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Plant Immunity and Pathogen Interfering Mechanisms: Effectors and Bodyguards

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Abstract There is an arm race between plants and their pathogens, by fungi, and bacteria, as well as between plants and insects. Plant proteases are hydrolytic enzymes, grouped on the basis of the catalytic amino acid, as serine, cysteine, aspartic acid, or metal dependent activity. Plant-fungi interactions, as well as plants with other invaders, have been elucidated in recent years, showing an evolutionary adaptation of hosts and invaders to produce proteases and evolve new protease inhibitors. Interactions between protease inhibitors and the target proteases provide information on the ways organisms interact and defend themselves from pathogens, recognizing symbionts from parasite organisms. A comparative analysis of protease inhibitors in plants with sequenced genomes have been recently performed. In the analysis of PIs, protease biochemical assays, protein-protein interaction studies and protease chips were used to analyze constitutive and inducible inhibitors under different conditions. Recently, activity-based protease profiling (ABPP) was used to differentiate enzymes tissue specificity, and roles in various physiological and pathological states. Specificities of PIs toward different protease (serine and cysteine proteases) can allow to selectively and differentially bind and detect various proteases. In this review we summarize the most recent knowledge on plant pathogens and the mechanisms they evolved to circumvent plant defences among which pathogen effectors, proteases and proteases inhibitors. Finally, we introduce the recent findings on pathogen bodyguards, proteins interfering with plant defence mechanisms or decoys, mimicking Transcription Activator Like Effectors (TALE). It is envisaged that further advances in understanding the function of pathogen effectors will provide new ways to improve plant immunity and mechanisms of defence against their pests.

Keywords Protease, Inhibitors, Interaction, Cell signaling, Apoptosis, Necrosis, Plant immunity, Fungi, Bacteria

1. Introduction

Proteases are hydrolytic enzymes, grouped on the basis of the catalytic amino acid, as serine, cysteine, aspartic acid, threonine, or metal dependent activity, grouped in the MEROPS database according to specificity and mechanism of activity. Studies on proteases have provided information on sequence-specific cleavage sites, target sequence-based inhibitors, and chemicals used in blocking their activity. Before the era of recombinant proteins expressed in *E. coli* or yeast, it was practice to assay the activity of proteases in plant tissues by making plant extracts. In these extracts, it is required to add protease inhibitors cocktails, to inactivate proteases with activity different from the target protease to be studied. In addition, plant extracts may be rich in protease inhibitors, therefore a protein denaturation protocol followed by protein renaturation (i.e. in-gel activity assay) or HPLC fractionation method was required. In the present days, studies are made possible by the use of enhanced expression (inducible, permanent or transient expression) and knock outs or plant mutants. Activity-based protease profiling (ABPP) is useful technique that enable to differentiate enzymes, their tissue specificity, and roles in various physiological and pathological states [1]. Specific assays for enzyme activity have been developed by means of click chemistry and biotin-tag fluorescent probes featuring an electrophlic trap. The method is based on chemical probes that react with the catalytic site of different enzymes in an activity-dependent manner. Labelled proteins are detected on 2-D polyacrylamide gels and identified by MALDI-TOF mass analysis. It was shown that specific activity of cysteine proteases could be identified during seed germination in Arabidopsis [2] and in tomato seedling undergoing hypersensitive reaction [3].

1.1. Plant Proteases

Basically, proteases are named for the amino acids within their active sites: serine proteases, cysteine proteases, aspartic acid or threonine proteases, and metalloproteases.

Plant genomes contain hundreds of genes classified as proteases, but for only one third of them a function is known.

In the *Arabidopsis thaliana* genome, the protease coding genes approach almost 900 transcripts [4], Roles of

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ubiquitin-26S proteasome system (UPS) in regulatory proteolysis in Arabidopsis-pathogen interactions [4], and in senescence [5] have been shown.

