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Plant–pathogen interactions: what is proteomics telling us?

Angela Mehta[1], Ana C. M. Brasileiro[1], Djair S. L. Souza[1,2,*], Eduardo Romano[1,*], Magnólia A. Campos[3,*], Maria F. Grossi-de-Sá[1,*], Marília S. Silva[4,*], Octávio L. Franco[5,6,*], Rodrigo R. Fragoso[4,*], Rosangela Bevitori[7,*] and Thales L. Rocha[1,*]

1 Embrapa Recursos Genéticos e Biotecnologia, Brasília, Brazil
2 Departamento de Biologia Celular, Universidade de Brasília, Brazil
3 Universidade Federal de Lavras, Brazil
4 Embrapa Cerrados, Planaltina, Brazil
5 Centro de Análises Proteômicas e Bioquímicas, Pós-Graduação em Ciências Genomicas e Biotecnologia, Universidade Católica de Brasília, Brazil
6 Departamento de Biologia, Universidade Federal de Juiz de Fora, Brazil
7 Embrapa Arroz e Feijão, Goiânia, Brazil

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Correspondence
A. Mehta, Embrapa Recursos Genéticos e Biotecnologia, PBI, PqEB Av. W 5 Norte Final, CEP 70770-900 Brasília, DF, Brazil
Fax: +55 61 3340 3658
Tel: +55 61 3448 4901
E-mail: amehta@cenargen.embrapa.br

*These authors contributed equally to this work

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Over the years, several studies have been performed to analyse plant–pathogen interactions. Recently, functional genomic strategies, including proteomics and transcriptomics, have contributed to the effort of defining gene and protein function and expression profiles. Using these 'omic' approaches, pathogenicity- and defence-related genes and proteins expressed during phytopathogen infections have been identified and enormous datasets have been accumulated. However, the understanding of molecular plant–pathogen interactions is still an intriguing area of investigation. Proteomics has dramatically evolved in the pursuit of large-scale functional assignment of candidate proteins and, by using this approach, several proteins expressed during phytopathogenic interactions have been identified. In this review, we highlight the proteins expressed during plant–virus, plant–bacterium, plant–fungus and plant–nematode interactions reported in proteomic studies, and discuss these findings considering the advantages and limitations of current proteomic tools.

Introduction

Plant–pathogen interactions have been studied extensively over the years from both the plant and pathogen viewpoints. An understanding of how plants and pathogens recognize each other and differentiate to establish either a successful or an unsuccessful relationship is crucial in this field of investigation. Looking at

Abbreviations
1DE/2DE, one-/two-dimensional electrophoresis; AHL, *N*-acyl homoserine lactone; Avr, avirulence; CWDE, cell wall-degrading enzyme; EST, expressed sequence tag; GST, glutathione *S*-transferase; MDL, mandelonitrile lyase; OPG, osmoregulated periplasmic glucan; OsPR-10, rice pathogenesis-related protein class 10; PBZ1, probenazole-inducible protein; PMMoV-S, pepper mild mottle tobamovirus Spanish strain S; PPV, plum pox potyvirus; PR, pathogenesis-related; Prx, peroxiredoxin; RLK, receptor-like protein kinase; RYMV, rice yellow mottle sobemovirus; SOD, superoxide dismutase; TLP, thaumatin-like protein; TMV, tobacco mosaic tobamovirus; TTSS, type III secretion system.

the defence mechanisms in plants, the recognition and signalling events that occur in plant cells in response to microorganism challenge need to be extremely rapid, reliable and specific, and are part of the strategy evolved by plants to survive attacks. The intracellular sensitive perception of pathogens and the recognition of pathogen-associated molecular patterns, such as lipopolysaccharides and flagellin, lead to the activation of the plant basal defence (or resistance), which is the first defence response, and trigger a generic mechanism consisting of plant cell wall thickening, papilla deposition, apoplast acidification and signal transduction and transcription of defence genes [1]. This generic basal defence mechanism has been observed in several incompatible plant–microorganism interactions, and is believed to corroborate the observation that most plants are resistant to invasion by the majority of pathogens. Therefore, successful pathogens must evolve mechanisms to interfere with or suppress basal defence to colonize the host and develop disease.

Superimposed on the basal defence, some plant varieties express resistance proteins that guard against this interference and trigger a specific, genetically defined hypersensitive response and subsequent programmed cell death. The function of the hypersensitive response is to contain the pathogen, and it is typified by various biochemical perturbations, known as generic plant responses, including changes in ion fluxes, lipid hyperperoxidation, protein phosphorylation, nitric oxide generation and a burst of reactive oxygen species and antimicrobial compounds. This rapid incompatibility response effectively puts an end to pathogen invasion and prevents further disease development [1].

With regard to plant pathogens, the capacity to overcome plant defence, by protecting themselves from the oxidative stress activated by the plant in response to pathogen perception, is of extreme importance. Therefore, pathogens induce several genes, such as catalases and superoxide dismutase (SOD), which are responsible for the inactivation of H_2O_2 and O_2^-. The importance of secretion pathways for pathogenicity has also been well established. Effector proteins expressed by the pathogen are predicted to collaborate in the suppression of basal resistance through the modification of specific host proteins. The secretion of extracellular enzymes, such as pectin esterases, polygalacturonases, xylanases, pectato lyases and cellulases, is another essential process for colonization and pathogenicity [2].

