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REVIEW

Polygalacturonase-Inhibiting Protein Is a Structural Component of Plant Cell Wall

M. A. Protsenko*, N. L. Buza, A. A. Krinitsyna, E. A. Bulantseva, and N. P. Korableva

Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, 119071 Moscow, Russia; fax: 954-2732; E-mail: protsenko@inbi.ras.ru

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Abstract—It is generally believed that plants "evolved a strategy of defending themselves from a phytopathogen attack" during evolution. This metaphor is used frequently, but it does not facilitate understanding of the mechanisms providing plant resistance to the invasion of foreign organisms and to other unfavorable external factors, as well as the role of these mechanisms in plant growth and development. Information on processes involving one of the plant resistance factors—polygalacturonase-inhibiting protein (PGIP)—is considered in this review. The data presented here indicate that PGIP, being an extracellular leucine-rich repeat-containing protein, performs important functions in the structure of plant cell wall. Amino acid residues participating in PGIP binding to homogalacturonan in the cell wall have been determined. The degree of methylation and the mode of distribution of homogalacturonan methyl groups are responsible for the formation of a complex structure, which perhaps determines the specificity of PGIP binding to pectin. PGIP is apparently one of the components of plant cell wall determining some of its mechanical properties; it is involved in biochemical processes related to growth, expansion, and maceration, and it influences plant morphology. Polygalacturonase (PG) is present within practically all plant tissues, but the manifestation of its activity varies significantly depending on physiological conditions in the tissue. Apparently, the regulation of PG functioning in apoplast significantly affects the development of processes associated with the modification of the structure of plant cell wall. PGIP can regulate PG activity through binding to homogalacturonan. The genetically determined structure of PGIP in plants determines the mode of its interaction with an invader and perhaps is one of the factors responsible for the set of pathogens causing diseases in a given plant species.

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Polygalacturonase-inhibiting protein (PGIP) is considered to be one of the components of the defense system in plants. It is found in vegetative tissues based on its action against polygalacturonase (PG)—an enzyme secreted by phytopathogenic organisms. PG cleaves polygalacturonic acid, which is a component of pectinaceous substances of the plant cell wall [1]. The possible participation of PGIP in reactions of plant resistance by inhibiting PG activity, which is one of the basic pathogenic factors, has drawn the attention of investigators. Moreover, it has been shown that PGIP promotes the PG-mediated formation of oligogalacturonides with higher degree of polymerization, which are known to stimulate protective reactions in plants [2]. PGIPs are found in virtually all tissues and organs of all plants investigated [3]. They are localized in the apoplast, and their content in tissues often corresponds to the degree of plant resistance to certain diseases [4, 5]. Infection, wounding, and treatment with some reagents (elicitors) stimulate the expression of PGIP genes. Therefore, it is possible to consider PGIP proteins being an important factor of plant resistance to adverse effects [6, 7]. However, PGIPs possessing certain specificity and acting on PGs of specific pathogens do not always prevent the development of disease [8]. PGIP content varies significantly in different plant tissues and changes during ontogenesis. Recently, data on the molecular structure of some PGIPs have been obtained. These studies suggest that PGIPs can bind pectin in the cell wall of plants and change its functioning. Questions remain on the role of PGIP in plants.

Abbreviations: eLRR) completely extracellular leucine-rich repeat-containing proteins; LRR) leucine-rich repeats; LRX) leucine-rich extensin; PEX1) pollen-specific extensin; PG) polygalacturonase; PGIP) polygalacturonase-inhibiting protein; PME) pectin methyl esterase.

* To whom correspondence should be addressed.

PGIP

Structure. PGIPs are members of a large class of plant proteins participating in intermolecular interactions and containing leucine-rich repeats (LRR) [9]. The experiments with transgenic plants where these genes were blocked or overexpressed have proved that LRR proteins play an important role in the processes of growth and development in plant tissues, and also in their interaction with invasive pathogens, since many products of *R* (resistance) genes belong to the group [10]. However, little is known about the functional mechanism of these proteins. Some of LRR proteins are localized in cytoplasm, and others penetrate the plasmalemma and have both intracellular and extracellular domains. PGIPs belong to a group of completely extracellular proteins (eLRR).

All known PGIPs are glycoproteins with the molecular weight around 40 kD. Their primary structures are similar [11]. The proteins consist of a central region containing LRR and two adjacent cysteine-rich domains. The molecules contain eight highly conservative cysteine residues—four at the N-terminus and four at the C-terminus. They form four disulfide bonds stabilizing the PGIP molecule and determining its secondary structure. A signal peptide consisting of 24-29 amino acid residues apparently participates in transport of the molecule through the endoplasmic reticulum and secretory systems into the intercellular space.

The LRR domain consists of 10 consecutive elements each containing 24 amino acid residues (xxLxLxxNxLt/sGxIPxxLxxLxxL) and forming a solenoid. Leucine (L) comprises about 15% of all amino acids of the PGIP molecule. The xxLxLxx repeats determine the formation of repeating structure, β-sheet/β-loop, where leucine residues form the hydrophobic center, and side chains of amino acids surrounding leucine are turned to solvent and interact with a ligand. Structural units are located in such a manner that all β-sheets and α-helices are parallel to the general axis, forming a non-globular saddle-shaped molecule, with bent parallel β-sheets covering the internal part of the saddle and helices surrounding its external surface. The presence of LRR repeats provides for the formation of a flexible structural framework for protein–protein interactions. The elasticity of the construction permits certain modification of the form of receptor site depending on requirements, the same way as antibodies can slightly change configuration upon binding with antigen [12]. Proteins with LRRs clearly have a unique structure capable of performing a wide variety of some important biological functions.

The crystal structure of a PGIP molecule from bean (*Phaseolus vulgaris*), PvPGIP2, has been studied in detail [12]. It forms a right-handed helix, which is typical for LRR proteins. Amino acid residues determining the specificity of PGIP interaction with PG are located in twelve β-sheets filling the internal concave side of the helix [13]. Twelve α-helices are located on the opposite side of the molecule (sheets B1). Unlike the majority of LRR proteins, PvPGIP2 has additional β-sheets (B2) connected with helices on the convex side by a β-loop. A characteristic feature of the PvPGIP2 molecule is the presence of two clusters of amino acid residues with opposite charges [14]. Negatively charged surface belongs to the concave side and, apparently, participates in PG binding. A positively charged site is located on the opposite side between two β-sheets.

