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Analytical Description and Future Prospects of Plant Antifungal Proteins

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Abstract
The fungal infection of crop plants causes yield losses which resulted in financial problems. Chemical fungicides cause environmental crisis and retains in food chain. The present article reviews various potential proteins with antifungal activity, which could lead to provide valuable gene pool for the development of transgenic plants that are resistant to fungal diseases. Most of the antifungal proteins are categorized and placed in different groups but some are still remained uncategorized and studied separately. This review is focused on types of plant antifungal proteins, their mechanisms of action, parameters and their potential applications for developing antifungal plants for future agricultural applications.

I. Introduction

The fungal infection to crop plants is one of the major causes of yield losses (Agrios, 2004). At present the most common method, used for controlling the damage and loss is application of agrochemicals. These fungicides are made up of either organic or inorganic chemicals, which by long use accumulate and persist in soil and become health hazards to both animals and human beings. Currently, much attention is being paid to identify and isolate genes that upon transfer could render target plants resistant to fungal diseases. It is well known that plants often respond to pathogen attack or to other environmental attack by synthesizing a group of proteins, commonly called "Pathogenesis Related Proteins" (PR-Proteins). These PR-Proteins include β-1,3 glucanases, chitinases, thaumatin-like proteins, protease inhibitors and many other antifungal proteins. In view of the importance of PR-Proteins in controlling the fungal diseases *in situ* in crop plants, the present article reviews various potential PR-Proteins which could lead to provide valuable gene pool for the development of transgenic plants that are resistant to fungal diseases. Besides PR-Proteins, many other antifungal proteins are also reported in different plants. These proteins are constitutively present in plants to strengthen the defence system of plants against many microbial diseases (Xie et al, 2016; Li et al, 2021; Chiu et al,2022). To develop plants resistant to fungal pathogens, plants have to be engineered to constitutively express one or more antifungal proteins.

II. PR-1 Proteins

Group 1 of pathogenesis-related (PR) proteins of tobacco contains three serologically related acidic proteins, PR-1a, PR-1b and PR-1c. Molecular weights of these proteins are approximately 15KD (Van Loon et al., 1987). cDNA (Cornelissen et al., 1986; Pfitzner and Goodman,1987; Cutt et al.,1988) and genomic clones (Cornelissen et al.,1987; Payne *et al*.,1988a; Oshima *et al*.,1990a,1990b) encoding these proteins have been isolated. Acidic PR-1 proteins accumulate extracellularly, as well as they are also located in calcium-oxalate containing vacuoles of specialized leaf cells called crystal idioblasts when tobacco leaves are infected with TMV (Dixon *et al.,*1991). Acidic PR-1 proteins are also strongly induced by spraying plants with salicylic acid solution (Hooft van Huijsduijnen *et al*., 1986). A basic protein of 14 KD, named p14 accumulates in tomato leaves, after infection with the fungus *Cladosporium fulvum* (De Wit and Vander Meer, 1986). P14 protein in tomato leaves also appeared when plants are infected with *Citrus exocortic* viroid (Vera and Conejero,1989). P14 is serologically related to PR-1 proteins (Nassuth and Sanger, 1986). A basic PR-1 protein having antifungal activity against rust fungus *Uromyces fabae* hyphae, was isolated from broad bean (*Vicia fabae*) leaves when treated with salicyclic acid or 2,6-dichloro-isonicotinic acid (DCINA), which induces resistance against the rust fungus (Rauscher *et al*.,1999).
Proteins serologically related to tobacco PR-1 proteins were detected in other plants also such as cowpea, potato, maize and barley (Nassuth and Sanger, 1986; White *et al*.,1987; Casacuberta *et al*.,1991).

III. Antifungal Proteins with Enzymatic Activity

In addition the above proteins , pathogen targeted hydrolytic enzymes are also synthesized in vegetative tissues of many plants(Kaufmann *et al*.,1987; Legrand *et al*.,1987; Lamb *et al*., 1993).These hydrolytic enzymes digest the fungal cell wall and protect the plant from infection (Broekaert et al.,1988:Schickler and Chet,

1997:Seetharaman, 1997).These hydrolases are β-1,3-glucanases and chitnases which are pathogen-related (PR) proteins of group-2 (PR-2) and group 3 (PR-3), respectively (Van de Rhee *et al.*,1984).

3.1 β-1,3-glucanase: PR-2 Proteins

β-1,3-glucanases are found in many plant species. The substrate β-1,3-glucan is found in the cell walls of several plant cell types (Fincher and Stone, 1981) and is a major constituent of the cell walls of many fungi (Wessels and Sietsma,1981). Thats why pathogen induced β-1,3-glucanases are thought to play a role in direct defence against pathogenic fungi (Godoy,1996; Youn and Hawang,1996). It has been also suggested that β-1,3-glucanases might function indirectly in inducing resistance by releasing oligosaccharide components from invading fungi which act as elicitors for the induction of the defense mechanisms of the plant (Boller and Metraux, 1988; Lamb *et al*.,1989; Mauch and Staehelin, 1989; Takeuchi *et al*., 1990). The pl8.4 isoform, Gb 2 of beta -1 3-glucanase constitutively present in hypocotyls and leaves of soyabeans but this form of glucanase completely disappeared after mercuric chloride treatment. Interestingly mercuric chloride treatment and *Phytophthora infestans f.sp.glycinea* infection led to the accumulation of distinct isoform of chitinase in soyabean tissues (Youn and Hwang,1996). A basic β-1,3-glucanase was isolated from resistant cultivar of pepper (*Capsicum annuum*) when inoculated with Phytophthora capsica (Egea *et al*., 1999). Five extracellular acidic β-1,3-glucanases named PR-2a (PR-2), PR-2b(PR-N), PR-2c(PR-O), PR-O´ and PR-Q^1were isolated from TMV- infected tobacco (Jamet and Fritig *et al*., 1986; Pierpoint, 1986; Vanloon *et al*., 1987; Fritig *et al.1989*). Acidic β-1,3-glucanases are also induced by salicylic acid treatment (Van den Bulcke *et al*., 1989, Bol *et al*., 1990). This basic β-1,3-glucanase can also be induced in tobacco leaves by treating with ethylene (Ohme- Takagi and Shinshi, 1990; Keefe *et al*.,1990) and pathogen attack (Kauffmann *et al*;1987 Vogeli-Lange *et al*., 1988; Meins and Ahl,1989). This enzyme was also isolated from potato after infection with *Fusarium solani* by Godoy *et al*., 1996, which showed in vitro antifungal activity. cDNA clones and genomic clones encoding β-1,3-glucanases from tobacco have isolated and characterized by several workers.

Characterization of genomic clones encoding acidic and basic beta-1, 3-glucanases showed that these genes have an intron at the end of the signal peptide sequence (Linthorst *et al*., 1990b; Ohme-Takagi and Shinshi,1990, Sperisen *et al.,*1991). The basic β-1,3-glucanase genes contain a C-terminal extension of 22 amino acids. The genomic clone for an acidic beta 1, 3-gucanase, corresponds to PR-2b, characterized by Linthorst *et al*.,1990 b. cDNA clones corresponding to PR-2a, Pr-2b, Pr-2c and Pr-Q^1 have been isolated (Linthorst *et al*.,1990 b; Payne *et al*.,1990a; Cote *et al*., 1991; Ward *et al*., 1991). PR-Q^1 was found to differ from PR-2a, b and c.

There seems to be a cooperative action between 1, 3 β-glucanase and 1,3-glucan synthase (callose synthase). The later enzyme is a plasma membrane localized wound-induced enzyme resulting in the rapid synthesis of callose which is then deposited at the wound site to prevent infection from spreading to other parts of the plant. This enzyme was purified to homogeneity from peanut seedlings (Kamath *et al*., 1997). It was also observed that there was significant increase in the level of extracellular endoglucanases supporting the earlier observations that endoglucanases may also be part of the defense mechanism which the plants use for protecting themselves against injury and thereby supplementing the action of 1, 3-β glucan synthase. It may also be pointed out that the wound induced proteins are thought to be involved in the plant defense because their transcripts are expressed after tissue damage and their sequences are related to proteins with defensive roles (Clarke *et al*., 1998)

3.2 Chitinases: PR-3 Proteins

Another family of PR proteins with enzymatic function is of chitinases of group 3 (PR-3). Chitin, the substrate of chitinases does not occur in plant cell walls, but it is present in cell walls of a number of phytopathogenic fungi (Wessels and Sietsma,1981). Fungal growth can be inhibited *in vitro* by chitinase (Schlumbaum *et al*., 1986). Broekaert *et al.,*1988, isolated chitinases from leaves of thorn apple (*Datura stramonium*), tobacco and from wheat embryos. All three chitinases exhibited enzymatic antifungal activities against *Trichoderma hamatum* and *Phycomyces blakesleeanus*. Chitinase activity was observed in protein fractions of cytoplasmic or exocellular origin, from roots, hypocotyls, cotyledons and leaves of healthy white lupin plants and six different isoforms were visualised (Burzynski et al., 2000).