With the increase in genomic and postgenomic studies, a large amount of information is available, and advances have been achieved in the understanding of defence mechanisms in plants, as well as the pathogenicity strategies employed by microbial pathogens. At present, the functional assignment of given proteins is considered to be the main challenge in postgenomic studies. Transcriptional changes do not reflect the complete cellular regulatory mechanism, as post-transcriptional processes which alter the amount of active protein, such as synthesis, degradation, processing and post-translational modification, are not taken into account. Thus, complementary approaches, such as proteome-based expression profiling, are needed to obtain a full picture of the regulatory elements. Moreover, several studies have revealed that the levels of mRNA do not necessarily predict the levels of the corresponding proteins in the cell [3]. The different stabilities of mRNAs and different efficiencies in translation can affect the generation of new proteins. Once formed, proteins also differ significantly in their stability and turnover rate, which makes proteomic investigation even more important.

Proteomics, or the analysis of the protein complement of the genome, provides experimental continuity between genome sequence information and the protein profile in a specific tissue, cell or cellular compartment during standard growth or different treatment conditions. Although the genome defines potential contributions to cellular function, the expressed proteome represents actual contributions. Moreover, by using proteomic approaches, differences in the abundance of proteins actually present at the time of sampling can be distinguished and different forms of the same protein can be resolved. The analysis of proteomes from organisms has been performed extensively by exploring the high resolution of two-dimensional electrophoresis (2DE) coupled with MS. These data, when complemented by *de novo* sequencing, allow the unequivocal identification of proteins involved in different biological functions. The proteomic approach is a fundamental method by which we can obtain an understanding and identification of the functions of proteins expressed in a given condition.

In this review, we highlight the proteins expressed during plant–virus, plant–bacterium, plant–fungus and plant–nematode interactions reported in proteomic studies, and discuss these findings considering the advantages and limitations of current proteomic tools.

Plant–virus interactions

For the success of plant infection, viruses must first be transmitted either mechanically or by a vector (transmission), replicate in plant cells (replication), subsequently move through plasmodesmata to neighbouring cells (cell-to-cell movement) and, finally, attain the vascular tissue to circulate systemically through the phloem to

the sink tissues of the host (vascular movement). After being unloaded from the phloem, viruses establish systemic infection through new cycles of replication and cell-to-cell/vascular movement. In both compatible (susceptible host) and incompatible (resistant host) interactions, viruses use plant host proteins to complete the steps of the infection process and suffer the influences of plant host proteins as a counteraction against the infection. The genes that encode these proteins have been studied extensively in numerous host–virus systems, mainly using transcriptional analysis [4].

Recently, 2DE and subsequent MALDI-TOF MS have been performed to analyse the induced expression of nuclear proteins in *Capsicum annuum* cv. Bugang (hot pepper) infected by tobacco mosaic tobamovirus (TMV) [5]. *C. annuum* cv. Bugang is hypersensitive response resistant against TMV-P_0 and susceptible to TMV-$P_{1.2}$ strains. A hypothetical protein and five annotated nuclear proteins (Table 1) were identified in hot pepper infected by TMV-P_0, including four defence-related proteins [14-3-3 protein (regulator of proteins involved in response to biotic stresses), 26S proteasome subunit (RPN7) (postulated to be involved in programmed cell death), mRNA-binding protein (may interact with viral RNA or interfere with plant RNA metabolism) and Rab11 GTPase (responsible for membrane trafficking/recycling and endocytosis/exocytosis)] and a ubiquitin extension protein.

Diaz-Vivancos *et al.* [6] used proteomic approaches to study the changes in enzymatic activity and protein expression in the antioxidative system within the leaf apoplast of *Prunus persica* cv. GS305 (peach) on plum pox potyvirus (PPV) infection. PPV infection provoked oxidative stress in peach leaf apoplast by increasing the antioxidant enzymatic activities and H_2O_2 contents. 2DE of apoplastic fluids from peach leaves infected with PPV, and subsequent MALDI-TOF MS analyses, revealed the identification of four proteins of the 22 analysed: one thaumatin-like and three mandelonitrile lyases (MDLs) (Table 1). Thaumatins are proteins involved in the plant response against fungal infection, and may equally be expressed in peach as a response to PPV infection [6]. MDLs are flavoproteins involved in the catabolism of (*R*)-amygdaline; however, to define their role in the peach plant–PPV interaction, further investigations must be performed.

Another study on plant–virus interaction was performed by Rahoutei *et al.* [7,8]. These authors demonstrated that the pepper mild mottle tobamovirus Spanish strain S (PMMoV-S) inhibits photosystem II electron transport, disturbing the oxygen-evolving complex, composed of the three proteins PsbP, PsbO and PsbQ, present within plant thylakoid membranes. PMMoV-S infection results in a lower expression of PsbP and PsbQ in the susceptible host *Nicotiana benthamiana* Domin (tobacco) relative to that in healthy

Table 1. Proteins expressed in plant–virus interactions and identified in plants using proteomic approaches.

Protein	Studied organism	Pathogen	Accession no.[a]	Reference
26S proteasome subunit RPN7	*C. annuum*	TMV-P_0	DQ975456	[5]
mRNA-binding protein	*C. annuum*	TMV-P_0	DQ991047	[5]
Rab11 GTPase	*C. annuum*	TMV-P_0	DQ975457	[5]
Ubiquitin extension protein	*C. annuum*	TMV-P_0	DQ975458	[5]
14-3-3 protein	*C. annuum*	TMV-P_0	DQ991045	[5]
Thaumatin-like protein	*Prunus persica*	PPV	AAM00215	[6]
R-(+)mandelonitrile lyase isoform MDL5 precursor	*Prunus serotina*	PPV	AAC61982	[6]
R-(+)mandelonitrile lyase isoform MDL4 precursor	*Pr. serotina*	PPV	AAD02266	[6]
Mandelonitrile lyase	*Pr. serotina*	PPV	CAA51194	[6]
PsbO (*N. benthamiana* isoform I)	*Pisum sativum*	PMMoV-S	P14226	[9]
PsbO (*N. benthamiana* isoform II)	*N. tabacum*	PMMoV-S	Q40459	[9]
PsbO (*N benthamiana* isoforms III, IV)	*Lycopersicon esculentum*	PMMoV-S	P23322	[9]
PsbP (*N. benthamiana* isoforms A, B, C)	*N. tabacum*	PMMoV-S	CAA39039	[9]
PsbP (*N. benthamiana* isoform D)	*N. tabacum*	PMMoV-S	CAA44292	[9]
Phenylalanine ammonia-lyase	*O. sativa*	RYMV	P14717	[11]
Mitochondrial chaperonin-60	*O. sativa*	RYMV	Q8H903	[11]
Aldolase C-1	*O. sativa*	RYMV	Q42476	[11]