Virtually all eLRR proteins are glycosylated. In these glycoproteins carbohydrates make up to 20% of the total molecular weight [15]. The sites of possible glycosylation are located in a part of the molecule that is exposed to solvent in an α-helix region and are connected to β-sheets close to the C-terminus. Two N-bound oligosaccharides are localized on D64 and D141. Their basic structure is similar to the typical plant complex N-glycan consisting of a central β-1,2-xylosylated pentasaccharide bearing α-1,3-fucose, which is connected with internal N-acetylglucosamine, and an external branch of an N-acetylglucosamine residue [11]. The quantity and position of glycosylation sites (N-x-S/T where "x" is any amino acid except P) are non-rigorously conserved in PGIPs from different plants and could possibly be important for the definition of interaction specificity of ligands. LRR sites in the central part of the molecule are not glycosylated and therefore could participate in interactions with other molecules. Glycosylation degree for the same protein could depend on the location of its expression. For example, expression of the *pPGIP* gene from pear (*Pyrus*) resulted in a protein with molecular weight of approximately 45 kD in fruits of the plant, while expression the same gene in leaves and fruits of transgenic tomato gave products of around 41 and 43 kD, respectively. These proteins had identical weight of ~33 kD after deglycosylation [16].

The details of PGIP structure are determined by genome features of a given plant species. The *pgip* genes encoding PGIPs have been cloned from different plant species [3]. They are organized in small families. For example, about five *pgip* genes controlling the biosynthesis of protein isoforms with different localization in different tissues at different stages of ontogenesis are found in bean. It has been proposed that a non-tuberous relative (*Solanum brevidens* Fill.) of the cultured potato has at least five genomic copies of *pgip* gene [17]. However, similarity of *pgip* genes in plants belonging to one family has been recorded. A dendrogram constructed on the basis of similarity analysis for nucleotide sequences reveals at least three subgroups containing *pgip* genes: monocotyledonous, legume, and representatives of others taxons [14].

PGIP localization in plants. PGIPs are attributed to completely extracellular LRR proteins (eLRR) since their molecules do not contain characteristic intracellular

and transmembrane domains. PGIP localization in the apoplast has been proven by experiments on its extraction from tissues by the method of vacuum infiltration [18, 19]. Apparently, PGIP molecules undergo processing in systems of endoplasmic reticulum and Golgi apparatus and are secreted into the apoplast, but their connection with cytoplasmic membrane is not found. Detailed data on PGIP incorporation in the structure of plant cell wall are absent. Its content in tissues is often estimated based on its inhibitory action on the activity of commercial preparations of polygalacturonase, which is determined by the number of reduced groups formed upon action of the enzyme on polygalacturonic acid [20], or by the diameter of the zone of pectin decomposition around a well in agarized medium containing pectin [21]. The strength of expression in different organs of plants [19] is also used.

The significant number of works is devoted to detection of PGIPs in bean plants, where its activity appeared to be the highest in the vegetative apex and flower [18]. It is low in roots, and it increases in stem on approaching the apex. The level of *pgip* transcription in etiolated plants is 5-6 times higher than that in light-grown ones [22]. In light-grown sprouts, a high content of *pgip* transcripts is found in between extending and mature sites of hypocotyl and in the basal part of the stem in adult plants. In bean pods PGIP content was 14 times higher than in etiolated hypocotyls [23]. In suspension culture of bean cells, the level of PGIP mRNA was higher than in leaves, hypocotyls, and flowers [24].

During vegetation of potatoes of Nevsky variety, PGIP content in leaves was decreased at the beginning of generative stage (the formation of inflorescences), but increased by the time of active blooming and during fructification and tuber formation [25]. In alfalfa calluses, PGIP content is much higher than in leaves and hypocotyls [26]. PGIP is present in pollen of petunia [27]. In tobacco sepals, genes of PGIP and of other "protective" proteins were expressed more intensively than in leaves [28]. PGIP content was very high in female flowers with ovary in muskmelon (*Cucumis melo* var. *cantalupensis*) [29]. Ripe melons contained a high quantity of PGIP in seed cells. High PGIP concentration is found in very unripe pear fruits, which decreased during ripening [30]. In tomato fruits, PGIP is expressed at early stages of development [16]. During increase in diameter of green fruits from 1.5 to 2.5 cm, the quantity of tPGIP mRNA decreased approximately 20-fold. It slightly rose in pink fruits and then continued to decrease upon ripening. The content of tPGIP mRNA was at least 200 times lower in stems than in grown green fruits [31]. Apple-tree fruits of six varieties differing in ripening terms were significantly different in PGIP content, which increased during fruit growth on the tree, decreased by the time of technical maturity, and increased during after-ripening [32]. Maximal activity of PGIP was observed in unripe raspberries, and it decreased during their ripening [33]. In wild strawberries the highest content of PGIP was found in ripe berries [34]. During storage of grapefruits the strength of PGIP binding to albedo cell walls decreased, since its extraction required buffer with lower ionic strength, i.e. containing 0.2 M NaCl instead of 1 M [35].

Therefore, it is apparent that PGIP content in plant tissues changes both during growth and in connection with change in physiological and biochemical activity. Unfortunately, features of cell walls in different tissues and during plant ontogenesis are poorly studied. For example, it is necessary to note the differences in wall structure of intensely stretching cells of etiolated sprouts and isodiametric loosely spread cells of calluses. Cell walls of tomato and avocado fruits loose structure and become gelatinous at maturation, and in mature apple fruits cells are separated one from another, preserving the wall structure [36]. In external tissues of melon fruits, cells are spread densely, unlike internal parts where they form a friable mass. It is likely that the role of PGIP could differ depending on the localization and physiological conditions of plant tissues.

Changes in PGIP content (expression) depending on external factors. The penetration of a phytopathogenic organism into plant tissue leads to a change in some processes including PGIP-related ones. During infection of bean with sort-incompatible race *Colletotrichum lindemuthianum* and subsequent reaction of hypersensitivity, fast intensive temporary accumulation of PGIP mRNA in cells adjacent to those infected take place. This phenomenon is not observed with the compatible combination of race and variety [37].

PGIP expression is stimulated by some abiotic stress factors [17, 38]. In *Brassica campestris* plants, expression of the *BcPGIP* gene was caused by mechanical wounding, cold, salinization, over-watering, and jasmonic acid [39]. Some other factors stimulating protective reactions in plants (salicylic acid, ethanol, H_2O_2) did not cause an increase in *BcPGIP* expression. In some cases, PGIP does not react to elicitors but reacts to wounding. In leaves of *Brassica napus*, mechanical damage was a more powerful inducer of *Bnpgip1* expression than infection with *Sclerotinia sclerotiorum* and cooling [40]. However, the induction of *Bnpgip2* expression was more intense during infection than after wounding. Treatment with jasmonic acid induced expression of these two genes. Thus, no effect of salicylic acid on their activity was observed. Possibly different factors cause unequal changes in the complex of cell wall polymers that finally leads to the induction of expression of different genes.

OTHER PROTEINS WITH eLRR

Though PGIP is found in virtually all plant species, other proteins with eLRR are also found in vegetative tis-

sues, and their effects on PG activity have not been revealed. They are also connected with pectin in the cell wall.