Three chitinases C1, C3 and C4 were isolated from in vitro grown chestnut plantlets. These chitinases inhibit the growth of *Cryphonectria parasitica* fungus *in vitro.* Nucleotide sequence analysis of the proteins suggested that C3 was a class I basic chitinase (Vannini *et al*., 1999). Somatin and chitinase appeared to be an active part of the defense mechanism of the sorghum caryopsis against grain mould, *Fusarium moniliforme* (Bueso *et al.,* 2000). Chitinase A isoenzyme (Molecular weight 28) was purified from the peel of yam (*Dioscorea opposita*) tubers by Arakane and Koga, 1999. Constitutive chitinase activity was detected in the culture filterate and enzyme extract of cells from a 6-day old rice cell culture, the amount of chitinase activity increased markedly in both the culture filterate and cell extracts after treatment of the culture with chitin. Therefore, soluble chitin fragments released from fungal cell walls through the action of constitutive rice chitinases can serve as biotic elicitors of defense-related responses in rice (Ren *et al.*, 1992). Chitin, chitosan and their oligomers are able to act

as elicitors which induce enhanced levels of chitinases in various plants. Lectins, which bind to N-acetyl-D-glucosamine strongly interfere with fungal chitin synthases and may be involved in plant pathogen interaction (Cohen 1993).

Chitinase and lysozyme which are capable of degrading the chitin component of fungal cell walls, were found in specialize vacuoles i.e. lutoids in latex of the commercial rubber tree. Acidic chitinase mol. mass 25.5 KD was purified (Martin, 1991). A basic chitinase having a molecular mass of approximately 32 KD was purified from *Arabidopsis thaliana,* which was synthesised in response to pathogen invasion and abiotic stress. The purified protein is an effective inhibitor of the growth of *Trichoderma reesei in vitro* (Verburg and Huynh, 1991).

Plant chitinases have been divided into three classes. Class I consists of the basic chitinases, containing hevein domain. Class II acidic chitinases lack the hevein domain but are otherwise similar to class I, like tobacco PR-3a and b, petunia chitinase (Linthorst *et al*., 1990c) and a barley seed chitinase (Leah *et al*., 1991) and acidic chitinase of potato (Buchter *et al*., 1997). Class III chitinases are lysozyme chitinases with conserved sequences different from Class I and Class II found in tobacco, cucumber, *Arabidopsis thaliana* and the rubber tree (Boller and Metraux, 1988; Samac *et al.*, 1990; Martin,1991; Lawton *et al*., 1992).

Nobrega *et. al.* (2005) isolated antimicrobial proteins from cowpea root exudates with inhibitory activity against *Fusarium oxysporum* and also purified a chitinase-like protein. Roots from cowpea seedlings contained β-1,3-Glucanases, chitinases and lipid transfer proteins (LTPs), all of which may potentially function as plant defence proteins. Immunolocalization of one of these proteins, chitinase, revealed its presence in the xylem cell wall vessel elements. These exudates also demonstrated an inhibitory effect on the growth of the fungus, *Fusarium oxysporum*, *in vitro*.

3.3 Comparative Study of Hydrolases of Different Classes

It was seen that class I, vacuolar chitinase and beta-1,3-glucanase isoforms were the most active against pathogenic fungus *Fusarium solani* germlings resulting in lysis of the hyphal tips and in growth inhibition. The class II isoforms of the two hydrolases exhibited no antifungal activity (Sela Buurlage *et al*., 1993). Whereas class II hydrolases, which are very homologous to class I hydrolases but which are localized extracellularly, are not antifungal, either alone or in combination with other proteins (Melchers, 1993). But exception is there, a basic chitinase Ch1 of molecular mass of 25 KD is a class II exochitinase was isolated from Castanea sativa Mill. Cotyledons. Ch1 has no cystine-rich hevein domain. But Ch1 inhibits the growth of the fungus *Trichoderma viride* (Collada *et al*., 1992).

Rice Chitinases are encoded by a small multigene family. These genes cht-1, cht-2 and cht-3 were isolated and characterized. All the three genes encode class I chitinase, but only cht-2 has a 130 bp intron and encodes a C-terminal peptide sequence similar to that known to function as a vacuolar targeting signal (Nishizawa *et al*., 1993). In tobacco vacuolar, basic chitinases differ from the homologous extracellular, acidic isoforms by the presence of a C-terminal extension. So, the C-terminal extension of tobacco chitinase- A is necessary and sufficient for the vacuolar localization of chitinases and therefore it comprises a targeting signal for plant vacuoles.

The vacuolar chitinases of class-I possess an N-terminal cysteine-rich domain. This N terminal domain is a chitin-binding domain was proved by Iseli et al., 1993. It was also seen that the both forms of basic chitinase-A of tobacco i.e. with and without N-terminal domain were capable of inhibiting the growth of *Trichoderma viride*, although the form with CB was about three times more effective than the one without it. Thus, the chitin binding N-terminal domain is not necessary for catalytic or antifungal activity of Chitinase (Iseli *et al*.,1993).

It was seen by Sticher *et al.*, 1992 that Type I chitinases are vacuolar enzymes and are not glycosylated and contain a small number of hydroxyproline residues restricted to a single, short peptide sequence. Although class I and class II chitinases are classified on the basis of domain structure and C-terminal extension sequences, it was seen by Araki et al., 1995 that class I chitinases are of high molecular weight and class II chitinases are of low molecular weight and the high molecular weight subclass i.e. class I chitinases are ancestral molecule in phylogenetic tree.

Simultaneous expression of a tobacco class I chitinase and a class I β1, 3-glucanase gene in transgenic tomato resulted in increased fungal resistance whereas transgenic tomato plants expressing either one of these genes were not protected against fungal (*Fusarium oxysporum*) infection. So, it is resulted that class I chitinases and class I beta 1, 3-glucanases act synergistically to inhibit the growth of fungi invitro (Jongedijk *et al.,*1995). Thus, the combination of a class I chitinase and a class I ,3-β-glucanase, which individually show little effect, exhibit a strong antifungal activity (Leah et al.,1991; Melchers *et al*;1993). Transgenic chestnut (Castanea sativa Mill.) plants with genes comprising lytic enzyme codes (glucanases and chitinases) were produced by Seabra and Pais, 1999, the presence of these genes leads to the degradation of fungal cell walls.

IV. PR-4 Proteins

Two to four almost neutral extracellular proteins with a molecular mass of 13-14.5 KD were detected in TMV-infected tobacco, which were placed in the PR-4 group (Van de Rhee *et al*., 1994). Whereas Pierpoint (1986) isolated a PR-protein from tobacco cultivar Xanthi-nc and designated as R^1. A basic, PR-4 type protein named P2

was purified from tomato leaves inoculated with *Cladosporium fulvum* (Joosten, *et al*.,1990). cDNA clones of tobacco and tomato PR-4 proteins were isolated using partial amino acid sequences from P2 (Linthorst *et al*.,1991). Two 24 KD proteins, named AP24 were purified from TMV-infected tobacco and *Phytophthora infestans* -infected tomato which inhibit the pathogenic fungus P infestans in vitro. These AP24 are Thaumatin like proteins of group PR-4 and cause lysis of fungal sporangia and inhibition of hyphal growth (Woloshuk *et al*.,1991).

A novel pathogen – and wound-inducible antifungal protein CBP 20 of 20 KD was purified from *Nicotiana tobacum* leaves inoculated with TMV, which exhibits antifungal activity against *Trichoderma viride* and *Fusarium solani* by causing lysis of the germ tube and /or growth inhibition. CBP 20 locate intracellularly and is the class I PR-4 type Protein (Ponstein *et al*.,1994).

V. Thaumatin-like Proteins: PR-5

Thaumatin-like proteins (TLP) were kept in PR-5 family. This name was given to these proteins, due to their structural similarity to thaumatin, a sweet-tasting, non-toxic protein that was first discovered from the fruit of the tropical plant *Thaumatococcus daniellii* (van der Wel & Loeve,1972). TLPs exhibit a broad range of biological activities, including antifungal activity. Different TLPs inhibit fungal growth through different mechanisms. They disrupt fungal membrane (Vigers, *et al., 1992)*., inhibiting fungal enzymes such as xylanase (Fierens, *et al.* 2007) and induce apoptosis by binding to specific fungal membrane receptors and hydrolyze β-1,3-glucans (Grenier *et al*. 1999). Osmotin and osmotin-like proteins are among the most studied TLPs having antifungal activity (Hakim, *et al.,* 2018)

. Osmotin and orthologs have been shown to exhibit broad-spectrum antifungal inhibitory effects (Lee *et al*.,2010). Osmotin isolated from tobacco cell suspensions can inhibit the hyphal growth of numerous pathogenic fungi *in vitro*, including species from *Bipolaris*, *Collectorichum, Fusarium*, *Kabatiella, Phytophthora*, and *Trichoderma (*Abad, *et al.* 1996*)*. Overexpression of osmotin in transgenic plants delayed disease symptoms from fungal pathogens (Bashir, *et al.,2020)*. Another osmotin-like protein from *Solanum nigerum* can inhibit the growth of phytopathogenic *Macrophomina phaseolina, Fusarium solani f. sp. glycines*, *Collectrichum glaesporioides* and *Collectrichum gossypii* var. *cephalosporioides* at the concentration between 0.1 µg/µL to 0.3 µg/µL (de A Campos, *et al. 2008)*

. Additionally, an osmotin-like protein from *Solanum nigrum* L. var *indica* was shown to inhibit fungal spore germination and permeabilize fungal hyphae *in vitro*. This protein is also stable and retains its antifungal activity at temperatures as high as 75 °C for 30 min and pH 3–8 (Chowdhury *et al*.2015).