The plant apoplast contains many proteases, as a first line of defense against effectors from fungi and oomycetes. Several proteases are inducible: the expression is triggered by sensing the pathogens and its effectors [6]. One of the most conserved proteases in plants are asparaginyl endopeptidases (AEP), such as Vacuolar Processing Enzymes (VPE).

These AEPs, required during tissue remodelling, cleave after asparagine residues, as well as after aspartic acid, possessing a caspase-like activity, being inhibited by YVAD-CHO aldehyde, a sequence specific caspase inhibitor [7]. The QACRG amino acid sequence in the active site of mammalian caspase-1 is similar to the E(A/G)CES sequence of the active site of VPEs, preserving the cysteine in the active site, that is also the target of cystatin inhibitors.

The sequence following the glycine, GxC/SxG, conserved in many proteases in viral genomes, shows the appearance of a second cysteine in proteases with a serine instead of cysteine in the GACRG, allowing the inhibition by plant cystatins [8].

Vacuolar Processing Enzyme (VPE) is involved in various physiological responses, as well as in cell death during Tobacco Mosaic Virus (TMV) infection, while other pathways bring to the expression of pathogenesis-related (PR) proteins [9]. The Hypersensitive Response (HR) is based on two processes, Programmed Cell Death (PCD) and induction of defence proteins, while VPE regulates only PCD [7]. Also a group of papain-type cysteine endopeptidases (CysEP), characterized by a localization signal (KDEL) for endoplasmic reticulum (ER) in their C-terminal, is involved in PCD in plants [10]. An oat subtilisin-like protease, saspase, with activity similar to caspases, was reported to hydrolyze sequence substrates such as DEVD [11]. Furthermore, metacaspases, cleaving at basic amino acid residues [12], containing domains pertaining to cysteine proteases, such as catalytic dyad His-Cys and the hemoglobinase fold [13], the proteasome-dependent threonine proteases [14], and phytaspases or saspases, have been identified and linked to specific plant processes and remodelling [15].

Studies in tomato showed that seven papain-like cysteine proteases (PLCPs), belonging to cathepsin family, are apoplast specific hydrolytic proteases**.** These proteins are synthesised as pre-proteases that after activation by pre-sequence cleavage during secretion are stored in the apoplast as mature 25–30 kDa proteins. Some PLCP carries a C-terminal granulin domain, having a size of 40 kDa. The mature PLCPs present a structure with two lobes that confine the substrate binding groove having the catalytic triad (Cys-His-Asn) in its centre.

Plant PLCPs are divided into nine subfamilies [16]. Three of the subfamilies are phylogenetically separate from the other six, sharing some feature with human cathepsin B, cathepsin F, and cathepsin H. The other subfamilies are cathepsin L-like proteases, that can be differentiated by the presence of two cysteines in the active site, such as SAG12-like protease in subfamily 6, or, in subfamily 3, CEP1-like protease, with a localization signal for the endoplasmic reticulum [16].

Tomato cysteine proteases, for instance Rcr3, Pip1, aleurain, and TDI-65, are necessary during basal host defence against fungal pathogens. Pip1 and Rcr3 are strongly induced by effectors during fungal development as well as by hormones such as salicylic acid [17-20].

The structural basis for avoidance of cell damage resides in the prodomain nature, i.e. the protease is activated by the cleavage of the pre-pro sequences, and by the extrusion on the outside of the cell by the release from the apoplast.

For the other proteases, in many cases an inhibitor is abundantly expressed within the cell compartment, so that the enzyme activity is blocked.

It was shown that dehydrin-5 (DHN-5) expression in durum wheat transgenic lines affects, probably by its protein-protein interaction property, the activity of some proteases, with increase in total cysteine protease activity, and decrease of total activity of aspartyl proteases, especially under salt stress conditions [21].