Antifreeze protein from carrots *Daucus carota* (DcAFP) interferes with tissue damage at low temperatures by changing the form of ice crystals and decreasing the temperature of crystallization [41, 42]. It is an eLRR protein, and its amino acid sequence is 60% similar to that of PvPGIP. Its inhibitory action on PG from two mycelial fungi, *Aspergillus niger* and *Alternaria alternata*, which are pathogenic for carrots, has not been revealed. It was shown that DcAFP differs from PGIP in the position of several non-conservative amino acid residues, which leads to changes in the electrostatic properties of the molecule surface at the site responsible for PG binding [43].

Structural proteins of cell wall, LRX (leucine-rich extensin) containing receptor-like domain, are encoded by *LRX* genes, which consist of a family of 11 genes in the genome of *Arabidopsis* [44]. Some of these are expressed in tissues of vegetative organs and others in reproductive organs. A mutation of *LRX* genes leads to the impaired formation of root threads. LRX proteins contain signal peptide, N-terminal domain, LRR domain, domain enriched with cysteine and consisting of three subdomains, and also a domain with characteristics of extensin—a subfamily of glycoproteins rich in oxyproline. LRX2 is expressed mainly in roots, and it is also connected with the growth pattern of root threads, on the tip. However, it is not expressed in another organ growing on the tip, the pollen tube, where LRX1 is expressed. LRX1 and LRX2 are different in five amino acids exposed to solvent, but, apparently, this does not significantly influence their functions. It is likely that these amino acids define the localization of proteins in different tissues. The extensin domain clearly provides immobilization of LRX proteins on cell wall. LRX proteins probably participate in the assembly of primary layer of cell wall on the tip of root thread and also of secondary layer along the lateral walls.

Protein PEX1 (pollen-specific extensin) is an extensin expressed exclusively in corn pollen; it is proline-rich and contains a LRR domain as well as a tyrosine residue in a C-terminal site that probably provides the binding of the protein with the cell wall [45]. The same extensins are found in pollen tubes of potatoes, tomatoes, and tobacco.

Secreted protein SHY participating in the regulation of the growth of petunia pollen tube has structure similar to that of PGIP, though its inhibitory action on PG of some phytopathogenic fungi was not observed [27]. Genes encoding corresponding proteins are present also in genomes of other plants, e.g. *Arabidopsis*, *Zea mays*, and representatives of the Solanaceae family.

Gene *OsFOR1* (*Oryza sativa* floral organ regulator 1) controls biosynthesis of a protein similar to PGIP that participates in the regulation of a number of organs in rice flowers and is capable of inhibiting the PG from *A. niger* [46]. PGIP can influence cell connections, and the participation of OsFOR1 protein in the regulation of cell adhesion in the meristem is probable.

There are clearly different proteins with eLRR in cell wall of plants, which are responsible for its structural features and functions. PGIP is one of these.

PECTIN

Structure. While being localized in apoplast, PGIP as well as some other eLRR proteins are bound to the pectin of cell wall. It is widely accepted that the basic component of pectin is a linear homogalacturonan consisting of 100-120 residues of α-D-galacturonic acid connected by α-1,4-glycoside bonds [47, 48]. The main chain of homogalacturonan is joined by branches—rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII) consisting of rhamnose, arabinose, and arabinogalactans connected to form branched chains. These three polysaccharide domains are covalently bound and form a pectin network in the matrix of primary cell wall and in the median plate preserving cell connection.

Carboxyl groups of galacturonic acid residues form methyl esters during biosynthesis in the system of cell endomembranes and become demethylated after secretion in the wall through the action of pectin methyl esterase (PME) [49]. Here methyl groups on adjacently located residues of galacturonic acid are cleaved, resulting in a block in homogalacturonan that does not contain methyl residues able to contact with other homogalacturonan chains through calcium cross-links. Hydroxyl groups could be acetylated or form bonds with other monosaccharides, e.g. xylose [50]. It has been shown that *in vitro* PGIP binds acetylated hydroxyl groups.

It should be noted that phytopathogenic organisms as well as plants secret PME; the PME of the former, unlike plant PME, randomly demethylates galacturonic acid residues [51]. Random demethylation is observed upon the action of PME from *Ralstonia solanacearum* on tomato tissues [52]. Such differences could cause the specificity of subsequent action of PG.

PGIP is bound to cell wall by ionic forces and interacts *in vitro* with partially or completely demethylated homogalacturonan via positively charged amino acid residues exposed to solvent [53]. Amino acid residues participating in the binding have been determined in the PvPGIP molecule (R183, R206, K230, R252). Here not only electrostatic forces but also the conformation of polysaccharide is important [54]. The degree of methylation and distribution pattern of homogalacturonan methyl groups are important for the formation of complex structure, which probably provides the specificity of PGIP binding to pectin [55].

Localization of demethylated pectin in cell wall. Pectin substances are the most abundant class of macromolecules in primary cell wall of plants, totaling in about 30-35%, and sometimes up to 50% of the matrix [50]. The presence of pectin in cell walls is mainly responsible for the connections between cells, providing the integrity and strength of a plant as a multicellular organism [56.]

Monoclonal antibodies to different epitopes of the pectin molecule provide data about homogalacturonan structure in plants [57]. Homogalacturonan epitopes that bind antibodies JIM5 and JIM7 are rather different in the degree of methylation and polymerization, though the features of their structure are not yet completely characterized [51]. Nevertheless, these antibodies are used for studying the localization of epitopes of pectin molecules in connection with their function in different regions of cell wall and in dependence on the age of plant tissue. Antibodies JIM5 poorly bind the completely demethylated homogalacturonan but reveal homogalacturonan that is demethylated by more than 40% (low-methylated pectin). They bind galacturonic acid regions containing not less than four successive non-methylated residues adjoining methylated residues [58]. Antibodies JIM7 bind homogalacturonan containing alternating through one methylated and one non-methylated residues in pectin demethylated by 15-80%.

After division of a parent cell in meristem, cells in plant tissues are bound to each other by the median lamina containing pectin [59]. Primary cell wall contains low-methylated epitopes binding JIM5 antibodies [57]. Presumably, such epitopes could connect PGIP.

In potato stolons, from which tubers are formed, JIM5 epitopes were concentrated in primary wall and median lamina of cells of epidermis and cortex [60]. A rising gradient of their concentration in the direction from the growing end of the stolon to its base is found. The etherification degree of carboxyl groups of galacturonic acid residues apparently depends on the degree of cell differentiation.

JIM5 epitopes are found in external walls of epidermal cells, median lamina, cortex parenchyma intracellular spaces, and cell walls of phloem of eucalyptus roots [61]. They are also present in the extracellular matrix of conductive channel of petunia pestle [62]. QUASIMODO mutation of *Arabidopsis*, which is characterized, in particular, by the impairment of cell adhesion in suspension culture, led to a decrease in the content of low-methylated homogalacturonan [63].