[a] Accession number from the organism of origin.

control plants. In *N. benthamiana* Domin–PMMoV-S interaction analysis, Perez-Bueno *et al.* [9] revealed, by 2DE immunoblotting and N-terminal sequencing of proteins from the thylakoid membranes, that there are four isoforms of PsbO and four isoforms of PsbP in *N. benthamiana* Domin (Table 1). These authors also showed that the expression of the four isoforms of PsbP decreases considerably in relation to PsbO proteins as the infection progresses. The fact that damage to the activity of the oxygen-evolving complex in virus-infected plants results in higher viral accumulation in the host may indicate the participation of PsbO in a basal resistance mechanism against viruses and in plant counteraction against the deleterious effects of viruses on photosynthetic activity [10].

Proteomic analysis was also performed to study the compatible interaction between *Oryza sativa* (rice) and rice yellow mottle sobemovirus (RYMV) [11]. This analysis led to the identification of a phenylalanine ammonia-lyase, a mitochondrial chaperonin-60 and an aldolase C (Table 1), but the role of these proteins during RYMV infection of rice remains to be determined. In another analysis of the same interaction, Brizard *et al.* [12] investigated RYMV–rice (susceptible *O. sativa indica* IR64) protein complexes (formed *in vivo* or *in vitro*) to identify plant proteins putatively involved in the virus–host interactions. SDS-PAGE analysis, followed by nano-LC-MS/MS, revealed the presence of 223 different proteins that fitted into three functional categories. In the metabolism category, a large number of enzymes involved in glycolysis, malate and citrate cycles were found, probably recruited by RYMV for the production of energy to support viral replication [12]. In the defence category, proteins involved in the generation and detoxification of reactive oxygen species were identified, presumably to maintain an oxido-reduction environment compatible with viral replication [12]. In the protein synthesis category, proteins involved in translation, elongation factors, chaperones, protein-disulfide isomerases and proteins involved in protein turnover with the 20S proteasome were observed [12]. Again these proteins may be recruited by RYMV to optimize the efficiency of viral infectivity [12]. Finally, in a recent proteomic study, the interaction of tomato fruits (*Lycopersicon esculentum*) with TMV was analysed. Of the 16 proteins identified, there were several pathogenesis-related (PR) proteins and antioxidant enzymes found to be expressed as a probable part of the plant resistance mechanism against viral infection [13].

Although proteomic approaches have shown the participation of several plant proteins (mentioned above) in virus replication, the involvement of plant factors in viral movement has never been demonstrated through proteomics. As viral movement in plants is tissue specific and involves various cell types which are difficult to isolate, such as leaf parenchyma (where cell-to-cell movement occurs) and phloem (where vascular movement occurs), the performance of proteomic assays of each separate tissue is hampered.

Plant–bacterium interactions

Bacteria rely on diverse secretion pathways in order to overcome plant defences and to establish successful colonization of the host plant. Five secretion systems (types I–V) have been reported in bacteria, which are distinguished by their constituent proteins [14]. The main secretion system used by pathogenic bacteria during infection is the type III secretion system (TTSS), which is involved in some of the most devastating diseases in animals and plants (for a review, see [15]). This system enables bacteria to directly inject proteins, called effectors or virulence factors, into the host cell and subvert cellular processes. TTSS is essential for pathogenicity and is conserved amongst Gram-negative bacteria; however, the proteins exported by this system are more variable [16,17]. The best-studied TTSS effectors are designated avirulence (Avr) proteins, which have been reported in several plant pathogens [18–21]. Other effectors have also been identified in different phytopathogenic bacterial species, including *Xanthomonas* outer protein (Xop) in *Xanthomonas* [22], Hrp outer protein (Hop) in *Pseudomonas* [23] and *Pseudomonas* outer protein (Pop) (based on a previous genus designation) in *Ralstonia* [24].

Another important system for bacterial pathogenicity is the type II secretion system, which is involved in the secretion of extracellular enzymes, toxins and virulence factors. Striking differences in the number and combinations of these enzymes in different pathogens are expected to be found.

Most of the data currently available on pathogenicity mechanisms in bacteria have been obtained by genomic studies. Few studies have employed the proteomic approach, which aims to identify the bacterial proteins putatively involved in pathogenicity. Mehta and Rosato [25] reported the analysis of *Xanthomonas axonopodis* pv. *citri* cultivated in the presence of the host *Citrus sinensis* leaf extract, and identified differentially expressed proteins, including a sulfate-binding protein, by NH_2 terminal sequencing (Table 2). The authors suggested that the induction of this enzyme may have been caused by the amino acids or different sugars present in the leaf extract. Tahara *et al.* [26] analysed the expressed proteins of *X. axonopodis* pv. *passiflorae*

Table 2. Proteins identified in phytopathogenic bacteria using proteomic approaches.