1.2. Plant Immunity and Hypersensitive Response (HR)

The first layer of plant defense is based on Pattern Recognition Receptors (PRR) on the membrane. These receptors (either receptor kinases or receptor-like proteins that associate with a kinase) recognize as a fingerprint specific microbial compounds, named pathogen associated molecular patterns (PAMPs), thus activating a local response named PAMP-triggered immunity (PTI) [22, 23]. In LRR proteins, the leucine-rich repeats determine the three dimensional structure: leucines are hydrophobic amino acids that when repeated periodically determine protein–protein interactions, such as in leucine zipper proteins. LRRs proteins have a α/β horseshoe fold structure [24, 25] composed of several 20–30 amino acid stretches highly rich in leucine. These repeats are able to fold together in a solenoid-like domain, namely the LRR domain, that acts as a scaffold to keep in place an active site directed to interact with a binding partner. In most of the cases, each domain assumes a beta strand-turn-alpha helix structure, and the structure has a parallel beta sheet on the internal side and an array of helices dominated by hydrophilic residues on the external side, exposed to solvent. The region between the helices and sheets, rich in leucines, is hydrophobic. In addition to pathogen effector receptors such as RLPs and Nucleotide binding domain (NBD)-LRRs, LRRs are present in polygalacturonase-inhibiting proteins (PGIP). Therefore, plants evolved this protein-protein interaction domain several times during the development of mechanisms in defense from enzymes and virulence effectors of fungal pathogens.

Pathogens secrete toxins and/or effector proteins able to hijack PTI signaling and to inactivate PRR-based defences, in order to allow nutrients availability, and to support

pathogen spread. Large repertoires of effector activities have been found for pathogens with different lifestyles. There are effectors in extracellular bacteria released in host cells by type III secretion system (TTSS); other effectors in oomycetes and fungi able to invaginate specialized feeding organelles, called haustoria, into host cells; and salivary proteins delivered to plant cells during aphid and nematode feeding. Effectors from evolutionarily diverse pathogens are highly specialised and specific for a limited number of plant proteins with activity and role linked to plant immunity.

In response to microbial evolution, plants developed pathogen-race specific receptors able to trigger resistance through the recognition of single effector, i.e. effector triggered immunity (ETI). It has been shown that PTI and ETI have similar anti-pathogen outputs, but the effector-triggered immune response is generally stronger and leads up to a programmed cell death (PCD) or to local necrosis and containment of pathogen spread, at the basis of the hypersensitive response (HR).

1.3. Defensive Effectors in Plant Pathogens. Effectors Interfering with Plant Immunity

Here is a list of roles of effectors grouped in defensive effectors in symbiotic bacteria that interfere with some component of the plant immune system to protect the symbiosis, and offensive effectors that subvert some physiological functions of the plant for the benefit of the symbiont, i.e. to increase nutrient availability. Host physiological networks may trigger plant immunity and cause cell death while suppressing defence functions to promote nutrition. In addition, for the symbionts, it is necessary to avoid host cell death, while for a hemibiotroph apoptosis may be beneficial or undesirable, depending on the timing of the infection.

In general, the mechanisms of defence of plants against pathogens involve numerous signals, starting with detection of pathogen-derived molecules (PAMPs), and their effectors, followed by signal transduction from receptors to transcription factors, to the production of antimicrobial molecules and plant cell death.

1.4. Chemical Inhibition of Proteases

Protease inhibitors can be specific to one protease or broad enough to affect many proteases. Most inhibitors are classified according to the type of protease they act upon (e.g., serine protease inhibitor) and may be reversible or irreversible. To shed more light on finding inhibitors to protect plant tissue extracts from proteolysis, here is some advice on how best to choose protease inhibitors and use them. Diazo methylketones, tosyl lysine and tosyl phenylalanine chloromethyl ketones (TLCK, TPCK) are mixed type inhibitors acting on serine and thiol proteases; phenylmethane sulfonyl fluoride (PMSF), and 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF), are sulfonylating agents reacting with the histidine of the catalytic triad within the serine protease's active site; benzamidine inhibits serine proteases. *N*-acetyl-L-leucyl -L-leucyl-L-argininal, or leupeptin, is a nature-based protease inhibitor inhibiting either cysteine, serine and threonine peptidases. The serine proteases are inactivated by PMSF, which reacts with serine in the active site; *p*-chloromercuribenzoic acid (PCMB), N-ethyl~maleimide (NEM), *N*-[*N*-(L-3-*trans*-carboxyirane-2-carbonyl)-L-leucyl] -agmatine and E-64 epoxide are thiol protease inhibitors.