The cited data indicate the doubtless participation of demethylated homogalacturonan in processes of adhesion and division of cells in plant tissues at different stages of development. Since the ability of PGIP to contact demethylated sites of homogalacturonan has been shown *in vitro*, it is logical to assume that it plays an important role in these processes *in planta*.

PG IN PLANTS

As mentioned above, PGIP was found as an inhibitor of PG from phytopathogenic organisms; however, PG is also secreted by plant cells. During growth and development of plants, pectin substances undergo changes leading to the destruction of cellular wall and maceration of tissues, which can be massive (in some juicy fruits) or strictly localized (in the dividing layer during organ abscission, fruit anthesis, division of pollen grains, promotion of pollen tube through pestle tissues) at the certain stages of plant development. A basic role in these processes is attributed to PG.

Endo-polygalacturonase (poly-[1,4-α-D-galacturonide]glycan hydrolase; EC 3.2.1.15) cleaves α-1,4-glycoside bonds between two non-methylated residues of D-galacturonic acid in polygalacturonic chains of a pectin molecule. Exo-PG (EC 3.2.1.67), which cleaves oligomeric fragments of polyuronide at the non-reducing end, is also known. It has been shown that PGIP acts mainly on endo-PG secreted by microorganisms.

Plant endo-PG is one of the most studied enzymes participating in transformations of pectin [64]. Homogalacturonan chain region containing more than four demethylated residues between two methyl groups is a subject of its action [58]. Therefore, variations in sizes and arrangement of demethylation sites define the character of polygalactouronan cleavage. The action of PG mainly results in cell detachment and tissue maceration.

The gene family encoding PG is rather large. These genes control the biosynthesis of protein isoforms participating in different processes [64]. The proteins are significantly different in amino acid sequences; moreover, greater similarity is noted between PG molecules from different plant species that participate in identical processes than between different PGs from the same plant. For example, PG participating in the maturation of tomato fruits has 41% amino acid sequence identity to PG from the separating layer of tomato and 60% identity to PG from melon fruits. Though in general PG molecules differ considerably, they contain highly conservative sites in the C-terminal region, which are characteristic of all PGs from plants and microorganisms. These sites are thought to be important for the catalytic functions of PG.

PG in plants has been most intensively studied during the maturation of juicy fruits. Sufficient differences in the character and rates of change in consistency of fruits have been found in different plants during their maturation [65]. They are caused to high degree by the mechanism of cell connection as well as by the initial structure of cell walls. Here pectin destruction can occur even at low enzyme activity. In fruits of transgenic tomatoes, 80% decrease in PG activity did not lead to a significant change in pectin cleavage. Hence, the intact plant contains an enzyme quantity that exceeds that necessary by at least five-fold [66].

During the abscission of organs characteristic for certain stages of plant development, the destruction of middle lamella takes place in a specialized separating layer of cells [64]. Here the enzymes destroying cell wall, in particular PG, are secreted. Different PG forms have been found in fruits and in the separating layer of leaves and flowers in tomato plants [67]. During the opening of fruits such as pod of cruciferous plants, beans of papilionaceous plants, and some others, the cell detachment from each other also takes place in a specialized separating layer [63]. Here pectin of middle lamella between cells of this layer is hydrolyzed and PG activity is observed [68].

PG plays an important role during plant pollination [64]. Its activity is found in anther and pollen grains. In pollen of *Populus deltoides* PG activity is 12 times higher than in tomato fruits [69]. A mutation impairing the cleavage of pectin leads to the inhibition of the division of pollen grains [70]. Selection toward decreasing of fruit crumbling to reduce the loss of rape seeds led to male sterility in plants because of impairment of anther opening [71]. It is interesting to note that in some plants PG is not expressed during non-compatible pollination [72].

The role of PG in plants is confirmed in experiments on transgenic plants. The expression of endo-PG from *A. niger* (AnPGII) in tobacco and *Arabidopsis* plants caused a decrease in the content of demethylated homogalacturonan and appearance of dwarf phenotype [73]. The overexpression of apple-tree PG resulted in the impairment of cell adhesion that led to the separation of leave epidermis from underlying cells, to premature subsidence of leaves due to the weakened connection between cells in a zone of petiole detachment, and to the impairment of stoma functions [74].

Intensive growth of plant tissues is provided not only by increase in number of cells, but also by their intensive stretching, accompanied by changes in the structure of cell wall in which an essential role belongs to PG. PG activity and the accumulation of PG mRNA are found in sprouting seeds and plant seedlings during the most intensive stretching in fast growing regions of cotyledon and stalk [64, 75, 76]. The formation of lateral stubs is also accompanied by the manifestation of PG activity, which apparently promotes the ingression of a newly formed stub between cells of previously existing tissues of root bark [77, 78].

So, PG is present in practically all plant tissues, but the manifestation of its activity differs strongly depending on physiological condition. It is likely that the regulation of PG functioning in apoplast substantially mediates the development of processes connected with change in the structure of cell wall.

Analysis of the presented data shows that upon binding with homogalacturonan, PG and PGIP form a sophisticated complex of cell wall components, whose interaction, caused by the features of their structure, accompanies a number of major processes in plant life.

PG OF PATHOGENS

PG is the first enzyme secreted by phytopathogenic microorganisms at the beginning of penetration into plant tissues [79]. It is considered to be one of major factors of pathogenesis, and numerous studies [80, 81] have been devoted to its investigation. PG participates in the penetration of a wide variety of different endobionts beginning from phytopathogenic bacteria and fungi that cause soft rots, and to nematodes, insects, mycorrhizal fungi, and parasitic higher plants.

The crystal structure of PG molecules secreted by phytopathogenic microorganisms (fungi and bacteria) has been studied [81]. The PG molecule forms a right-handed β-helix from parallel sheets with a hydrophobic center and randomly oriented amino acids exposed to solvent, which form loops of different length.

Practically all known PGs have the following conservative amino acid residues: N-191, N-212, N-213, R-267, and K-269; these are located together in a cavity and are able to form the active center of the molecule as revealed from the analysis of tertiary structure. Mutations of these amino acids in PG molecules of phytopathogenic fungi *A. niger* and *Fusarium moniliforme* led to full loss of enzyme activity [82]. The presence of H-188 at the edge of the active site is characteristic for all known PGs from phytopathogenic fungi. PGs isolated from plant tissues and possessing a lower activity have P in this position as well as W behind position 270. The replacement of H-188 with P or the introduction of W lead to a decrease in enzymatic activity of PG, which becomes comparable to PG activity in plants. Binding with a substrate leads to conformational changes in regions of the molecule remote from the binding site [83].