VI. Protease Inhibitors: PR-6

Plant protease inhibitors (PIs) were named as PR-6. These are important proteins involved in many plant biological processes, including seed germination, protease-related house-keeping functions and defence against biotic and abiotic stresses (Rustgi et al., 2018). PIs are normally found in ample quantities in seeds and tubers. Plants in the Solanaceae family generally have exceptionally high levels of PIs (Platé *et al*., 1993) some of them have antifungal activity. For instance, potatoes encode several PIs ranging from 4.1 to 39 kDa that exhibit broad-spectrum antifungal activities (Bártová et al, 2019). Potide-G, a Kunitz-type (Kunitz soybean trypsin inhibitor is a type of protein contained in legume seeds which functions as a protease inhibitor) PI isolated from potato tubers. This protein is of size 5.5 kDA and inhibits pathogenic fungi *Candida albicans* and *Rhizoctania solani in vitro* even when heated to 70 °C for 20 min and also exhibits antiviral and antibacterial activities (Kim *et al.,2006)*. Similarly, the potato protease inhibitors I and II (PPI–I and PPI-II) can inhibit the growth of various fungi, including *B. cinerea (*Hermosa et al. 2006), *Fusarium solani* and *Fusarium oxysporum* (Bártová et al, 2019) . *Both PPI-I and II are heat stable, which can* maintain their ability to inhibit *F. solani* and *F. oxysporum* growth *in vitro* under temperature as high as 100 °C (Bártová *et al.* 2018). Komarnytsky *et al*., 2011 found that the extraction of bioactive PPIs from potatoes is laborious and of low yields. They have also been heterologously expressed in *Saccharomyces cerevisiae,* yet the antifungal activity of the purified protein was not examined (Fischer *et al*.,2015).

Another PI of interest is the Bowman-Birk protease inhibitor (BBI), which is typically under 20 kDA (Qi *et al*., 2005; Gitlin-Domagalska *et al*., 2020). It contains seven conserved disulfide bonds, and inhibits trypsin and chymotrypsin, which are common enzymes and pathogenic fungi utilize them, when infecting plants (Singh and Rao, 2002). The BBI gene is induced during plant immune responses and overexpression of this gene in plants confers improved disease resistance against fungal pathogens (Grosse-Holz *et al*.,2016). BBIs from the legume (Fabaceae) or cereal (*Poaceae*) family have a double or single inhibitory loop respectively (James *et al.,* 2017)

and synthetic peptides that contain only the disulfide-linked, 9-residue long loop have shown to retain their trypsin and chymotrypsin inhibitory activity (Inhibitor *et al*., 1977). This short, truncated form of the protein may be of interest for the development of antifungal agents of smaller molecular weight for easier production and higher stability, compared with larger protein agents. Aside from the small size, BBI is thermostable with the

ability to withstand 100 °C for 10 min, tolerates a wide pH range from 1.6 to 8.0, is not allergenic, and is approved by the FDA for human consumption (Losso,2008). Additionally, unlike some other candidates to be engineered as antifungal agent, BBI has passed phase II human clinical trials and is highly unlikely to be toxic, especially given its prevalence in soy products. BBIs have already been successfully utilized as an exogenously applied antifungal agent *in vitro*. One study identified that a BBI-type trypsin-chymotrypsin inhibitor purified from broad beans can inhibit the growth of *B. cinerea, F. oxysporum,* and *M. arachidicola* at a dose as low as 60 µg per plate (Ye et al.,2001). Plant BBIs have often been isolated from a variety of seeds such as those from *Vigna mungo (*Prasad et al., 2010 a), *Cajanus cajan* Prasad et al., 2010b)*, and* Clitoria *fairchildiana* (Dantzger, *et al.* 2015) and have been tested for their insecticidal properties. Rice BBI has also been expressed in *E. coli* and retained the inhibitory activity. However, the titer is relatively low at 20 mg/L, likely due to the presence of the disulfide bonds that make it prone to forming inclusion bodies (Li *et al.,1999)*. In addition, care should be taken when developing BBI as an antifungal agent, as it is a multifunctional PI with a relatively broad activity towards various proteases (Farady and Craik, 2010), and may affect beneficial microbiota and fungi in the soil and plants.

VII. Plant Defensins: PR-12

Plant defensins are cysteine – rich peptides which inhibit the growth of a broad range of fungi at micromolar concentrations (Broekaert *et al*.,1995). Antifungal activity assays of these defensins were carried out with *Alternaria brassicola, Ascochyta pisi, Botrytis cinerea, Fusarium culmorum* and *Verticillium dahliae* (Samblanx et al., 1996). Rs. -AFP1 and Rs-AFP-2 antifungal defensins were isolated from radish seeds by Terras et al., 1992, 1993. Even transgenic tomato cv Belyi Naliv carrying defensin gene Rs-AFP2 from radish seeds was developed by Parashina *et al*. 1999 using vector pH 22Kneo. The transgenic plants thus obtained displayed stronger resistance to *Alternaria solani* infection than untransformed control plants (Parashina *et al*., 1999). Cystatin from chestnut also contributes to plant defense against plant pathogenic fungi. Purified chestnut cystatin inhibited the growth of *Botrytis cinerea*, *Colletotrichum graminicola* and *Septoria nodorum*, but not that of the saprophyte *Trichoderma viride* (Pernas *et al*., 1999). A basic antifungal defensin having a signal peptide of 47 amino acids with a molecular mass of 5300KD was also isolated from *Helianthus annuus* (Sunflower) flowers by Urdangarin *et al*., 2000.

VIII. Non-Specific Lipid Transfer Proteins: PR-14

Three proteins IWF 1 and IWF 2(Nielsen et al.,1996) and IWF 4 (Nielsen et al.,1997) were isolated from the intercellular washing fluid (IWF) of sugarbeet leaves. These proteins showed antifungal activity against *Cercospora beticola*, the causal agent of leaf spot disease in sugarbeet. These proteins are related to the family of plant non-specific lipid transfer proteins (ns LTPs). IWF1 and IWF 2 are basic monomeric proteins of 91 amino acid residues, 89 of which are identicals. On the other hand, IWF 4 has 30 amino-acids residues and is highly basic (Nielsen *et al*., 1996, 1997) in nature. Xiaochun Ge *et. al.* (2003) also studied the antifungal activity of a rice lipid transfer protein.

An Antifungal Protein is Purified from Pearl Millet Seeds Shows Sequence Homology to Lipid Transfer Proteins and this protein has a molecular mass of 25 kDa. The N-terminal sequence of the protein (25 residues) shows homology to non-specific lipid transfer proteins (LTPs) of cotton, wheat and barley. The purified LTP inhibited mycelial growth of Trichoderma viride and the rice sheath blight fungus, *Rhizoctonia solani* in vitro (Velazhahan *et. al*. 2001).

IX. Lectins (carbohydrate-binding proteins)

Lectins are carbohydrate-binding proteins that bind glycans of glycoproteins, glycolipids or polysaccharides with high affinity (Goldstein and Hayes ,1978). Most of the plant lectins are secretory proteins which are accumulated either in vacuoles or in the cell wall and intercellular spaces. When fungal hyphae grow into plant tissues, they may disrupt cellular compartmentation, causing the release of vacuolar lectins that may inhibit further hyphae growth (Chrispeels and Ralkjel,1991). Lectins with antifungal activity have a chitin binding domain of 43 amino acids as part of their structures (Chrispeels and Ralkjel,1991) and chitin is present in cell walls of many plants pathogenic fungi (Wessels and Sietsma,1981). This chitin binding domain is found in wheat germ agglutinin (WGA), the lectin of wheat (*Triticum aestivum*) and in the homologous lectins from barley and rice and also in nettle lectin. The C-terminal propeptide is necessary for correct sorting of these lectins to the vacuole (Broekaert *et al.,* 1990). WGA and barley lectin are 36 KD-dimers composed of two identical 18 KD subunits. In cultivated rice species the majority of the 18 KD-subunits cleaved into two subunits of 8 KD and 10 KD (Stinissen *et al*.,1984). The mature proteins of all Gramineae lectins have four homologous domains of 43 amino acids and there is about 50% amnio acid sequence identity between these cereal lectin domains and hevein (rubber plant antifungal proteins) so these domains have become known as hevein domain. Mirelman et al., 1975 proposed that WGA plays a role in defense of seedlings against fungal attack. They observed that WGA inhibits spore germination and hyphal growth of *Trichoderma viride.*

Small lectin UDA, Urtica dioica agglutinin or nettle lectin abundantly present in the rhizomes of the stinging nettle (Peumans *et al*.,1983) also have a chitin binding domain. UDA has been shown to have strong antifungal properties against several chitin-producing fungi (Broekaert *et al*., 1989). With some fungi (Botrytis cinerea) UDA is more effective than chitinase, whereas with other fungi (*Trichoderma hematum)* the reverse is true (Broekaert *et al*., 1989) Protein and cDNA sequencing have shown that mature UDA is composed of two hevein domains and is processed from a precursor protein. The precursor contains a signal peptide, two in tandem hevein domains, a hinge region and a carboxyl-terminal chitinase domain. It was seen that UDA is encoded by a large gene family (Does *et al*.,1999). A novel lectin with antifungal and antiproliferative activities was isolated from *Sophora alopecuroides* seeds by Li *et al*. (2012). Das Graças *et al*. (2002), isolated and partial characterized a novel lectin from *Talisia esculenta* seeds that interferes with fungal growth. M. Ghosh (2009), purified a lectin-like antifungal protein from the medicinal herb, *Withania somnifera*.

