense system of plants. 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 open new ways for the plant genetic engineering technology for crop improvement.

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