Upon pathogen penetration into plant tissue, the availability of homogalacturonan as a substrate for its PG plays the same role as the quantity and activity of the enzyme [65]. During plant ontogenesis, pectin demethylation takes place leading to the formation of charged surfaces that modulate the balance of pH and ions and limit the mobility of charged proteins [84]. An important role in this process belongs to PME, which demethylates pectin [49]. When bound to homogalacturonan, PGIP can interfere with its cleavage by blocking sites of the molecules subject to enzyme action. A change in pH in the apoplast as a result of blocking of carboxyl groups by PGIP molecules can change the activity of enzymes localized in the cell wall [65]. Hence, PGIP bound to homogalacturonan can participate in the regulation of PG functioning, in particular by interfering with its action as a pathogenic factor.

INTERACTION OF PGIP WITH PG

The main data profile on inhibitory action of PGIP is obtained from intensive investigations of model systems

using PG from phytopathogenic fungi [85]. In the cases where the inhibition is found, PGIP reversibly forms a strong complex with PG at concentration ratio of 1 : 1. It was shown that amino acids in the PGIP molecule defining the specificity and affinity to fungal PG are located inside the conservative site, xxLxLxx, which forms β-sheet/β-loop structure exposed to the solvent [13, 86]. Proteins PvPGIP1 and PvPGIP2 from *P. vulgaris* differ only in eight amino acid residues located inside or near this site. The first is unable to interact with PG from *F. moniliforme* and interacts with PG from *A. niger*, the second interacts with both PGs. Single mutations of amino acid residues of PvPGIP2 to the corresponding residues of PvPGIP1 cause the loss of affinity to PG from *F. moniliforme*. On the other hand, the single mutation of K in position 253 of PvPGIP1 to the corresponding amino acid from PvPGIP2 (Q) was enough to provide the protein with the ability to interact with PG from *F. moniliforme*. Therefore, sequence variability in β-sheet/β-loop structure influences ligand binding and has a functional value for the PGIP molecule, defining the selectivity of PG recognition. Information on structural features that provide LRR proteins with the ability to specifically interact with ligands will probably allow manipulations with cell functions controllable by these proteins.

The PG molecule forms multiple contacts with PGIP [81]. In PG from *F. moniliforme*, amino acids K-269 and R-267, located inside the active site where a substrate is bound, as well as residue H-188 located on the edge of active site, directly participate in the formation of a complex with PGIP from bean, PvPGIP2 [13]. Mutations leading to the replacement of these amino acids resulted in the loss of enzymatic activity but did not interfere with the binding to PvPGIP2.

It is assumed that PGIP binds pectin by the "convex" side of the molecule. Thus its "concave" side, containing the PGIP binding site, is free and can participate in the interaction with other proteins of cell wall or with PG [14].

The kinetics of the inhibitory action of PGIP on PG varies regarding the competition for substrate [15, 87, 88]. In some inhibitor–enzyme pairs, the inhibition is competitive, and in others it is incompetitive; inhibition of mixed type that indicates the participation of different sites in the interaction is also observed [87, 89]. During the interaction of PG–substrate complex with PGIP, an inhibitor binds the enzyme in the region of the molecule remote from the substrate-binding site and limits conformational changes in PG. Probably such an interaction can explain the incompetitive inhibition of PG. The activating action of PGIP, which can be the consequence of a structural modification of PG molecule during the allosteric binding of an inhibitor, has also been noted [90]. These differences question whether PG of pathogens is really the unique target of an inhibitor [52].

The degree and the site of glycosylation of the inhibitor molecule are very important for the interaction of PGIP and PG. Bean PGIP that was expressed in tomato plants had lost the ability to inhibit PG from *F. moniliforme* [89]. On the other hand, citrus PGIP, expressed in *Escherichia coli*, did not have the posttranslational modification associated with the glycosylation, but its inhibitory activity did not disappear. In the presence of this PGIP, the activity of PG from *A. niger* was decreased almost to 60% [91].

Glycosylation at some sites influences the interaction. The investigation of Cf-9 structure, an eLRR protein that is connected with tomato resistance to *Cladosporium fulvum*, has shown that many glycosylation sites, especially in the region of α-helix, are important for the activity of the protein [92]. Glycosylation in the cavity formed by β-sheets and apparently causing the specificity blocks its participation in resistance.

It could not be ruled out that upon the penetration of a pathogen PGIP bound to pectin is released as a result of complex formation with fungal PG, providing the availability of homogalacturonan to the enzyme.

So far, the direct action of PGIP on PG from plants has not been observed. However, such investigations are not numerous, and that have been performed mainly with PG and PGIP isolated from the same plants. The expansion of experimental studies will probably provide additional data. It was found that PGIP from bean does not act on PG from the same plant and some others, but inhibits exo-PG from corn pollen [93]. PG from tomato fruits is not inhibited by its own PGIP, which is in agreement with the normal softening of plant fruits overexpressing PGIP [16, 89].

Influence of PGIP on disease progression. The inhibition of PG from phytopathogenic organisms by PGIP preparations gives certain optimism regarding its use for the increase of plant resistance to diseases.

The expression of PGIP from pear led to a decrease in injury of transgenic tomato plants by the pathogen *Botrytis cinerea* [16]. However, transgenic tomato plants expressing bean PGIP did not become more resistant to the infection with pathogen whose PGs were treated with PGIP [89]. The overexpression of endogenic *PGIP* genes in *Arabidopsis* led to the reduction of diseases symptoms [94]. The speed of distribution of the fungus *Monilia fructigena* on apple fruits changed in inverse proportion to PGIP content [32, 95]. The action of PGIP from apple fruits on PG from some other phytopathogenic fungi has been observed [96, 97].

Quite often fungal PG injuring a certain plant species is insensitive to the action of PGIP from the same plant. However, such regularity is not always observed. It appeared that PGIP from apple fruits acted differently on fungal PG both pathogenic and non-pathogenic for apples [8]. Besides, some fungi caused the disease of fruits irrespectively of the inhibitory action of PGIP on the enzyme.

The expression of apple tree PGIP in potato led to plant juvenilization [98]. Here an increase in resistance to

the infection with *Verticillium dahliae* was noted, which apparently was not connected with the inhibition of PG activity. Increased PGIP content is characteristic for young tissues. Probably the reverse effect also takes place: overexpression of PGIP leads to changes in plant metabolism characteristic for earlier stages of development.

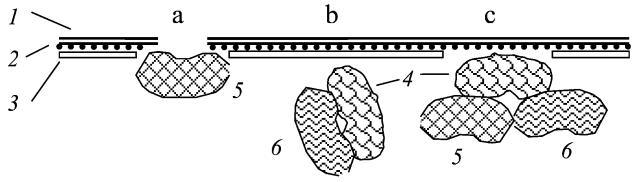
An increase in plant resistance upon PGIP overexpression is probably caused by the specific binding not only with pathogenic PG, but also with plant homogalacturonan. The following data provide evidence in favor of this hypothesis: the expression of some PGIPs are induced during wounding or the action of oligouronides (i.e. during endogenous changes), but does not occur upon the action of external factors such as pathogen metabolites or jasmonic or salicylic acids [99].