Protein	Studied organism	Plant/condition	Accession no.[a]	Reference
Sulfate-binding protein	*X. axonopodis* pv. *citri*	*Citrus sinensis* (leaf extract)	PO2906	[25]
Inorganic pyrophosphatase	*X. axonopodis* pv. *passiflorae*	*Passiflorae edulis* (leaf extract)	AAM38285.1	[26]
Outer membrane protein	*X. axonopodis* pv. *passiflorae*	*Pa. edulis* (leaf extract)	AAM38389.1	[26]
Outer membrane protein A (OmpA)	*Dickeya dadantii* (syn. *E. chrysanthemi*)	*Saintpaulia ionantha* (leaf extract)	18822	[27]
Type III secretory pathway, porin component (HrcC)	*D. dadantii* (syn. *E. chrysanthemi*)	*Sa. ionantha* (leaf extract)	20864	[27]
Oligogalacturonate specific porin (KdgN)	*D. dadantii* (syn. *E. chrysanthemi*)	*Sa. ionantha* (leaf extract)	15523	[27]
Oligogalacturonate specific porin (KdgM)	*D. dadantii* (syn. *E. chrysanthemi*)	*Sa. ionantha* (leaf extract)	19629	[27]
Polygalacturonase X (pehX)	*E. chrysanthemi*	*Chrysanthemum* leaves (leaf extract)	14958	[31]
Avr-like protein	*E. chrysanthemi*	*Chrysanthemum* leaves (leaf extract)	19143	[31]
Metalloprotease A	*E. chrysanthemi*	*Chrysanthemum* leaves (leaf extract)	20373	[31]
Cellulase	*E. chrysanthemi*	*Chrysanthemum* leaves (leaf extract)	18772	[31]
OmpA-related protein	*X. campestris* pv. *campestris*	Culture media	AAM42288	[32]
Cellulase	*X. campestris* pv. *campestris*	Culture media	AAM42791	[32]
Superoxide dismutase	*X. campestris* pv. *campestris*	Culture media	AAM41557	[32]
Arabinogalactan endo-1,4-β-galactosidase	*X. campestris* pv. *campestris*	Culture media	AAM42894	[32]
GroEL (60 kDa chaperonin)	*X. campestris* pv. *campestris*	Culture media	AAM39839	[32]

[a] Accession number from the organism of origin.

during the interaction with the host *Passiflorae edulis* leaf extract, and identified an inorganic pyrophosphatase and an outer membrane protein upregulated in the presence of leaf extract, also by NH_2 terminal sequencing. It was proposed that the outer membrane protein identified may have an important role in pathogenicity [26].

Plant extracts have also been used as a stress condition in the analysis of outer membrane proteins of the soft rot pathogen *Dickeya dadantii* (syn. *Erwinia chrysanthemi*) by 2DE and MALDI-TOF MS analyses [27]. Several proteins were identified, such as the porin OmpA, involved in binding to specific host cell receptor molecules [27], HrcC, a member of the PulD/pIV superfamily of proteins that function in outer membrane translocation of type II and type III secretion pathways [28], and the oligogalacturonate-0 specific porins KdgM and KdgN [27].

The *E. chrysanthemi* proteome was further analysed by comparing *E. chrysanthemi* wild-type and osmoregulated periplasmic glucan (OPG)-defective mutant cells, which show a loss of virulence, by 2DE. Several proteins differentially expressed in the mutant cells, essential for cellular processes such as protein folding and degradation and carbohydrate metabolism, were identified [29]. The authors concluded that *E. chrysanthemi* responds to OPG deficiency by activating cellular processes that protect the cell against environmental stresses, which suggests that the opgG strain is impaired in the perception of its environment [29].

In a 2DE-mediated proteomic study of *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis, it was observed that *X. fastidiosa* did not produce significant changes in heat shock protein expression when compared with *X. axonopodis* pv. *citri* [30]. However, it was found that *X. fastidiosa* constitutively expressed several stress-inducible proteins, such as HspA and GroeS, which were induced in *X. citri* under stress conditions. The authors suggested that the constitutive expression of these proteins may help *X. fastidiosa* cope with sudden environmental changes and stresses.

Secretome analysis is a primary field of study of bacterial pathogenicity, which may reveal new virulence proteins. As a result of the high importance of secreted proteins in the bacterial infection process, the *E. chrysanthemi* secretome was analysed and revealed an upregulation of several pectate lyases expressed in the presence of leaf extract of *Chrysanthemum* [31]. These enzymes play a crucial role in *E. chrysanthemi* infection, and the occurrence of several isoforms may

permit pathogenicity to a variety of different conditions and hosts [31]. A polygalacturonase X, which is another cell wall-degrading enzyme (CWDE), was also identified using MALDI-TOF analysis [31]. Similarly, several secreted proteins involved in various functions were identified in the *Xanthomonas* secretome [32], including outer membrane proteins, proteins involved in trace element acquisition, degrading enzymes, metabolic enzymes, proteins involved in maintenance and folding, and proteins with other functions (Table 2).

Other proteomic studies have reported global protein expression and reference maps of important bacterial plant pathogens, including *X. fastidiosa* [33] and *Agrobacterium tumefaciens* [34]; however, proteomic studies of the direct interaction of these pathogens with the plant or plant extracts are still at an initial stage.