The mechanisms of activity of protease inhibitors may be various. During plant extract preparation experiments, it may help to approach inhibitor choice in a more focused manner. Protease inhibitors may behave as tight-binding reversible or pseudo-irreversible inhibitors, and prevent substrate access to the active site through steric hindrance. Other inhibitors may exert their effects through modification of an amino acid in the protease's active site. The specificity of the target sequence containing the P' site of protease attack has been exploited in the studies on caspase inhibitors, using different sequence specific targets (YVAD) followed by coumaryl groups that release fluorescence after proteolysis or by aldehyde groups that inhibit the proteolytic cleavage. An assay for detection of proteases was set up using a biotinylated irreversible caspase-1 inhibitor (biotin-xVAD-fmk) to detect VPE, either as mature protease or its pre-protease, in virus infection of tobacco leaves [7].

1.5. Plant Protease Inhibitors

Serine protease inhibitors from the Kunitz family are present in many higher plants. Kunitz inhibitors are trypsin or papain inhibitors, an activity that is exerted through overlapping protease binding sites. Potato (*Solanum tuberosum* L.) tubers, as many other Solanum species, contain various Kunitz-type protease inhibitors (PKPIs), with a size of 23-24 kDa, [26-29], originated from gene duplication, having a tertiary structure, the β-trefoil fold, with a tree-like shape. Potato tubers contain also a serine protease inhibitor (PSPI) with a Janus-type multifunctionality, binding simultaneously trypsin and chymotrypsin [30, 31]. Recently, Gebhardt and colleagues have tested the activity of recombinant Kunitz inhibitors and other protease inhibitors (PI-I and PI-II) from potato tubers, and showed the inhibition *in vitro* of invertase, lipoxygenase, HIV protease, β secretase (BACE), elastase and cathepsin K [32].

Legumain-inhibiting cystatins, or phytocystatins [33], wit ha size of 12-16 kDa, are cysteine protease inhibitors. Phytocystatins are bifunctional inhibitors targeting different proteases through several mechanisms, for instance by steric hindrance, or mimicking the substrate in the case of AEP/VPE proteases. The inhibition of serine and cysteine protease by cystatins [34] relies on a tripartite wedge, formed by the N-terminus and two hairpin loops, a structure that contain the conserved QxVxG motif. This wedge is inserted into the active site of the serine protease in a tight but reversible interaction. The inhibition of VPE/AEP protease by phytocystatins is based on a similarity with the substrate,

in which an Asn residue enters the protease active site wit the support of an α-helix in the C-terminal [35].

Bifunctional inhibitors have been classified as Janus-type inhibitors, possessing two inhibition domains, able to bind different target proteases; or as multidomain proteins, assembling several inhibitor domain in series, such as the potato multicystatin (PMC), and tomato multicystatin impairing the spread of fungal pathogens in vitro [36], and macrocyclic cystine knot peptides (knottins) that possess either inhibitory and non-inhibitory roles. The cystine knot structure is formed by a hairpin of two antiparallel β-strands in which three cysteines are linked by three disulphide bridges. Squash inhibitors, abundant in the seeds of *Cucurbitaceae,* are macrocyclic knottins of less than 50 amino acids, with several disulphide bridges, inhibiting trypsin proteases. The squash inhibitors are originated from multidomain precursors containing several repeat units [37, 38].