It is likely that other eLRR proteins binding pectin as well as PGIP, even though not rendering direct inhibitory action on PG from phytopathogenic organisms, can still shield homogalacturonan regions from the action of the enzyme. In that case the accumulation of these proteins in tissues will cause an increase in plant resistance to infection with at least some pathogens. The selection of pathogens whose PG does not bind PGIP and is capable of cleaving polymers of cell wall despite the presence of PGIP apparently occurs. Such pathogens appear to be capable of causing the diseases of corresponding plants.

Proteins of cell wall and apoplast represent a significant part of the plant proteome though they are poorly studied in comparison with the complex of proteins of chloroplasts, mitochondria, or other cell compartments [100, 101]. Extracellular proteins localized outside cytoplasmic membrane are rather conditionally distinguished into structural proteins immobilized on cell wall and soluble apoplast proteins, which are easy to extract and often possess enzymatic activity.

PGIP is likely to be one of the components of cell wall defining some of its mechanical properties and mediating the biochemical processes connected with growth, extension, and maceration and influencing plant morphology (Scheme). According to structural features of the molecule, it binds demethylated sites of homogalacturonan in pectin molecule that can provide an increase in the durability of cell wall and also regulate the process of its extension. The binding of PGIP with carboxyl residues formed as a result of mechanical separation of walls of adjacent cells during the formation of intracellular spaces or other cavities is also possible. Here high PGIP content can cause more intensive maceration.

An organism penetration into plant tissues (bacterium, fungus, parasitizing higher plant, etc.) introduces additional components in the complex of cell wall polymers. The degree of compatibility of these components with the processes in such a complex defines the opportunity and duration of coexistence of two organisms. PGIP is apparently one of the important factors in this system. The impairment of a balance in the system of polymers of cell wall upon the action of some factors (pathogenic PG, elicitors, oligouronides, etc.) leads to the induction of processes restoring the balance, in particular, of PGIP biosynthesis.



Scheme of the interaction of homogalacturonan, PGIP, and PG: a) PGs of plant or pathogen hydrolyze homogalacturonan; b) PGIP inhibits the activity of pathogenic PG; c) PGIP shields homogalacturonan site, interfering with the action of PG from plant or pathogen. *1*) Homogalacturonan; *2*) carboxyl groups; *3*) methyl groups; *4*) PGIP; *5*) plant PG; *6*) pathogen PG

The role of PGIP in resistance to pathogens is apparently a secondary one, as well as, possibly, roles of other plant proteins connected with pathogenesis. PGIP structure inherited in a plant defines the character of its interaction with PG of intruding alien organism and is one of the factors responsible for the spectrum of pathogens causing diseases of a given plant species.

The need to increase studies for the accumulation of information on PGIP localization and action in plant tissues regarding the features of their morphology and biochemical processes taking place in cell wall is obvious. It will help to define the role of PGIP in a sophisticated complex of polymers comprising cell wall of plant and also to estimate opportunities and ways of its use for increasing the resistance of plants to the influence of pathogenic organisms and other adverse factors.

REFERENCES

1. Albersheim, P., and Anderson, A. J. (1971) *Proc. Natl. Acad. Sci. USA*, **68**, 1815-1819.
2. Darvill, A., Bergmann, C., Cervone, R., de Lorenzo, G., Ham, K.-S., Spiro, M. D., York, W. S., and Albersheim, P. (1994) *Biochem. Soc. Symp.*, **60**, 89-94.
3. De Lorenzo, G., D'Ovidio, R., and Cervone, F. (2001) *Annu. Rev. Phytopathol.*, **39**, 313-335.
4. Abu-Goukh, A. A., and Labavitch, J. M. (1983) *Physiol. Plant Pathol.*, **23**, 123-135.
5. Favaron, F., Castiglioni, C., D'Ovidio, R., and Alghisi, P. (1997) *Physiol. Mol. Plant Pathol.*, **50**, 403-417.
6. Di, C., Zhang, M., Xu, S., Cheng, T., and An, L. (2006) *Crit. Rev. Microbiol.*, **32**, 91-100.
7. Federici, L., di Matteo, A., Fernandez-Recio, J., Tsernoglou, D., and Cervone, F. (2006) *Trends Plant Sci.*, **11**, 65-70.
8. Glinka, E. M., Protsenko, M. A., Bulantseva, E. A., and Sal'kova, E. G. (2001) *Prikl. Biokhim. Mikrobiol.*, **37**, 607-611.
9. Kajava, A. V. (1998) *J. Mol. Biol.*, **277**, 519-527.