With regard to plant defence responses, direct evidence of the involvement of target proteins has also been provided by proteomic studies. Although few, the reports outlined below clearly show the importance of proteomic approaches, which can aid significantly in the understanding of plant–bacterium interactions. Jones *et al.* [3], in the same study, analysed the proteomic and transcriptomic profiles of *Arabidopsis thaliana* leaves during early responses (1–6 h postinoculation) to the challenge by *Pseudomonas syringae* pv. *tomato*. They compared the proteomic changes in *A. thaliana* in response to the *P. syringae* pv. *tomato* highly virulent strain DC3000, which results in successful parasitism, a DC3000 *hrp* mutant, which induces basal resistance, and a transconjugant of DC3000 expressing *avrRpm1*, which triggers a gene-for-gene-based resistance. Two subsets of proteins, which consistently showed clear differences in abundance after various challenges and time intervals, were glutathione *S*-transferases (GSTs) and peroxiredoxins (Prxs). Both of these groups of antioxidant enzymes were considered to have probable significant roles in the regulation

Table 3. Proteins expressed in plant–bacterium interactions and identified in plants using proteomic approaches.

Protein	Studied organism	Pathogen	Accession no.[a]	Reference
Glutathione *S*-transferase	*A. thaliana*	*P. syringae*	At2g47730 At4g02520 At1g02930 At1g02920	[3,35]
Peroxiredoxin	*A. thaliana*	*P. syringae*	At5g06290 At3g52960 At3g11630	[3,35]
Peroxiredoxin, chloroplast	*O. sativa*	*X. oryzae* pv. *oryzae*	AM039889	[36]
Glyceraldehyde 3-phosphate dehydrogenase	*O. sativa*	*X. oryzae* pv. *oryzae*	S33872	[36]
Triosephosphate isomerase, cytosolic (EC 5.3.1.1)	*O. sativa*	*X. oryzae* pv. *oryzae*	P46226	[36]
Thaumatin-like protein	*O. sativa*	*X. oryzae* pv. *oryzae*	P31110	[36]
Superoxide dismutase	*O. sativa*	*X. oryzae* pv. *oryzae*	S29146	[36]
Alcohol dehydrogenase 1	*O. sativa*	*X. oryzae* pv. *oryzae*	CAA34363	[37]
Quinone reductase	*O. sativa*	*X. oryzae* pv. *oryzae*	NP_916411	[37]
Prohibitin	*O. sativa*	*X. oryzae* pv. *oryzae*	NP_916591	[37]
Hypersensitive-induced response	*O. sativa*	*X. oryzae* pv. *oryzae*	AAK54610	[37]
Ascorbate peroxidase	*O. sativa*	*X. oryzae* pv. *oryzae*	XP_470658	[37]
Zinc finger and C2 domain protein-like	*O. sativa*	*X. oryzae* pv. *oryzae*	XP_478243	[37]
Low molecular weight heat shock protein	*O. sativa*	*X. oryzae* pv. *oryzae*	NP_912354	[37]
Universal Stress Protein	*O. sativa*	*X. oryzae* pv. *oryzae*	AAP53941	[37]
Remorin 1	*Lycopersicon hirsutum*	*Clavibacter michiganensis* ssp. *michiganensis*	4731573	[38]
Phospholipid hydroperoxide glutathione peroxidase	*L. hirsutum*	*Cl. michiganensis* ssp. *michiganensis*	31872080	[38]
Pathogenesis-related 3 (endochitinase precursor)	*L. hirsutum*	*Cl. michiganensis* ssp. *michiganensis*	Q05540	[38]
Glutathione *S*-transferase	*L. hirsutum*	*Cl. michiganensis* ssp. *michiganensis*	TC116034	[38]
Ascorbate peroxidase	*L. hirsutum*	*Cl. michiganensis* ssp. *michiganensis*	6066418	[38]

[a] Accession number from the organism of origin.

of redox conditions within infected tissue (Table 3). These results were further related to changes in the expression profiles for the corresponding GST and Prx genes, identified by Affymetrix GeneChip analysis. In general, a good correlation was observed between changes obtained at the transcript and protein levels for the Prx family, but not for the GST family. Only for the PrxB protein was the decrease observed in the spot intensity following pathogen challenge clearly related to transcriptional suppression. These observations were used to highlight the complexity of comparative proteomics and transcriptomics, even when derived from the same inoculation system.

As a follow-up study, the same group [35] examined the global proteomic profile in three subcellular fractions (soluble protein, chloroplast- and mitochondria-enriched) of *A. thaliana* responding to the same three *P. syringae* pv. *tomato* DC3000 strains. This was the first report to associate post-translational events (1–6 h postinoculation) occurring before significant transcriptional reprogramming. In total, 73 differential spots representing 52 unique proteins were successfully identified, and were representative of two major functional groups: defence-related antioxidants and metabolic enzymes. The results show that several chloroplast systems are modified during all aspects of the defence response. Components of the Calvin–Benson cycle are rapidly altered during basal defence, and some of these changes are reversed by type III effectors. Photosystem II has emerged as a target of resistance signalling. Mitochondrial porins appear to be modified early in basal defence, with specific alterations to other components in response to AvrRpm1. Finally, the interplay between redox status and glycolysis, with probable links to lipid signalling [through glyceraldehyde 3-phosphate dehydrogenase, some GSTs, lipase and NADH: quinone oxidoreductase (NQR)], may coordinate communication between organelles. Significant changes to photosystem II and to mitochondrial porins seem to occur early in basal defence. Rapid communication between organelles and the regulation of primary metabolism through redox-mediated signalling are supported by these results.

To investigate the role of defence-responsive proteins in the rice–*Xanthomonas oryzae* pv. *oryzae* interaction, Mahmood *et al.* [36] applied a proteomic approach. Cytosolic and membrane proteins were fractionated from the rice leaf blades 3 days postinoculation with incompatible and compatible *X. oryzae* pv. *oryzae* races. From 366 proteins analysed by 2DE, 20 were differentially expressed in response to bacterial inoculation (Table 3). Analyses clearly revealed that four defence-related proteins [PR-5, probenazole-inducible protein (PBZ1), SOD and Prx] were induced for both compatible and incompatible *X. oryzae* pv. *oryzae* races, wherein PR-5 and PBZ1 were more rapid and showed higher induction in incompatible interactions and in the presence of jasmonic acid.