X. Vicilin Derived Proteins

Many antimicrobial peptides (AMPs) have been identified in plants; Vicilin Derived Proteins are one of them. These peptides are highly divergent at the primary sequence and have the ability to form disulfide bonds, tandemly repeated amino acid sequences and a net charge at pH 7.

Various vicilin-derived proteins of basic nature were isolated from cotton and sequenced. These are 9-11 kD and 46.3 KD Proteins and exhibit selective growth inhibitory activity in vitro against the filamentous fungi *Botrytis cinerea, Alternaria brassicola, Chalara elegans* and *Fusarium oxysporum* (Chung et al.,1997). Xie et al., 2016 identified a Cys repeat within a vicilin (seed storage protein) of a wild legume, *Centrosema virginianum*. Cleavage of the vicilin protein during germination would generate a vicilin derived Cys peptide (VDCP and it generate a vicilin derived Cys peptide (VDCP) during seed germination. Transgenic tobacco plants that expressed cloned sequences encoding the Cysteine repeat unit from *C. virginianum*, *Theobroma cacao* and *Gossypium hirsutum were developed by them*. Extracts from fully expanded leaves were tested for antimicrobial activity against a fungal pathogen, *Botrytis cinerea (*Xie *et al*., 2016).

XI. Ribosome Inactivating Proteins (RIPS)

The plant RIPs inhibit protein synthesis in target cells by specific modification of 28 s rRNA, inhibiting elongation step in protein synthesis (Stripe *et al*., 1992). RIPs are specific towards ribosomes of other plants and do not affect the ribosomes of plants in which they are produced (Cornelissen and Melchers,1993). At least 34 fungal species are sensitive to some RIPs or RIP-Like Proteins. This includes a wide variety of species belonging to various families of basidiomycetes and ascomycetes which includes *Phytophthora infestans, Trichoderma reesei, Fusarium sporotrichioides, Botrytis cinerea etc.* However, about 16 fungal species have shown resistance to this RIP when tested on agar plates, including *Phycomyces blakesleeanus*, *Alternaria alternariae* and *Neurospora crassa.* Transgenic plants have been designed that carry the gene for a RIP and are resistant to pathogenic fungi plants have also been obtained from some important crops such as wheat (*Triticum aestivum* L.), potato (*Solanum tuberosum* L.), Indian mustard (*Brassica juncea* (L.) Czern.), black gram (*Vigna mungo* (L.) Hepper), maize (*Zea mays* L.), rice (*Oryza sativa* L.), or creeping bentgrass (*Agrostis stolonifera* L.), (Iglesias, 2024).

XII. DUF26-Containing Proteins

Signal transduction mechanisms are present in plants as well as in other eukaryotes. They are involved in the regulation of cell functions, the cell–cell coordination system and the exchange of information between cells and the environment. Proteins with extracellular domains and large gene families encoding secretory proteins in plants play an important role in sensing environmental changes and development through signal transduction mechanisms. The DUF26 domain (PF01657) belongs to the extracellular domain. Its core contains a conserved cysteine motif (C-8X-C-2X-C), which exists in three plant proteins. The first is a cysteine rich receptor like secreted protein (CRRSP). The most typical CRRSP is Gnk2 from *Ginkgo biloba* leaves, which has an antifungal activity as mannose binding lectin in vitro. Two maize CRRSPs have also been shown to bind mannose and participate in the defense against fungal pathogens. The second is a cysteine rich receptor kinase (CRK) that has a typical DUF26 structure in the extracellular region, forming a large RLK subgroup in plants and playing a role in the response of Arabidopsis to stress. The third DUF26 domain-containing protein is plasmodesmata localized protein (PDLP). PDLP contains two DUF26 domains and a transmembrane helix in its extracellular domain, but lacks a kinase domain. They are related to plasmodesmata and participate in intercellular signal transduction, pathogen response, systemic signal transduction, and callose deposition control. However, the specific biochemical function of the DUF26 domain-containing gene in plants is still unclear (Huang *et al*., 2022).

XIII. Other Proteins

Gastrodia antifungal protein I (GAFP-I) was purified from corms of the orchid (*Gastrodia elata*) which plays an important role in the defence mechanism of plants in restricting infection by *Armillaria mellea*. GAFP-I has molecular weight of 13958 Da, and is a single polypeptide of 129 amino acids, rich in Asn(19) and Gly(14),with a disulfide bridge between the 29th and 52nd amino acids. The N-terminal 7 amino acid sequence was used to synthesize a degenerate primer. mRNA from Gastrodia corm was amplified to give a specific cDNA of about 600 bp through a 3'-RACE experiment with the degenerate primer. The sequence analysis of cDNA contained a 429 bp region encoding GAFP-1 consisting of 129 amino acids and a C-terminal extending peptide of 14 amino acids. The determined amino acid sequence was 98.4% identical to the one deducted from the nucleotide sequence of the cDNA (Hu *et al*.,1999) The cDNA which encodes the antifungal protein KP4 was isolated from *Ustilago maydis*-infecting virus. Then this cDNA was inserted behind the ubiquitin promoter of maize and genetically transferred to wheat varieties particularly susceptible to stinking smut (*Tilletia tritici*) disease. The transgene was integrated and inherited over several generations of seven transgenic lines, three showed antifungal activity against U. maydis due to the presence of KP4-transgene. KP4-transgenic, soil grown wheat plants exhibit increased endogenous resistance against stinking smut (Clausen *et al*.,2000). Ye *et. al*. (2002) isolated Cicerin and arietin, two novel peptides from chickpea with different antifungal potencies.

Jeenkeawpieam *et. al*., (2020) examined the antifungal activity of AFPs isolated from a Thai medicinal plant, *Rhinacanthus nasutus,* against the human pathogenic fungus *Talaromyces marneffei*. This dimorphic fungus causes systemic infections in immunocompromised individuals and is endemic in Southeast Asian countries. The *R. nasutus* crude protein extract inhibited the growth of *T. marneffei*. The anti-*T. marneffei* activity was completely lost when treated with proteinase K and pepsin, indicating that the antifungal activity was dependent on a protein component.

XIV. Conclusion

Plants often respond to pathogen attack or to other environmental stresses by synthesizing a group of proteins which constitute the important component of the plant defence system against the pathogen attack. These proteins include plant defensins which are cysteine-rich peptides, vicilin derived proteins, lectins having chitin-binding domain, PR-1 and PR-4 proteins of tobacco, ribosome inactivating proteins (RIPs) that inhibit the elongation step of proteins synthesis, non-specific lipid transfer proteins and beta-1,3-glucanse and chitinase. The PR-proteins exhibit varying degree of bio-specificity towards pathogens. In general beta 1, 3-glucanase and chitinase act synergistically. Thaumatin-like proteins (TLP) are in PR-5 family and they show antifungal activity. Different TLPs inhibit fungal growth through different mechanisms. Plant protease inhibitors (PIs) were named as PR-6 are important proteins involved in many plant biological processes and also have antifungal characteristic feature. The DUF26 domain (PF01657) containing plant proteins has an antifungal activity as found in *Ginkgo biloba* leaves**.** Many other antifungal proteins are also found in different plants. Bio-chemical and molecular characterization of antifungal proteins would provide useful information for the development of transgenic plants resistant to diseases caused by the pathogenic fungi.