10. Martin, G. B., Bogdanove, A. G., and Sessa, G. (2003) *Annu. Rev. Plant Biol.*, **54**, 23-61.
11. Mattei, B., Bemalda, M. S., Federici, L., Roepstorff, P., Cervone, F., and Boffi, A. (2001) *Biochemistry*, **40**, 569-576.
12. Di Matteo, A., Federici, L., Mattei, B., Salvi, G., de Lorenzo, G., Tsernoglou, D., and Cervone, F. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 10124-10128.
13. Leckie, F., Mattei, B., Capodicasa, C., Hemmings, A., Nuss, L., Aracri, B., de Lorenzo, G., and Cervone, F. (1999) *EMBO J.*, **18**, 2352-2363.
14. D'Ovidio, R., Mattei, B., Roberti, S., and Bellincampi, D. (2004) *Biochim. Biophys. Acta*, **1696**, 237-244.
15. Lafitte, C., Barthe, J. P., Montillet, J. L., and Touze, A. (1984) *Physiol. Plant Pathol.*, **25**, 39-53.
16. Powell, A. L. T., van Kan, J., ten Have, A., Visser, J., Greve, L. C., Bennett, A. B., and Labavitch, J. M. (2000) *Mol. Plant–Microbe Interact.*, **13**, 942-950.
17. Krinitsyna, A. A., Speranskaya, A. S., Poltronieri, P., Santino, A., Bogacheva, A. M., Buza, N. L., Protsenko, M. A., and Shevelev, A. B. (2006) *Genetika*, **42**, 477-486.
18. Salvi, G., Giarrizzo, F., de Lorenzo, G., and Cervone, F. (1990) *Plant Physiol.*, **136**, 513-518.
19. Gazendam, I., Oelofse, D., and Berger, D. K. (2004) *Physiol. Mol. Plant Pathol.*, **65**, 145-155.
20. Milner, J., and Avigard, G. **(**1967) *Carbohydr. Res.*, **4**, 359-3621.
21. Dingle, J., Reid, W. W., and Solomonos, G. L. **(**1953) *J. Sci. Food Agric.*, **4**, 149-153.
22. Devoto, A., Clark, A. J., Nuss, L., Cervone, F., and de Lorenzo, G. (1997) *Planta*, **202**, 284-292.
23. Pressey, R. (1996) *Phytochemistry*, **42**, 1267-1270.
24. Toubart, P., Desiderio, A., Salvi, G., Cervone, F., Daroda, L., de Lorenzo, G., Bergmann, C., Darvill, A. G., and Albersheim, P. (1992) *Plant J.*, **2**, 367-373.
25. Buza, N. L., Krinitsyna, A. A., and Protsenko, M. A. (2005) *Materials of Int. Sci.-Pract. Conf. "Urgent Problems of Protection of Potato, Fruit and Vegetable Cultures from Diseases, Vermins, and Weeds"*, Minsk, pp. 392-396.
26. Degra, L., Salvi, G., Mariotti, D., de Lorenzo, G., and Cervone, F. (1988) *J. Plant Physiol.*, **133**, 364-366.
27. Guyon, V., Tang, W., Monti, M. M., Raiola, A., de Lorenzo, G., McCormick, S., and Taylor, L. P. (2004) *Plant J.*, **39**, 643-654.
28. Thornburg, R. W., Carter, C., Powell, A., Mittler, R., Rizhsky, L., and Horner, H. T. **(**2003) *Plant Syst. Evol.*, **238**, 211-218.
29. Fish, W. W. (2005) *Eur. J. Plant Pathol.*, **111**, 67-76.
30. Abu-Goukh, A. A., Strand, L. L., and Labavitch, J. M. (1983) *Physiol. Plant Pathol.*, **23**, 101-109.
31. Stotz, H. U., Contos, J. J. A., Powell, A. L. T., Bennett, A. B., and Labavitch, J. M. (1994) *Plant Mol. Biol.*, **25**, 607-617.
32. Buza, N. L., Krinitsyna, A. A., Protsenko, M. A., and Vartapetyan, V. V. (2004) *Prikl. Biokhim. Mikrobiol.*, **40**, 104-108.
33. Johnston, D. J., Ramanathan, V., and Williamson, B. (1993) *J. Exp. Bot.*, **44**, 971-976.
34. Mehli, L., Schaart, J. G., Kjellsen, T. D., Tran, D. H., Salentijn, E. M. J., Schouten, H. J., and Iversen, T. H. (2004) *New Phytologist*, **163**, 99-110.
35. D'Hallewin, G., Schirra, M., Powell, A. L. T., Greve, L. C., and Labavitch, J. M. (2004) *Physiol. Plant.*, **120**, 395-404.
36. Redgwell, R. J., MacRae, E. A., Hallett, I., Fischer, M., Perry, J., and Harker, R. (1997) *Planta*, **203**, 162-173.
37. Nuss, L., Mahe, A., Clark, A. J., Grisvard, J., Dron, M., Cervone, F., and de Lorenzo, G. (1996) *Physiol. Mol. Plant Pathol.*, **48**, 83-89.
38. Li, R., Rimmer, R., Min, Yu, Sharpe, A. G., Serguin-Swartz, G., Lydiate, D., and Hegedus, D. D. (2003) *Planta*, **217**, 299-308.
39. Ahsan, N., Yoon, H.-S., and Jo, J. (2005) *Plant Sci.*, **169**, 1081-1089.
40. Devoto, A., Leckie, F., Lupotto, E., Cervone, F., and de Lorenzo, G. (1998) *Planta*, **205**, 165-174.
41. Worrall, D., Elias, L., Ashford, D., Smallwood, M., Sidebottom, C., Littford, P., Talford, J., Holt, C., and Bowles, D. (1998) *Science*, **282**, 115-117.
42. Meyer, K., Keil, M., and Naldrett, M. J. (1999) *FEBS Lett.*, **447**, 171-178.
43. Zhang, D.-Q., Wang, H.-B., Liu, B., Feng, D.-R., He, Y.-M., and Wang, J.-F. (2006) *Acta Genet. Sin.*, **33**, 1027-1036.
44. Baumberger, N., Steiner, M., Ryser, U., Keller, B., and Ringli, C. (2003) *Plant J.*, **35**, 71-81.
45. Stratford, S., Barnes, W., Hohorst, D. L., Sagert, J. G., Cotter, R., Golubiewski, A., Showalter, A. M., McCormick, S., and Bedinger, P. **(**2001) *Plant Mol. Biol.*, **46**, 43-56.
46. Jang, S., Lee, B., Kim, C., Kim, S.-J., Yim, J., Han, J.-J., Lee, S., Kim, S.-R., and An, G. (2003) *Plant Mol. Biol.*, **53**, 357-369.
47. Albersheim, P., Darvill, A. G., O'Neill, M. A., Schols, H. A., and Voragen, A. G. J. (1996) in *Pectins and Pectinases* (Visser, J., and Voragen, A. G. J., eds.) Elsevier Science BV, Amsterdam, pp. 47-55.
48. Perez, S., Mazeau, K., and Herve du Penhoat, C. (2000) *Plant Physiol. Biochem.*, **38**, 37-55.
49. Micheli, F. (2001) *Trends Plant Sci.*, **6**, 414-419.
50. Willats, W. G. T., McCartney, L., Mackie, W., and Knox, J. P. (2001) *Plant Mol. Biol.*, **47**, 9-27.
51. Wydra, K., and Beri, H. (2006) *Physiol. Mol. Plant Pathol.*, **68**, 41-50.
52. Di Matteo, A., Bonivento, D., Tsernoglou, D., Federici, L., and Cervone, F. (2006) *Phytochemistry*, **67**, 528-533.
53. Sakamoto, T., Bonnin, E., and Thibault, J.-F. (2003) *Biochim. Biophys. Acta*, **1621**, 280-284.
54. Spadoni, S., Zabotina, O., di Matteo, A., Mikkelsen, J. D., Cervone, F., de Lorenzo, G., Mattei, B., and Bellincampi, D. (2006) *Plant Physiol.*, **141**, 557-564.
55. Willats, W. G. T., Marcus, S. E., and Knox, J. P. (1998) *Carbohydr. Res.*, **308**, 149-152.
56. Jarvis, M. C., Briggs, S. P. H., and Knox, J. P. (2003) *Plant Cell Environ.*, **26**, 977-989.
57. Willats, W. G. T., Limberg, G., Buchholt, H. C., van Alebeek, G.-J., Benen, J., Christensen, T. M. I. E., Visser, J., Voragen, A., Mikkelsen, J. D., and Knox, J. P. (2000) *Carbohydr. Res.*, **327**, 309-320.
58. Clausen, M. H., Willats, W. G. T., and Knox, J. P. (2003) *Carbohydr. Res.*, **338**, 1797-1800.
59. Roberts, J. A., Elliott, K. A., and Gonzalez-Carranza, Z. H. (2002) *Annu. Rev. Plant Biol.*, **53**, 131-158.
60. Bush, M. S., Marry, M., and Huxham, I. M. (2001) *Planta*, **213**, 869-880.
61. Salerno, M.-I., Gianinazzi, S., Arnould, C., and Gianinazzi-Pearson, V. (2004) *J. Gen. Plant Pathol.*, **70**, 153-158.