Studying the same rice–*X. oryzae* pv. *oryzae* interaction, Chen *et al.* [37] analysed proteins from rice plasma membrane to study the early defence responses involved in XA21-mediated resistance. XA21 is a rice receptor kinase, predicted to perceive the *X. oryzae* pv. *oryzae* signal at the cell surface, leading to the 'gene-for-gene' resistance response. They observed a total of 20 proteins differentially regulated by pathogen challenge at 12 and 24 h postinoculation, and identified at least eight putative plasma membrane-associated and two non-plasma membrane-associated proteins (Table 2) with potential functions in rice defence.

Proteins from the wild tomato species *Lycopersicon hirsutum* that are regulated in response to the causal agent of bacterial canker (*Clavibacter michiganensis* ssp. *michiganensis*) were identified by comparing two partially resistant lines and a susceptible control line in a time course (72 and 144 h postinoculation) experiment [38]. Using 2DE and ESI-MS/MS, 26 differentially regulated tomato proteins were identified, 12 of which were directly related to defence and stress responses (Table 3).

Proteomic analysis was also used to detect the responses of the model legume *Medicago truncatula* to the pathogenic bacterium *Pseudomonas aeruginosa* in the presence of known bacterial quorum-sensing signals, such as *N*-acyl homoserine lactone (AHL) [39]. The fast and reliable detection of bacterial AHL signals by plant hosts is essential to make appropriate responses to the pathogen. Therefore, *M. truncatula* is able to detect very low concentrations of AHL from *P. aeruginosa*, and responds in a global manner by significant changes in the accumulation of 154 proteins, 21 of which are related to defence and stress responses.

As phosphorylation plays a central role in the initiation of the plant response to bacterial signals, phosphoproteomics (large-scale analysis of phosphoproteins) is a powerful strategy to better understand the events that occur rapidly in the host after bacterial perception [40]. Although it has been shown that the phosphorylation pathway of proteins changes rapidly after signal perception, relatively few of these phosphoproteins have been identified in plant species. By using a phosphoproteome approach, early changes in proteins potentially phosphorylated during the bacterial defence response have been described, and include dehydrin, chaperone, heat shock protein and glucanase [41,42]. The phosphorylation of these proteins is probably part of the early basal plant defence response.

Plant–fungus interactions

Considerable advances have been achieved in the last 10 years in the identification of the determinants of plant–fungus interactions. Currently, more than 25 fungal genomes have been elucidated, including human and plant pathogens, such as *Aspergillus fumigatus* and *Magnaporthe grisea*, respectively (http://www.broad.mit.edu/annotation/fgi/). A key challenge in modern fungal biology is to analyse the expression, function and regulation of the entire set of proteins encoded by the revealed fungal genomes.

When pathogenic fungi start the infection process, secreted and intracellular proteins are up- or downregulated, improving the predation ability of fungi [43,44]. In this field, several proteomic studies have been carried out in order to understand fungal pathogenicity. These include pioneering studies, aimed at an understanding of the dimorphic transition from budding to filamentous growth [45] as well as appressorium construction [46]. Appressorium formation is believed to be an important event in the establishment of a successful interaction between the pathogen *Phytophtora infestans* and its host plant potato [46]. Although most spots were not identified, some proteins involved in amino acid biosynthesis, including methionine and threonine synthases, were obtained (Table 4).

Proteomic analyses have also been used to study wheat leaf rust, caused by the fungus *Puccinia triticina* [47]. Rust diseases cause a significant annual decrease in the yield of cereal crops worldwide [48]. In order to better understand this problem at the molecular level, the proteomes of both host and pathogen were evaluated during disease development. A susceptible line of wheat infected with a virulent race of leaf rust was compared with mock-inoculated wheat using 2DE (with isoelectric focusing, pH 4–8) and MS analysis [47]. The fungus differentially expressed 22 different proteins during pathogen infection, including proteins with known and hypothetical functions.

Another approach, which has been frequently employed for the study of fungal proteins, involves the analysis of the exoproteome, also known as the secretome [49]. In this context, *Fusarium graminearum*, a devastating pathogen of wheat, maize and other cereals, was grown on hop (*Humulus lupulus*) cell walls. Using 1DE and 2DE, followed by MS analyses, 84 fungal secreted proteins were identified [49]. Amongst the identified proteins were cellulases, glucanosyltransferases, endoglucanases, phospholipases, proteinases and chitinases (Table 4). It was observed that 45% of the proteins observed in *F. graminearum* grown in the presence of hop cells were strictly involved in cell wall degradation and indirectly related to carbon and nitrogen absorption. When this same fungus was grown in a medium containing glucose, however, the enzyme patterns were totally different, showing that fungi are capable of regulating their secretion according to the presence of substrate [49].

A cell wall proteome was also proposed for *Phytophthora ramorum*, the causal agent of sudden oak death [50]. This study showed an inventory of cell wall-associated proteins based on MS sequence analysis. Seventeen proteins were identified, all of which were authentic secretory proteins. Functional classification based on homology searches revealed six putative mucins, five putative glycoside hydrolases, two transglutaminases, one annexin-like protein and one Kazal-type protease inhibitor [50], clearly suggesting that cell wall proteins are also important for fungal pathogenicity (Table 4).