Serpin and α-macroglobulin domains in promiscuous inhibitors exploit the same binding site to inhibit one of the target proteases. These inhibitors, different in structure, act as a mouse trap for the protease, due to a conformational change occurring after protease binding. Serpins inhibit the target proteases as a suicide inhibitor. Serine and cysteine proteases recognise the serpin reactive loop, and form an acyl-enzyme intermediate. Cleavage triggers a a conformational modification in the serpin, with the active site of the protease irreversibly binding the inhibitor. Similarly, CrmA inhibits animal caspases through the formation of inhibition complexes following the cleavage of target amino acid sequences. The trapping mechanism is shared by many serpins, with different protease specificity, targeting a range of Ser and Cys proteases [39, 34]. α-macroglobulins protease inhibition is based on an exposed bait region that recognise specifically one protease among several types: upon cleavage by the protease, the inhibitor undergoes a conformational change. In this way, the protease remain trapped inside the macroglobulin protein cage. The protease cannot bind to other proteins, while is accessible to small molecule probes [40]. The change in the bait region structure is at the basis of multifunctionality in macroglobulins [40].

Bifunctional protease inhibitors such Bowman–Birk inhibitors (BBIs) are single proteins that can inhibit two distinct proteases, using active sites to block trypsin and chymotrypsin [35]. BBIs contain two inhibition sites, localised in two regions at the opposed terminal of a beta-sheet core and function via the Laskowski mechanism: the inhibitor acts as a 'substrate with limited proteolysis' [41]. A reactive peptide bond on this limited substrate is bound by the protease, forming an acyl intermediate showing a high association constant. The proteolytic cleavage and the dissociation from the catalytic site is low. There is an equilibrium between the protease and inhibitor on the left side of the reaction equation, and the complex on the right. The intact and the cleaved inhibitor can bind the protease, and the reaction is reversible. Bifunctional inhibitors, such as barley α-amylase and subtilisin inhibitor (BASI) can form a complex with two enzymes, such as α-amylase and a protease.

In the analysis of PIs, protease biochemical assays, protein-protein interaction studies and protease chips were used to analyze constitutive and inducible inhibitors under different conditions. The specificities of PIs toward different protease (serine and cysteine proteases) can allow to selectively and differentially bind and detect various proteases.

Kunitz-type protease inhibitors have been applied in several protein chip studies. Potato tubers under *A. carbonarius* infection induce and accumulate several classes of PIs with different specificities, such as trypsin/ chymotrypsin inhibitors, followed by papain, ficin and cathepsin B inhibitors [29]. Recent studies showed the usefulness of these PIs as inhibitors of 5-lipoxygenase, HIV protease, β secretase (BACE), elastase and cathepsin K [32]. A comparative analysis of protease inhibitors in plants with sequenced genomes have been recently performed [42].

1.6. Fungal Proteases/Hydrolases and Interaction with Plant Protease Inhibitors

The main secreted proteases have been identified in *Fusarium* species. Fungalysin metalloproteases, subtilisin, polyglycine hydrolases, chymotrypsin, papain, among others.

The most studied fungal proteases in fungi, oomycete and bacteria plant pathogens, are AvrPphB in *P. syringae* pv. p*haseolicola*, AvrRpt2 in *P. syringae* pv. *tomato*, XopD in *X. campestris* pv. *vesicatoria*, HopX1 in *P. syringae* pv. *tabaci*, protease IV in *P. aeruginosa*, AprA in *P. syringae* pv. *tomato* [43]. The effector proteins of HopX1 family (also known as AvrPphE) are synthesised in *P. syringae* pathovars [43] (Pogany et al., 2015). It was shown that HopX1 from *Pseudomonas syringae* pv. *tabaci* (Pta) is a cysteine protease able to cleave Jasmonate ZIM domain (JAZ) protein, an inhibitor of Myc transcription factor activation by Jasmonate-isoleucine (JA-Ile). HopX1 activates the jasmonic acid pathway while suppressing salicylic acid response [44]. This pathway is in a different way exploited also by coronatine producing *Pseudomonas syringae*. CORONATINE INSENSITIVE1 (COI1) E3 ubiquitin ligase targets proteins to the proteasome degradation pathway. COI1 is an F-box protein taking part of an Skp1-Cullin-F-box (SCF) complex. SCF complex interacts with COI1 and bring the formation of the ternary complex COI1-JA-Ile-JAZ.