62. Lenartowska, M., Rodriguez-Garcia, M. I., and Bednarska, E. (2001) *Hort. Planta*, **213**, 182-191.
63. Leboeuf, E., Guillon, F., Thoiron, S., and Lahaye, M. (2005) *J. Exp. Bot.*, **56**, 3171-3182.
64. Hadfield, K. A., and Bennett, A. B. (1998) *Plant Physiol.*, **117**, 337-343.
65. Brummell, D. A., and Harpster, M. H. (2001) *Plant Mol. Biol.*, **47**, 311-340.
66. Smith, C. J. S., Watson, C. F., Ray, J., Bird, C. R., Morris, P. C., Schuch, W., and Grierson, D. (1988) *Nature*, **334**, 724-726.
67. Kalaitzis, P., Solomos, T., and Tucker, M. L. (1997) *Plant Physiol.*, **113**, 1303-1308.
68. Roberts, J. A., Whitelaw, C. A., Gonzalez-Carranza, Z. H., and McManus, M. T. (2000) *Ann. Bot.*, **86**, 223-235.
69. Pressey, R., and Reger, B. J. (1989) *Plant Sci.*, **59**, 57-62.
70. Rhee, S. Y., and Somerville, C. R. (1998) *Plant J.*, **15**, 79-88.
71. Sander, L., Child, R., Ulvskov, P., Albrechtsen, M., and Borkhardt, B. (2001) *Plant Mol. Biol.*, **46**, 469-479.
72. Dearnaley, J. D. W., and Daggard, G. A. (2001) *Sex Plant Reprod.*, **13**, 265-271.
73. Capodicasa, C., Vairo, D., Zabotina, O., McCartney, L., Caprari, C., Mattei, B., Manfredini, C., Aracri, B., Benen, J., Knox, J. P., de Lorenzo, G., and Cervone, F. (2004) *Plant Physiol.*, **135**, 1294-1304.
74. Atkinson, R. G., Schroder, R., Hallett, I. C., Cohen, D., and MacRae, E. A. (2002) *Plant Physiol.*, **129**, 122-133.
75. Pressey, R., and Avants, J. K. (1977) *Plant Physiol.*, **60**, 548-553.
76. Zang, Z., Pierce, M. L., and Mort, A. (2007) *Phytochemistry*, **68**, 1094-1103.
77. Sitrit, Y., Downie, B., Bennet, A. B., and Bradford, K. J. (1996) *Plant Physiol.*, **111**, 161 (Abstr. No. 752) Suppl.
78. Peretto, R., Favaron, F., Bettini, V., de Lorenzo, G., Marini, S., Alghisi, P., Cervone, F., and Bonfante, P. (1992) *Planta*, **188**, 164-172.
79. Jones, T. M., Anderson, A. J., and Albersheim, P. (1972) *Physiol. Plant Pathol.*, **2**, 153-166.
80. Vasil'eva, K. V., Gladkikh, T. A., and Davydova, M. A. (1984) in *Biochemistry of Immunity, Rest, and Aging of Plants* (Berezin, I. V., ed.) [in Russian], Nauka, Moscow, pp. 105-124.
81. Herron, S. R., Benen, J. A., Scavetta, R. D., Visser, J., and Jumak, F. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 8762-8769.
82. Federici, L., Caprari, C., Mattei, B., Savino, C., di Matteo, A., de Lorenzo, G., Cervone, F., and Tsernoglou, D. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 13425-13430.
83. King, D., Bergmann, C., Orlando, R., Benen, J. A., Kester, H. C., and Visser, J. (2002) *Biochemistry*, **41**, 10225-10233.
84. Carpita, N. C., and Gibeaut, D. M. (1993) *Plant J.*, **3**, 1-30.
85. Sakamoto, T., Bonnin, E., and Thibault, J.-F. (2003) *Biochim. Biophys. Acta*, **1621**, 280-284.
86. Caprari, C., Mattei, B., Basile, M. L., Salvi, G., Crescenzi, V., de Lorenzo, G., and Cervone, F. (1996) *Mol. Plant–Microbe Interact.*, **9**, 617-624.
87. Stotz, H. U., Bishop, J. G., Bergmann, C. W., Koch, M., Albersheim, P., Darvill, A., and Labavitch, J. M. **(**2000) *Physiol. Mol. Plant Pathol.*, **56**, 117-130.
88. Barmore, C. R., and Nguyen, T. K. (1985) *Phytopathology*, **75**, 446-449.
89. Desiderio, A., Aracri, B., Leckie, F., Mattei, B., Salvi, G., Tigelaar, H., van Roekel, J. S. C., Baulcombe, D. C., Melchers, L. S., de Lorenzo, G., and Cervone, F. (1997) *Mol. Plant–Microbe Interact.*, **10**, 852-860.
90. Kemp, G., Stanton, L., Bergmann, C. W., Clay, R. P., Albersheim, P., and Darvill, A. (2004) *Mol. Plant–Microbe Interact.*, **17**, 888-894.
91. Nalumpang, S., Gotoh, Y., Tsuboi, H., Gomi, K., Yamamoto, H., and Akimitsu, K. **(**2002) *J. Gen. Plant Pathol.*, **68**, 118-127.
92. Van der Hoorn, R. A. L., Wulff, B. B. H., Rivas, S., Durrant, M. C., van der Ploeg, A., de Wit, P. J. G. M., and Jonesb, J. D. G. (2005) *Plant Cell*, **17**, 1000-1015.
93. Cervone, F., de Lorenzo, G., Pressey, R., Darvill, A. G., and Albersheim, P. (1990) *Phytochemistry*, **29**, 447-449.
94. Ferrari, S., Vairo, D., Ausubel, F. M., Cervone, F., and de Lorenzo, G. (2003) *Plant Cell*, **15**, 93-106.
95. Wu, Q., Szakacs-Dobozi, M., Hemmat, M., and Hrazdina, G. (1995) *Plant Physiol.*, **102**, 219-225.
96. Yao, C. L., Conway, W. S., and Sams, C. E. (1993) *Phytopathology*, **85**, 1373-1377.
97. Brown, A. E. (1984) *Phytopathol. Z.*, **111**, 122-132.
98. Gazendam, I., Oelofse, D., and Berger, D. K. (2004) *Physiol. Mol. Plant Pathol.*, **65**, 145-155.
99. Federici, L., di Matteo, A., Fernandez-Recio, J., Tsernoglou, D., and Cervone, F. (2006) *Trends Plant Sci.*, **11**, 65-70.
100. Lee, S.-J., Saravanan, R. S., Damasceno, C. M. B., Yamane, H., Kim, B.-D., and Rose, J. K. C. **(**2004) *Plant Physiol. Biochem.*, **42**, 979-988.
101. Gorshkova, T. A. (2007) *Plant Cell Wall as a Dynamic System* [in Russian], Nauka, Moscow.