Another fungal exoproteome was analysed in order to gain a more thorough understanding of the phytopathogenic fungus *Sclerotinia sclerotiorum* [51]. Extracted secreted proteins collected from liquid culture were separated using 2DE and annotated following ESI-Q-TOF MS/MS. Fifty-two secreted proteins were identified by MALDI-MS/MS peptide sequencing, and many of the annotated secreted proteins were cell wall-degrading enzymes that had been identified previously as pathogenicity or virulence factors of *S. sclerotiorum*. However, one of the identified proteins, α-L-arabinofuranosidase, which is involved in the virulence process of *S. sclerotiorum*, was not detected by EST studies, clearly demonstrating the merit of performing proteome-level research [51].

With regard to plant responses, although only a few proteomic studies have focused on plant–pathogen interactions, the plant–fungus association has been the most studied using this approach. In such studies, several proteins involved in diverse biological processes, including defence and stress responses, signal transduction, photosynthesis, electron transport and metabolism, have been found. Some examples reporting these proteins are mentioned below.

The *Ma. grisea*–rice interaction is a model system for understanding plant disease because of its great economic importance, and also because of the genetic and molecular genetic tractability of the fungus [52]. What makes this an important system is that both genomes have been sequenced and a rice proteome database is available (http://gene64.dna.affrc.go.jp/RPD/main.html). A pioneering study on rice proteomics was performed to analyse the protein profile after

Table 4. Proteins identified in phytopathogenic fungi using proteomic approaches.

Protein	Studied organism	Plant/condition	Accession no.[a]	Reference
Methionine synthase (*Pi-met1*) gene	*Phytophtora infestans*	*Solanum tuberosum*	NP_660391	[46]
Threonine synthase	*Ph. infestans*	*So. tuberosum*	8439546	[46]
Chitinase	*F. graminearum*	*Humulus lupulus*	–	[49]
Serine proteinase	*F. graminearum*	*Hu. lupulus*	–	[49]
Leucine aminopeptidase	*F. graminearum*	*Hu. lupulus*	–	[49]
Lipases	*F. graminearum*	*Hu. lupulus*	–	[49]
Pectate lyase	*F. graminearum*	*Hu. lupulus*	–	[49]
α-Arabinofuranidase	*F. graminearum*	*Hu. lupulus*	–	[49]
Ceramidase	*F. graminearum*	*Hu. lupulus*	–	[49]
Chitin deacetylase	*F. graminearum*	*Hu. lupulus*	–	[49]
β-Glucosidase	*F. graminearum*	*Hu. lupulus*	–	[49]
Polygalacturonidase	*F. graminearum*	*Hu. lupulus*	–	[49]
Trypsin	*F. graminearum*	*Hu. lupulus*	–	[49]
Aspartyl proteinase	*F. graminearum*	*Hu. lupulus*	–	[49]
Xyloglucanase	*F. graminearum*	*Hu. lupulus*	–	[49]
Carboxypeptidase	*F. graminearum*	*Hu. lupulus*	–	[49]
α-Amylase	*F. graminearum*	*Hu. lupulus*	v	[49]
Mucin	*Ph. ramorum*	Oak	73547	[50]
Glucanase	*Ph. ramorum*	Oak	74257a 74257b 72319 83680	[50]
Transglutaminases	*Ph. ramorum*	Oak	53744 83169	[50]
Exopolygalacturonase	*S. sclerotiorum*	Culture media	gi32454433 gi1483221 gi2196886	[51]
Cellobiohydrolase 1 catalytic domain	*S. sclerotiorum*	Culture media	gi20986705	[51]
Acid protease	*S. sclerotiorum*	Culture media	gi6984107	[51]
Aspartic proteinase precursor: aspartyl proteinase	*S. sclerotiorum*	Culture media	gi12002205	[51]

[a] Accession number from the organism of origin.

Ma. grisea infection, and was conducted using infected leaf blades fertilized with various levels of nitrogen [53]. Rice plants grown with high levels of nitrogen nutrient are more susceptible to infection by the blast fungus [54]. Although this study failed to establish any correlation between nitrogen application and disease resistance, leaf proteins revealed some minor changes when plants grown under different levels of nitrogen were compared [55]. Twelve proteins, including the rice thaumatin-like protein (TLP) (PR-5), were identified with accumulation changes at different levels of nitrogen.

Another study of the same interaction was performed by Kim *et al.* [56] using rice suspension-cultured cells. Twelve proteins from six different genes were identified, including the rice pathogenesis-related protein class 10 (OsPR-10), isoflavone reductase-like protein (PBZ1), glucosidase and putative receptor-like protein kinase (RLK), which had not been reported previously in suspension-cultured rice cells (Table 5). The authors followed with another proteome study using rice leaves, where they identified eight proteins newly induced or with increased expression [57]. The identified proteins belonged to several groups of PR proteins, and included two RLKs, two β-1,3-glucanases (Glu1, Glu2), TLP, peroxidase (POX 22.3), PBZ1 and OsPR-10 (Table 5). Although the proteins identified by Kim *et al.* [56,57] are most probably involved in the plant response to fungal attack and plant resistance/susceptibility, the purpose and function of each was not investigated in these preliminary and exploratory studies.

Another rice–fungus interaction study reported recently was that of sheath blight, caused by the fungus *Rhizoctonia solani*. Lee *et al.* [58] investigated rice sheath leaves after infection with this fungus, and the

Table 5. Proteins expressed in plant–fungus interactions and identified in plants using proteomic approaches.