REFERENCES

[1]. Abad L.R., Matilde Paino D'Urzo, Dong Liu, Meena L. Narasimhan, Moshe Reuveni, Jian Kang Zhu, Xiaomu Niu, Narendra K. Singh, Paul M. Hasegawa, Ray A. Bressan (1996), "Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization" Plant Sci, 118 pp. 11-23 https://cris.haifa.ac.il/en/publications/antifungal-activity-of-tobacco-osmotin-has-specificity-and-involv

[2]. Agrios G. (2004), Plant pathology: fifth edition pp. 1-922 ,9780080473789

[3]. Arakane Y. and Koga D. (1999) Purification and Characterization of a Novel Chitinase Isozyme from Yam Tuber. Bioscience, Biotechnology and Biochemistry, 63 (1): 1895-1901

[4]. Araki T. and Torikata T. (1995) Structural classification of plant chitinases: Two subclasses in class l and class II chitinases. Bioscience, Biotechnology and Biochemistry, **59 (2)** :336-338.

[5]. Bártová V., Bárta J., Vlačihová A., Šedo O., Zdráhal Z., Konečná H., Stupková A. & Josef Svajner J. (2018), "Proteomic characterization and antifungal activity of potato tuber proteins isolated from starch production waste under different temperature regimes" Appl Microbiol Biotechnol, 102 pp. 10551-10560

[6]. Bártová V., Bárta J. and M. Jarošová (2019),"Antifungal and antimicrobial proteins and peptides of potato (Solanum tuberosum L.) tubers and their applications" Appl Microbiol Biotechnol, 103 pp. 5533-5547

[7]. Bashir M.A. , Cristian Silvestri [2], Touqeer Ahmad [1], Ishfaq Ahmad Hafiz [1], Nadeem Akhtar Abbasi [1], Ayesha Manzoor [3], Valerio Cristofori [2], Eddo Rugini (2020), "Osmotin: a cationic protein leads to improve biotic and abiotic stress tolerance in plants" Plants, 9 p. 992 https://pubmed.ncbi.nlm.nih.gov/32759884/

[8]. Bol J.F., Linthrost H.J.M. and Cornelissen B.J.C. (1990) Plant pathogenesis related proteins induced by virus infection. Annual Review of Phytopathology**, 28**: 113-138.

[9]. Boller T. and Metraux J.P. (1988) Extracellular localization of chitinases in cucumber. Phycological and Molecular Plant Pathology, **33:** 11-17.

[10]. Broekaert, W.F., Lee, H.I., Kush, A., Chua, N.H. and Ralkhel N.V . (1990) Wound- induced accumulation of mRNA containing a hevein sequence in laticifers of rubber tree (Hevea brasiliensis). Proceeding of the National Academy of Science USA, **87:** 7633-7637.

[11]. Broekaert W.F., Parijs J. Van, Allen A.K. and Peumans W.J. (1988) Comparison of some molecular, enzymatic and antifungal properties of chitinases thornapple, tobacco and wheat. Phycological and Molecular Plant Pathology, **33** (3) : 319-331.

[12]. Broekaert W.F., Trerras F.R.G., Cammue B.P.A. and Osborn R.W. (1995) Plant Defensins: Novel antimicrobial peptides as components of the host defence system. Plant physiology, **108:** 1353-1358.

[13]. Broekaert W.F., Van Parijis J., Leyna F., Joos H. and Peumans W.J. (1989) A chitin-binding lectin from stinging nettle rhizomes with antifungal properties. science **345;** 1100-1102.

[14]. Buchter R., Stromberg, A., Schmelzer, E. and Kombrink E. (1997) primary structure and expression of acidic (class 11) chitinase in potato. Plant Molecular Biology, **35**(6): 749-761

[15]. Bueso, F.J., Waniska, R.D., Rooney, W.L. and Bejosano F.P. (2000) Activity of antifungal proteins against mould in sorghum caryopses in the field. Journal of Agriculture and food chemistry, **48** (3) 810-816.

[16]. Burzynski A., Pislewska M. and Wojtaszek P. (2000) Active chitinases in the apoplastic fluids of healthy white lupin (Lupinus albus L.) plants. Acta. Physiological Plantarum, **22** (1): 31-38.

[17]. Casacuberta J.M., Puigdomenech P. and San Segoundo B. (1991) A gene coding for a basic pathogenesis – related (PR-like) Protein from Zea mays. Plant Molecular Biology, **16**: 527-536.

[18]. Chiu T., Theo Poucet and Yenram Li (2022) "The potential of plant proteins as antifungal agents for agricultural applications" Dec.7 (4): 1075-1083

[19]. Chowdhury S., Basu A. and Kundu S. (2015), "Cloning, characterization, and bacterial over-expression of an osmotin-like protein gene from Solanum nigrum L. With antifungal activity against three necrotrophic fungi" Mol Biotechnol, 57 pp. 371-381

[20]. Chrispeels M.J. and Ralkhel N.V. (1991) "Lectins, lictin genes, and their role in plant defense" The plant Cell, **3**: 1-9.

[21]. Chung R.P.T., Neumann G.M. and Polya G.M. (1997) Purification and characterization of basic proteins with in virto antifungal activity from seeds of cotton, Gosypium hirsutum. Plant Science Limerick, **12**(1): 1-16.

[22]. Clarke H.R.G., Lawrence S.D., Flaskerud J., Korhnak T.E., Gordon M.P., Davis J.M. (1998) Chitinase accumulates systematically in wound poplar trees. Physiologia Plantarum **103**: 154-161.

[23]. Clausen M., Krauter R., Schachermayr G., Potrykus I. and Sautter C. (2000) "Antifungal activity of a virally encoded gene in transgenic wheat" Nature Biotechnology, **18**: 446-449.

[24]. Cohen E. (1993) Chitin synthesis and degradation as targets for pesticide action. Archives of Insect Biochemistry and Physiology, **22** (1/2): 245-261.

[25]. Collada C., Casado R., Fraile A. and Aragoncillo C. (1992) Basic endochitinases are major proteins in Castanea sativa cotyledons. Plant Physiology, **100**: 778-783.

[26]. Cornellissen B.J.C. and Melchers L.S. (1993) Strategies for control of fungal diseases with transgenic plants. Plant Physiol., **101**: 709-712.

[27]. Cornellissen B.J.C., Hooft van Huijsduijnen R.A.M., Van Loon L.C. and Bol J.F. (1986) Molecular characterization of messenger RNAs for "pathogenesis-related" proteins 1a, 1b and 1c, induced by TMV infection of tobacco. The EMBO Journal, **5**: 37-40.

[28]. Cornellissen B.J.C., Horowitz J., Van Kan J.A.L., Goldberg R.B. and Bol J.F. (1987) Structure of tobacco genes encoding pathogenesis-related proteins from the PR-1 group. Nucleic Acids Research, **15**: 6799-6811.

[29]. Cote F., Cutt J.R. Asselin A. and Klessing D.F. (1991) Pathogenesis related acid β-1, 3-glucanase genes of tobacco are regulated by both stress and developmental signals. Molecular Plant-Microbe Interactions, **4**: 173-181.

[30]. Cutt J.R., Dixon D.C., Carr J.P. and Klessing D.F. (1988) Isolation and nucleotide sequence of cDNA clones for the pathogenesis-related proteins PR1a, PR1b and PR1c of Nicotiana tobacum cv. Xanthinc induced by TMV infection. Nucleic Acids Research, **16**: 9861.

[31]. Dantzger M., Vasconcelos I. M. , Valéria Scorsato , Ricardo Aparicio [4], Sergio Marangoni [5], Maria Lígia Rodrigues Macedo, (2015), "Bowman–Birk proteinase inhibitor from Clitoria fairchildiana seeds: isolation, biochemical properties and insecticidal potential" Phytochemistry, 118 pp. 224-235

[32]. Das Graças F.M. , V.M. Gomes, R.E. Corsini, O.L. Machado, S.G. De Simone, J.C. Novello, S. Marangoni, M.L.R. Macedo (2002), "Isolation and partial characterization of a novel lectin from Talisia esculenta seeds that interferes with fungal growth" Plant Physiol. Biochem., 40 pp. 61-68

[33]. De Wit P.G.M. and Van der Meer F.E. (1986) Accumulation of the pathogenesis-related tomato leaf protein P 14 as an early indicator of incompatibility in the interaction between Cladosporium fulvum (syn. Fulvia fulva) and tomato. Physiological and Molecular Plant Pathology, **28**: 203-214.

[34]. Dixon D.C., Cutt J.R. and Klessing D.F. (1991) Differential targeting of the tobacco PR-1 pathogenesis-related proteins to the extracellular space and vacuoles of crystal idioblasts. The EMBO Journal, **10** : 1317-1324.

[35]. Does M.P., Ng D.K., Dekker H.L., Peumans W.J., Houterman P.M., Damme E.J.M. van, Cornellissen B.J.C. and Van Demme E.J.M. (1999) Characterization of Urtica dioica agglutinin isolectins and the encoding gene family. Plant Molecular Biology **39**(2): 335-347.

[36]. Egea C., Dickinson M.J., Candela M. and Candela M.E. (1999) beta-1, 3- glucanases isoenzymes and genes in resistant and susceptible pepper (Capsicum annuum) cultivars infected with Phytophthora capsica, Physiologia Plantarum, **107**(3): 312-318.