Plants produce proteinase inhibitors and pathogens resistance (PR) proteins to block the activity of fungal enzymes. In wheat, WAMPs are hevein-like antimicrobial peptides with inhibitor activity on fungalysin [45]. Hevein, a chitin-binding domain, is used to study carbohydrate-peptide interactions. WAMPs contain a Ser at position 36, and inhibit the activity of fungalysin (Fv-cmp), a Zn-metalloproteinase, isolated from *Fusarium verticillioides*. This protease is able to truncate corn and Arabidopsis class

IV chitinases by cleaving within the Gly-Cys site located in the chitin-binding domain of the plant chitinase. The presence of Ser36 confers to WAMP resistance to proteolysis by Fv-cmp [45].

UPI is an Unusual serine Protease Inhibitor found in barley and in faba bean.

Several tissue-specific protease inhibitors show elicitor inducibility and accumulation during fungal invasion, such as potato Kunitz-type PIs in leaves and tubers [29].

Recently, a biochemistry team has performed assays for ininhibition of insect peptidases, describing in detail the specific activity of the most used compounds, as well as proteomic tools, such as substrates, inhibitors, and activity-based probes [46].

1.7. Plant Enzymes-Fungal Inhibitors, Fungal Enzymes-Plant Inhibitors Interactions

Polygalacturonases and xylanases are fungal enzymes involved in cell wall degradation, and as such are recognised as PAMPs by specific receptors. Plants counterfight fungal attack by expressing xylanase inhibtors (XIP) and polygalacturonase inhibitors (PGIP) containing Leucine Rich Repeat (LRR) domains [47, 48].

Glucanases as beta-1-glucanase are plant enzymes that degrade fungal walls. *Phytophtora soyae* (*P. sojae*) secretes glucanase inhibitor protein-1 (GIP1), which targets endo β-1,3-glucanases in soybean.

Other fungal proteins have decoy function and antagonistic activity, such as chitin binding proteins that block chitinases and avoid chitin binding by CBD-chitinases in plants.

1.8. Fungal Pathogen Effectors. Role of Effectors of Virulence during Plant-Pathogen Interactions and Involvement in Tissue Invasion

The pathogen effectors are microbial proteins or secondary metabolites that subvert host physiology for the advantage of the microbe [49]. Effectors represent adaptation to hosts, evolved from genes and functions from saprotrophic ancestors and plant symbionts, from molecules used to suppress ecological competitors.

Botrytis and *Pythium* are necrotrophic pathogens, that destroy plant tissues with limited species specificity [49]. The pathogenicity is based on degrading enzymes or toxic metabolites, with a limited number of effectors produced, and cell killing protein toxins. Other fungi have a highly specialized life cycle and restricted host range. The fungi start a growth within the plant apoplast without any symptom, then pathogens produce metabolites and toxins targeting specifically gene products, i.e., a single gene of the pathogen interacts with a single gene of the plant to induce susceptibility [49]. Biotrophic pathogenic fungi, such as rust, powdery mildew, or white rust and downy mildew oomycetes, show host specificity and dependence on the host plant for metabolites. In this case, evolution toward pathogenicity has led to genome shrinking with loss of genes involved in nutrient acquisition, with expansion of effector genes [49].

The type III effector proteins are dependent on secretion machinery and are delivered into the host plant to manipulate host defence. To protect the effectors from host proteases, fungi evolved several mechanisms of protease inhibition [50].

P. infestans, in tomato infection, produces two cysteine protease inhibitors, EPIC1 and EPIC2b, that inhibit two proteases, C14 and Pip1 [51, 52]. Oomycetes can produce up to 12–15 Kazal type serine protease inhibitors. In *P. infestans*, EPI1 and EPI10 were characterized in depth [53]. Induced during infection, EPI1 and EPI10 interact and inhibit P69B cysteine protease in tomato apoplast [52]. In maize, fungal cysteine proteinase inhibitor Pit2 binds and inhibits CP2, CP1A and CP1B proteases. AvrP123, in *Melampsora lini*, was identified as a Kazal-like proteinase inhibitor [54]. As an exception to these findings, Kazal class inhibitors are lacking in many fungal genomes.