[37]. Farady C.J. and Craik C.S. (2010), Mechanisms of macromolecular protease inhibitors" Chembiochem pp. 11 2341-2346 https://pubmed.ncbi.nlm.nih.gov/30276716/

[38]. Fierens E., Sigrid Rombouts, Kurt Gebruers, Hans Goesaert,[*] Kristof Brijs,[*] Johnny Beaugrand, Guido Volckaert, Steven Van Campenhout, Paul Proost, Christophe M. Courtin and Jan A. (2007), "Delcour TLXI, a novel type of xylanase inhibitor from wheat (Triticum aestivum) belonging to the thaumatin family" Biochem J, 403 p. 583 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1876379/

[39]. Fincher G.B. and Stone B.A. (1981) Metabolism of noncellulosic polysaccharides. Encylopaedia of Plant Physiology, **13 B**, 68-132.

[40]. Fischer M., Kuckenberg M., Kastilan R., J. Muth J. and Gebhardt C. (2015), "Novel in vitro inhibitory functions of potato tuber proteinaceous inhibitors",Mol Genet Genom, 290 pp. 387-398

[41]. Fritig B., Rouster S., Kauffmann S., Stinzi A., Geoffroy P., Kopp M. et al. (1989) Virus-induced glycanhydrolases and effects of oligosaccharide- signals on plant-virus interactions. In Signal Molecules in Plants and Plant Microbe Interactions, H36, edited by B. Lutenberg 161-168. New York: Plenum Press.

[42]. Ghosh M. (2009), "Purification of a lectin-like antifungal protein from the medicinal herb, Withania somnifera" Fitoterapia, 80 pp. 91-95

[43]. Gitlin-Domagalska A., Maciejewska A. and Dębowski D. (2020), "Bowman-Birk inhibitors: insights into family of multifunctional proteins and peptides with potential therapeutical applications", Pharmaceuticals, 13 pp. 1-40

[44]. Godoy A.V., Olivieri F.P. and Casalongue C.A. (1996) Induction of hydrolytic enzymes and antifungal in vitro activity in S. tuberosum subsp. andigena after infection with Fusarium solani f. sp, eumartu. Potato Research **39**(2): 259-266.

[45]. Goldstein L.I. and Hayes C.E. (1978) The lectins: carbohydrate-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem., **35** : 127-340.

[46]. Grenier J., Potvin C., Trudel J. and Asselin A. (2002) "Some thaumatin-like proteins hydrolyse polymeric β-1,3-glucans" Plant J, 19 pp. 473-480 **https://doi.org/10.1046/j.1365-313X.1999.00551.x**

[47]. Grosse-Holz F.M., van der R. A. and Hoorn L. (2016), "Juggling jobs Roles and mechanisms of multifunctional protease inhibitors in plants" New Phytol, 210 pp. 794-807

[48]. Hakim , Abid Ullah , Amjad Hussain , Muhammad Shaban, Aamir Hamid Khan , Muna Alariqi , Summia Gul, Zhang Jun, Sun Lin , Jianying Li , Shuangxia Jin, Muhammad Farooq Hussain Munis (2018), "Osmotin: a plant defense tool against biotic and abiotic stresses" Plant Physiol. Biochem. PPB, 123 pp. 149-159 https://pubmed.ncbi.nlm.nih.gov/29245030/

[49]. Hermosa M.R. , Turrà D. , Fogliano V., Monte E. and Lorito M. (2006), "Identification and characterization of potato protease inhibitors able to inhibit pathogenicity and growth of Botrytis cinerea" Physiol Mol Plant Pathol, 68 pp. 138-148

[50]. Hooft van Huijsduijnen R.A.M., Alblas S.W., De Riijk R.H. and Bol J.F. (1986) Induction by salicylic acid of pathogenesis-related proteins and resistance to alfalfa mosaic virus infection in various plant species. Jour. General Virology, **67**: 2135-2143.https://www.jstor.org/stable/42951699

[51]. Hu Z., Huang Q.Z., Liu X.Z. and Yang J.B. (1999) Primary structure and cDNA cloning of the antifungal protein GAFP-I Gastrodia elata. Acta- Botanica-Yunnanica, **21**(2) : 131-138.

[52]. Huang C. (Cheng), Dianwen Wang , Hongping Chen, Wei Deng, Dazhou Chen, Pin Chen and Jilin Wang (2022) "Genome-Wide Identification of DUF26 Domain-Containing Genes in Dongxiang Wild Rice and Analysis of Their Expression Responses under Submergence" Curr. Issues Mol. Biol. **2022**, 44, 3351–3363. https://doi.org/ 10.3390/cimb44080231

[53]. Iglesias R., Citores L., Claudia C. Gay and José M. Ferreras (**2024**), "Antifungal Activity of Ribosome-Inactivating Proteins" Toxins , 16(4), 192; **https://doi.org/10.3390/toxins16040192**

[54]. Inhibitor B.-B., Nishino N., Aoyagi H and T. Kato (1977) "Studies on the synthesis of proteinase inhibitors I. Synthesis and activity of nonapeptide fragments of soybean" J Biochem, 82

[55]. Iseli B., Boller T. and Neuhaus J.M. (1993) The N-terminal cysteine-rich domain of tobacco class I chitinase is essential for chitin binding but not for catalytic or antifungal activity. Plant Physiology, **103**: 221-226. **ISSN: 2319-7706 Volume 2 Number 8, pp. 41-48**

[56]. James A.M. , Jayasen A. S. , Jingjing hang · Oliver Berkowitz , David Secco , Gavin J Knott , James helan , Charles S Bond , Joshua S Mylne (2017), "Evidence for ancient origins of bowman-birk inhibitors from Selaginella moellendorffii" Plant Cell, 29 pp. 461-473

[57]. Jamet E. and Fritig B. (1986) Purification and characterization pf 8 of the pathogenesis-related proteins in tobacco leaves reacting hypersensitively to tobacco mosaic virus. Plant Molecular Biology, **6**: 69-80.

[58]. Jebor M. A., Al-Saadi1 A., Behjet R. H., Al-Terehi1M., Jeenkeawpieam J., Yodkeeree S., Andrianopoulos A., Roytrakul S., and Pongpom M. (2020) "Antifungal Activity and Molecular Mechanisms of Partial Purified Antifungal Proteins from Rhinacanthus nasutus against Talaromyces marneffei" Journal of Fungi, 6(4), 333; **https://doi.org/10.3390/jof6040333**

[59]. Jongedijk E., Tigelaar H., Roekal S.C. van, Bres-Vloemans S.A., Dekker I., Elzen P.J.M., van den, Cornellissen J.C., Melchers L.S., Cassells A.C. and Jones P.W. (1995) Synergistic activity of chitinases and beta – 1, 3- glucanases enhances fungal resistance in transgenic tomato plants. Euphytica **85**: 1-3, 173-180.

[60]. Kauffmann S., Legrand M., Geoffroy P. and Fritig B. (1987) "Biological function of pathogenesis-related proteins. Four PR Proteins have 1, 3-β-glucanase activity". The EMBO Journal **6**: 3209-3212.

[61]. Keefe D., Hinz U. and Meins F. (1990) "The effect of ethylene on the cell-type specific and intracellular localization of β-1, 3-glucanase and chitinase in tobacco leaves". Planta, **182**: 43-51.

[62]. Kim M.H., Park S. C., Kim J. Y., Lee S. Y., Lim H.T., Cheong H., Kyung-Soo Hahm K. S. and Park Y. (2006), "Purification and characterization of a heat-stable serine protease inhibitor from the tubers of new potato variety 'Golden Valley", Biochem Biophys Res Commun, 346 pp. 681-686

[63]. Komarnytsky S., Cook A. and Raskin I. (2011), "Potato protease inhibitors inhibit food intake and increase circulating cholecystokinin levels by a trypsin-dependent mechanism" Int J Obes, 35 pp. 236-243

[64]. Lamb C.J., Lawton M.A., Dron M. and Dixon R.A. (1989) "Signals and transduction mechanisms for activation of plant defenses against microbial attack". Cell, **56** : 215-224.

[65]. Lamb C.J., Ryals J.A., Ward E.R., Dixon R.A., You C.B., Chen Z.L. and Ding Y. (1993) "Emerging strategies for enhancing crop resistance to microbial pathogens". Current Plant Science and Biotechnology in Agriculture, Vol. 15, 45-60.

[66]. Lawton K., Ward E., Payne G., Moyer M. and Ryals J. (1991) Acidic and basic class III chitinase messenger RNA accumulation in response to TMV infection of tobacco. Plant Molecular Biology, **19**: 735-743.

[67]. Leah R., Tommerup H., Svendsen I. and Mundy J. (1991) Biochemical and molecular characterization of three barley seeds proteins with antifungal properties. Jour. Biological Chemistry, **266**: 1564-1573.

[68]. Lee H., Damsz B., Woloshuk C.P. , Bressan R.A. and Narasimhan M.L. (2010), "Use of the plant defense protein osmotin to identify fusarium oxysporum genes that control cell wall properties" Eukaryot Cell, 9 pp. 558-568

[69]. Legrand M., Kauffmann S., Geoffroy P. and Fritig B. (1987) Biological function of "pathogenesis-related" proteins: four tobacco PR-proteins are chitinases. Proceedings of the National Academy of Sciences USA., **84**: 6750-6754.