Cystatin-like EPIC proteins, secreted by the oomycete *Phytophthora infestans* (Pinf), target the C14 protease in *Solanaceae*. In Arabidopsis, pathogen *Hyaloperonospora arabidopsidis* (Hpa) produces EPIC-like cystatins targeting RD21 cysteine protease. Differently from the Pinf-*Solanaceae* pathosystem, the *rd21* mutants were susceptible to *Botrytis cinerea* [55].

Many pathogens possess and produce protease inhibitors able to protect the effectors from cleavage [51]. Many effector proteins secreted into the apoplast are rich in cysteine residues [54-57] forming disulfide bridges, that increase protein stability in a protease-rich environment. Among identified fungal effectors, Avr9 in *C. fulvum* is a protease inhibitor. Avr9 has a cysteine-knot structure resembling a carboxypeptidase inhibitor [58]. Avr9 is recognised by High Affinity Binding Sites (HABS) on plasma membrane, therefore triggering activation of the LRR receptor *Cf-9*.

During infection, *Cladosporium fulvum* produces several effectors. Both Rcr3 and Pip1 plant proteases are inhibited by Avr2 from *C. fulvum*. Avr2 inhibits tomato cysteine proteases, including Rcr3, Pip1, aleurain, and TDI-65, important in basal host defence. *Avr2* encodes a preprotein of 78 amino acids, and its mature form is a 58-amino acid protein with eight cysteine residues. The binding of Avr2 to Rcr3 causes the recognition of the complex by tomato *Cf-2* immune receptor [59]. When Cf-2 is secreted into the plant cell, Avr2 binds to an allele of Rcr3, Rcr3pimp, causing Effector Triggered Immunity (ETI) [17, 18].

Avr2 inhibits *Arabidopsis* cysteine proteases except B3 cathepsin. In a biochemical study, XCP1, XCP2 and CPR1 showed high Avr2 affinity, while Responsive to Dehydration 21A (RD21A) and aleurain and aleurain-like thiol proteases had low Avr2 affinity [43]. XCP2, RD21A and Responsive to Dehydration 21B (RD21B) were identified using yeast two-hybrid assays as interacting partners of PIRIN2 protease inhibitor in Arabidopsis [43]. PIRIN2 inhibits the autolytic degradation of XCP2, therefore it stabilises XCP2. The interaction between XCP2

and PIRIN2 is required for susceptibility to *Ralstonia solanacearum*, a bacterial pathogen colonizing the xylem, facilitated by proteolysis of cellular contents in leaves or vessel elements [60].

Cladosporium fulvum effector Avr4. CfrAvr4 binds and hides chitin from chitinases. However, the presence of the effector is sensed triggering the activation of *Cf-4*. In addition, effector proteins from *Ustilago maidis* can block plant immune responses by inducing expression of cystatins, counteracting the expression of cysteine proteinase C69 [61-65].

Recently, several scientists pointed out to the role of microbial decoys, proteins mimicking the interaction domain of a protein partner, thus impeding its accessibility, or enzymes and proteins interfering with a plant defence mechanism, such as Transcription Activator Like Effectors (TALE) mimics [66-69]. Such decoys have been named bodyguards, in that they are able to protect virulence factors from the action of resistance genes and plant defence pathways.

2. Conclusions

Plant-pathogen interactions mechanisms have been detailed in recent years. There is an evolutionary adaptation of hosts and invaders to produce proteins evolved to counteract host recognition and signaling.

In recent years we have seen huge advances exploiting the biotechnology methods, with characterization of protein-protein interactions. Further advances in understanding structure and function of effectors and response modulators in plant pathogens will allow to discover the mechanisms of adaptation of plant defences with novel resistance genes and alleles and understanding of the signaling pathways leading to plant immunity.

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