[70]. Li N., , Qu L. J., Liu Y., Li Q., Gu H and Z Chen "The refolding, purification, and activity analysis of a rice Bowman-Birk inhibitor expressed in Escherichia coli" Protein Expr Purif, 15 (1999), pp. 99-104 https://pubmed.ncbi.nlm.nih.gov/10024476/

[71]. Li T., X. Yin, D. Liu, X. Ma, H. Lv, S. Sun (2012), "Isolation and characterization of a novel lectin with antifungal and antiproliferative activities from Sophora alopecuroides seeds" Acta Biochim. Biophys. Sin., 44 pp. 606-613

[72]. Linthorst H.J.M., Melchers L.S., Mayer A., Van Roekel J.S.C., Cornellissen B.J.C. and Bol J.F. (1990 b) Analysis of gene families encoding acidic and basic β-1, 3-glucanases of tobacco. Proceedings of National Academy of Science USA, **87**: 8756-8760.

[73]. Linthorst H.J.M., Van Loon L.C., Van Rossum C.M.A., Mayer A., Bol J.F., Van Roekel J.S.C. et al. (1990 C). Analysis of acidic and basic chitinases from tobacco and petunia and their constitutive expression in transgenic tobacco. Molecular Plant Microbe Interactions, **3**: 252-258.

[74]. Losso J.N. (2008), "The biochemical and functional food properties of the Bowman-Birk inhibitor" Crit Rev Food Sci Nutr, 48 pp. 94-118

[75]. Magnólia de A Campos, Marilia S Silva, Cláudio P Magalhães, Simone G Ribeiro, Rafael PD Sarto, Eduardo A Vieira & Maria F Grossi de Sá (2008), "Expression in Escherichia coli, purification, refolding and antifungal activity of an osmotin from Solanum nigrum" Microb Cell Factories, 7 pp. 1-10 https://microbialcellfactories.biomedcentral.com/articles/10.1186/1475-2859-7-7

[76]. Martin M.N. (1991) The latex of Hevea brasiliensis contains high levels of both chitinases and chitinase / lysozymes. Plant-Physiology, **95**: 469-476.

[77]. Mauch F. and Staehelin L.A. (1989) Functional implications of the subcellular localization of ethylene-induced chitinase and β-1, 3-glucanases in bean leaves, The Plant Cell, **1**, 447-457.

[78]. Meins F. and Ahl P. (1989) Induction of chitinase and β-1, 3-glucanases in tobacco plants infected with Pseudomonas tobaci and Phytophthora parasitica var. nicitianae. Plant Science **61**, 155-161.

[79]. Melchers L.S., Ponstein A.S., Sela-Buurlage M.B., Vhoemans S.A. and Cornellissen B.J.C. (1993) in B. Fritig and M. Legrand, eds. Mechanisms of Plant Defense Response, Kluver Academic Publishers, Dordracht, The Netherlands.

[80]. Mirelman D., Galun E., Sharon N. and Lotan R. (1975) Inhibition of fungal growth by wheat germ agglutinin. Nature **255**, 414-416.

[81]. Muzard J., Fields C., John O'mahony J. and G.U. Lee (2012), "Probing the soybean Bowman−Birk inhibitor using recombinant antibody fragments" Journal of Agricultural and Food Chemistry 10.1021/jf3004724

[82]. Nassuth A. and Sanger H.L. (1986) Immunological relationships between "pathogenesis related" leaf proteins from tomato, tobacco and cowpea. Virus Research, **4**: 229-242.

[83]. Nielsen K.K., Nielsen J.E., Madrid S.M. and Mikkelsen J.D. (1996) New antifungal proteins from sugarbeet (Bert vulgaris L,) showing homology to non-specific lipid transfer proteins. Plant Molecular Biology **31**(3), 539-552.

[84]. Nishizawa Y., Kishimoto N., Saito A. and Hibi T. (1993) Sequence variation, differential expression and chromosomal location of rice chitinase genes. Molecular and General Genetics, **241**, (1-2), 1-10.

[85]. Nóbrega F.M., Santos I.S., Da Cunha M., Carvalho A.O. and Gomes V.M. (May 2005) "Antimicrobial proteins from cowpea root exudates: inhibitory activity against Fusarium oxysporum and purification of a chitinase-like protein" in Plant and Soil Vol. 272, No. ½, pp. 223-232

[86]. Ohme-Takagi M. and Shinshi H. (1990) Structure and expression of a tobacco β-1, 3-glucanase gene. Plant Molecular Biology, **15**, 941-946.

[87]. Ohshima M., Harada N., Matsuoka M. and Ohashi Y. (1990a) The nucleotide sequence of pathogenesis-related (PR) 1b protein gene of tobacco. Nucleic Acids Research, **18**, 181.

[88]. Ohshima M., Harada N., Matsuoka M. and Ohashi Y. (1990b) The nucleotide sequence of pathogenesis-related (PR) 1c protein gene of tobacco. Nucleic Acids Research, **18**, 182.

[89]. Parashina E.V., Shadenkov A.A., Navrova N.V. and Avetisov V.A. (1999) The use of the defensive peptide (defensin) gene from radish seeds in order to improve the resistance of tomatoes towards diseases caused by fungi. Biotekhnologica **15**(6), 35-41.

[90]. Payne G., Parks T.D., Burkhart W., Dicher S., Ahl P., Metraux J.P. et al., (1988a) Isolation of the genomic clone for pathogenesis-related protein 1a from Nicotiana tobacum c.v. Xanthi-nc. Plant Molecular Biology, **11**, 89-94.

[91]. Payne G., Ward E., Gaffrey T., Ahl-Goy P., Moyer M., Harper A. et al., (1990a) Evidence for a third structural class β-1, 3-glucanase in tobacco. Plant Molecular Biology, **15**, 797-808.

[92]. Pernas M., Lopez-Solanilla E., Sanchez-Monge R., Salcedo G. and Rodriguez-Palenzuella P. (1999) Antifungal activity of a plant cystatin. Molecular Plant Microbe Interactions, **12**(7), 624-627.

[93]. Pfitzer U.M. and Goodman H.M. (1987) Isolation and characterization of cDNA clones encoding pathogenesis-related proteins from tobacco mosaic virus infected tobacco plants. Nucleic Acids Research, **15**, 4449-4465.

[94]. Pierpoint W.S. (1986) The pathogenesis-related proteins of tobacco leaves. Phytochemistry, **25**, 1595-1601.

[95]. Platé N.A., Valuev L.I., Valueva T.A. and Chupov V.V. (1993), "Biospecific haemosorbents based on proteinase inhibitor. I. Synthesis and properties", Biomaterials, 14 pp. 51-56

[96]. Ponstein A.S., Bres-Vloemans S.A., Sela Buurlage M.B., Elzen P.J.M. van den, Melchers L.S. and Cornellissen B.J.C. (1994) A novel Pathogen-and wound- inducible tobacco (Nicotiana tobacum) protein with antifungal activity. Plant Physiology, **104**(1), 109-118.

[97]. Prasad E.R., Dutta-Gupta A. and Padmasree K. (2010a), "Purification and characterization of a Bowman-Birk proteinase inhibitor from the seeds of black gram (Vigna mungo)" Phytochemistry, 71 pp. 363-372

[98]. Prasad E.R., Merzendorfer H., Madhurarekha C., Dutta-Gupta A. and Padmasree K. (2010b), "Bowman-Birk proteinase inhibitor from Cajanus cajan seeds: purification, characterization, and insecticidal properties" J Agric Food Chem, 58 pp. 2838-2847, Published By: Springer

[99]. Qi R.F., Song Z.W. and Chi C.W. (2005), "Structural features and molecular evolution of Bowman-Birk protease inhibitors and their potential application" Acta Biochim Biophys Sin, 37 pp. 283-292

[100]. Rauscher M., Adam A.L., Wirtz S., Guggenheim R., Mendgen K. and Deising H.B. (1999) PR-1 protein inhibits the differentiation of rust infection hyphae in leaves of acquired resistant broad bean. Plant Journal, **19**(6), 625-633.

[101]. Ren Y.Y. and West C.A. (1992) Elicitation of diterpene biosynthesis in rice (Oryza sativa L.) by chitin. Plant Physiology, **99**(3), 169-1178.

[102]. Rustgi S., Boex-Fontvieille E., Reinbothe C., von Wettstein D. and Reinbothe S. (2018), "The complex world of plant protease inhibitors: insights into a Kunitz-type cysteine protease inhibitor of Arabidopsis thaliana" Communicative & Integrative Biology, Volume 11, 2018 - Issue 110.1080/19420889.2017.1368599

[103]. Samac D.A., Hirinaka C.M., Yallalay P.E. and Shah D.M. (1990) Isolation and characterization of genes encoding basic and acidic chitinase in Arabidopsis thaliana. Plant Physiology, **93**, 907-914.

[104]. Samblanx G.W., Fernan dez-de, Carmen A., Sijtsma L., Plasman H.H., Schaaper W.M.M., Posthuma G.A., Fant F., Meloen R.H., Broekaert W.F. and Van Amerongen A. (1996) Antifungal activity of synthetic 15-mer peptides based on the Rs-AFP2 (Raphanus sativus antifungal protein 2) sequence. Peptide Research **9**(6), 262-268.

[105]. Schickler H. and Chet I. (1997) Heterologous chitinase gene expression to improve plant defence against phytopathogenic fungi. Journal of industrial Microbiology and Biotechnology, **19** (3), 196-201.

[106]. Schlumbaum A., Mauch F., Vogeli U. and Boller T. (1986) Plant chitinases are potent inhibitors of fungal growth. Nature, **324**, 365-367.

[107]. Seabra R. and Pais M.S. (1999) Genetic transformation of European chestnut (Castanea sativa Mill.) Silva Lusitana, **7** (2), 199-232.

[108]. Seetharaman K., Whitehead E., Keller N.P., Wanishka R. D. and Rooney L.W. (1997) In vitro activity of sorghum seed antifungal proteins against grain mold pathogens. Journal of Agricultural and Food Chemistry, **45** (9) 3666-3671.

[109]. Sela-Buurlage M.B., Ponstein A.S., Bres-Vloemans S.A., Melchers L.S., Elzen P.J.M. van den and Cornellissen B.J.C. (1993) Only specific tobacco (Nicotiana tobacum) chitinase and beta 1,3- glucanases exhibit antifungal activity. Plant Physiology, **101**, 857-863.

[110]. Singh R.R. and Appu Rao A.G. (2002), "Reductive unfolding and oxidative refolding of a Bowman-Birk inhibitor from horsegram seeds (Dolichos biflorus): evidence for 'hyperreactive' disulfide bonds and rate-limiting nature of disulfide isomerization in folding" Biochim Biophys Acta Protein Struct Mol Enzymol, 1597 pp. 280-291

[111]. Sperisen C. Ryals J. and Meins F. (1991) Comparison of cloned genes provides evidence for intergenomic exchange of DNA in the evolution of a tobacco glucan endo – 1, 3 – glucosidase gene family. Proceedings of the National Academy of Science USA, **88**, 1820-1824.

[112]. Sticher L., Hofsteenge J., Milani A., Neuhaus J.M. and Meins F. (1992) Vacuolar chitinases of tobacco-a new class of hydroxy-proline-containing proteins. Science, **257**, 655-657.

[113]. Stinissen H.M., Peumans W.J. and Chrispeels M.J. (1984) Subcellular site of lectin synthesis in developing rice embroys. EMBOJ, 3, 1979-1985.

[114]. Stripe F., Barbieri L., Batelli L.G., Soria M., Lappi D.A. (1992) Ribosome-inactivating proteins from plants: present status and future prospects. Biotechnology, **10**, 405-412.

[115]. Takeuchi Y., Yashikawa M., Takeba G., Tamaka K., Shibata D. and Horino O. (1990) Molecular cloning and ethylene induction of encoding a phytoalexin elicito-releasing factor, β-1, 3- endoglucanase, in soybean. Plant Physiology, **93**, 673-682.

[116]. Terras F.R.G., Schoofs H.M.E., De Bolle M.F.C., Van Leuven F., Rees S.B., Vander-leyden J., Cammue B.P.A. and Broeksert W.F. (1992) Analysis of two novel classes of antifungal proteins from radish (Raphanus sativus L.) seeds. J. Biol. Chem, **267** : 15301-15309.

[117]. Terras F.R.G., Torrekens S., Van Leuven F., Osborn R.W. Vanderleyden J., Cammue B.P.A. and Broekaert W.F. (1993) A new family of basic cysteine-rich plant antifungal proteins from Brassicaceae species. FEBS Lett **316**, 233-240.

[118]. Urdangarin M.C., Norero N.S., Broekaert W.F. and Canal L. de-la (2000) A defensin gene expressed in sunflower inflorescence. Plant Physiology and Biochemistry, Paris, **38** :3, 253-258.

[119]. Van de Rhee M.D., Linthorst H.J.M. and Bol J.F. (1994) Pathogen induced gene expression in stress-induced Gene Expression in Plants, edited by Basra A.S., pp. 249-284. Switzerland: Harwood Academic Publishers.

[120]. Van den Bulcke M., Bauw G., Castresama C., Van Montagu M. and Vandeker ckhove J. (1989) Characterization of vacuolar and extracellular β-(1,3)-gulcanases of tobacco: evidence for a strictly compramentalized lant defence system. Proceedings of the National Academy of Science USA, **86**, 2673-2677.

[121]. van der Wel H.and K. Loeve V (1972), "Isolation and characterization of thaumatin I and II, the sweet-tasting proteins from Thaumatococcus daniellii benth" Eur J Biochem, 31 pp. 221-225

[122]. Van Loon L.C., Gerritsen Y.A.M. and Ritter C.E. (1987) Identification, purification and characterization of pathogenesis-related proteins from virus-infected Samson NN tobacco leaves. Plant Molecular Biology, **9**, 593-609.

[123]. Vannini A., Caruso C., Leonardi L., Rugini E., Chiarot E., Caporale C. and Buonocore V. (1999) Antifungal properties of chitinases from Castanea sativa against hypovirulent and virulent strains of the chestnut blight fungus Cryphonectria parasitica. Physiological and Molecular Plant Pathology, **155**: 29-35.

[124]. Velazhahan R., Radhajeyalakshmi R., Thangavelu R. and S. Muthukrishnan (2001) "An antifungal protein purified from pearl millet seeds shows sequence homology to lipid transfer proteins",Biologia Plantarum, Volume 44, pages 417–421, https://doi.org/10.1023/A:1012463315579

[125]. Vera P. and Conejero V. (1989) The induction and accumulation of the pathogenesis-related P69 proteinase in tomato during Citrus exocortis viroid infection and after chemical treatments. Physiological and Molecular Plant Pathology, **34**, 323-324.

[126]. Verberg J.G. and Huynh Q.K. (1991) purification and characterization of an antifungal chitinase from Arabidopsis thaliana. Plant Physiology, **95**: 450-455.

[127]. Vigers A.J., Wiedemann S., Walden K. Roberts, Michel Legrand , Claude P. Selitrennikoff and Bernard Fritig (1992), "Thaumatin-like pathogenesis-related proteins are antifungal" Plant Sci, 83 pp. 155-161 https://www.sciencedirect.com/science/article/abs/pii/016894529290074V

[128]. Ward E.R., Payne G.B., Moyer M.B., Williams S.C., Dincher S.S., Sharkey K.C. et al., (1991) Differential regulation of β-1,3-glucanase messenger RNAs in response to pathogen infection. Plant Physiology, **96**, 390-397.

[129]. Wessels J.G.H. and Sietsma J.H. (1981) Fungal Cells Walls, a survey. Encyclopaedia of Plant Physiology **13 B**, 352-394.

[130]. White R.F., Rybicki E.P., Von Wechmar M.B., Dekker J.L. and Antoniw J.F. (1987) Detection of PR-1 type proteins in Amaranthaceae, Chenopodiaceae, Gramineae and Solanaceae by immunoelectroblotting. Journal of General Virology, **68**: 2043-2048.

[131]. Xiaochun Ge1, Jichao Chen, Chongrong Sun and Kaiming Cao (2003) "Preliminary study on the structural basis of the antifungal activity of a rice lipid transfer protein" Protein Engineering vol. 16 no. 6 pp. 387-390, DOI: 10.1093/protein/gzg055

[132]. Xie Z., Saha N. and Chlan C. (2016), "Antimicrobial Activity of a Cys-Rich Peptide Derived from a Centrosema virginianum Vicilin" American Journal of Plant Sciences, **7**, 92-107. http://dx.doi.org/10.4236/ajps.2016.71011

[133]. Ye X.Y., Ng T.B. and Rao P.F. (2001), "A bowman–birk-type trypsin-chymotrypsin inhibitor from broad beans" Biochem Biophys Res Commun, 289 pp. 91-96

[134]. Ye XY, Ng TB and Rao PF. (2002 May) "Cicerin and arietin, novel chickpea peptides with different antifungal potencies. Peptides". 23(5):817-22. doi: 10.1016/s0196-9781(02)00005- PMID: 12084511.

[135]. Youn Y.S. and Hwang B.K. (1996) "Differential induction and accumulation of beta 1,3- glucanases and chitinase isoforms in soybean hypocotyls and leaves after compatible and incompatible infection with Phytophthora megasperma f. sp. Glycinea". Physiological and Molecular Plant Pathology. **48**(3): 179-192.

[136]. Zaidan H. K., and Al-Saadi A.K. M. (2013) "Characterization and antimicrobial activity of barley grain (Hordeum vulgare) extract" International Journal of Current Microbiology and Applied Sciences