Protein	Studied organism	Pathogen	Accession no.[a]	Reference
Peroxidases (PR-9)	*O. sativa*	*Ma. grisea*	AAC49818	[57]
	O. sativa	*Rhizoctonia solani*	gi32879781	[58]
	Triticum aestivum	*F. graminearum*	AAL08496	[59]
	Tomato	*F. oxysporum*	–	[62]
	A. thaliana	*Fusarium* elicitor	At1g07890	[75]
β-1,3-Glucanases (PR-2)	*O. sativa*	*Ma. grisea*	BBA77783	[57]
	O. sativa	*R. solani*	gi4884530	[58]
	T. aestivum	*F. graminearum*	AAD28734	[59]
	Zea mays	*F. verticillioides*	–	[61]
	Tomato	*F. oxysporum*	AAA03617	[62]
Thaumatin-like protein (PR-5)	*O. sativa*	*Ma. grisea*	–	[53]
	O. sativa	*Ma. grisea*	T04165	[57]
	T. aestivum	*F. graminearum*	CAA66278	[59]
	Tomato	*F. oxysporum*	AAM23272	[62]
Chitinase (PR-3)	*O. sativa*	*R. solani*	gi55168113	[58]
	T. aestivum	*F. graminearum*	BAB82472	[59]
	Tomato	*F. oxysporum*	CAA78845	[62]
Glutathione *S*-transferase	*T. aestivum*	*F. graminearum*	CAC94005	[59]
	Z. mays	*F. verticillioides*	2288968	[61]
	A. thaliana	*Fusarium* elicitor	At1g02930	[75]
Glyceraldehyde 3-phosphate dehydrogenase	*O. sativa*	*R. solani*	gi166702	[58]
	T. aestivum	*F. graminearum*	XP493811	[59]
	Z. mays	*F. verticillioides*	Q09054	[61]
Pathogenesis-related class 10	*O. sativa*	*Ma. grisea*	T14817	[56]
	O. sativa	*Ma. grisea*	AF416604	[57]
	M. truncatula	*Aphanomuces euteiches*	P93333	[60]
Fructose-bisphosphate aldolase	*Z. mays*	*F. verticillioides*	P08440	[61]
	A. thaliana	Fungal elicitor	At3g52930	[75]
Probenazole-induced protein	*O. sativa*	*Ma. grisea*	T02973	[56]
	O. sativa	*Ma. grisea*	T02973	[57]
Adenosine kinase	*Z. mays*	*F. verticillioides*	AJ012281	[61]
Superoxide dismutase (Cu–Zn)	*Z. mays*	*F. verticillioides*	P23346	[61]
Glutamate dehydrogenase	*T. aestivum*	*F. graminearum*	AAB51596	[59]
Thioredoxin	*T. aestivum*	*F. graminearum*	CAA06735	[59]
Disease-resistance-response protein pi 49	*M. truncatula*	*Aphanomuces euteiches*	PI4710	[60]
20S proteasome β unit	*O. sativa*	*R. solani*	gi50933089	[58]
Chaperonin 60 β percursor	*O. sativa*	*R. solani*	gi34897924	[58]
Receptor-like protein kinase	*O. sativa*	*Ma. grisea*		[56]
	O. sativa	*Ma. grisea*	AAL87185	[57]
14-3-3-like protein	*O. sativa*	*R. solani*	gi7271253	[58]

[a] Accession number from the organism of origin.

results revealed six proteins whose relative abundance varied significantly in the resistant and susceptible lines, and 11 additional proteins which were identified in abundance in the response of the resistant line only. These proteins have been reported previously to be involved in antifungal activity, signal transduction, energy metabolism, photosynthesis, protein folding and degradation, and antioxidation (Table 5), indicating a common pathway for both stress and non-stress plant functions.

Many other efforts have focused on the plant response to fungal attack. *Fusarium* head blight, caused mainly by *F. graminearum*, is one of the most destructive diseases of wheat, and the interaction between them has been investigated [59]. Zhou *et al.* [59] found 33 plant proteins which were expressed in response to *F. graminearum* in wheat spikes (Table 5). These proteins were divided into two groups, each related to defence response or metabolism. The authors suggested that several of these proteins were

Table 6. Proteins expressed in plant-parasitic nematode species identified by proteomic approaches.

Protein	Studied organism	Accession no.[a]	Reference
β-1,4-endoglucanase 2 precursor	*H. schachtii*	AJ299387	[69]
No known homologue	*H. schachtii*	–	[69]
Calreticulin precursor	*Ml. incognita*	–	[70]
Tropomyosin	*Ml. incognita*	–	[70]
Myosin regulatory light chain 2	*Ml. incognita*	–	[70]
ATP synthase β chain	*Ml. incognita*	–	[70]
Chaperonin protein HSP-60	*Ml. arenaria–Ml. javanica–Meloidogyne* sp.	AAA28077	[71]
Actin protein 4, isoform c	*Ml. arenaria–Ml. javanica–Meloidogyne* sp.	Q8I9k0	[71]
Translation initiation factor eIF-4A	*Ml. incognita*	S26281	[71]
Enolase	*Ml. incognita*	Q8MU59	[71]

[a] Accession number from the organism of origin.

directly involved in mounting the plant defence against infection by protecting against the oxidative burst inside the plant cell. Such a burst can be caused in plant cells by invading fungus.

Although most reports have focused on the leaf proteome, some studies have also analysed other tissues and organs. Using 2DE, the root protein profiles of *M. truncatula* were analysed after *Aphanomyces euteiches* pathogen infection during a time course experiment [60]. The majority of the induced proteins belonged to the PR-10 family, whereas others corresponded to putative cell wall proteins and enzymes of the phenylpropanoid–isoflavonoid pathway (Table 5). Another study focused on *Zea mays* embryos in response to the fungus *Fusarium verticillioides* [61]. The proteins identified included PR proteins, antioxidant enzymes and proteins involved in protein synthesis, folding and stabilization.

Another interesting study was performed to investigate the molecular details of the interaction between the xylem-colonizing plant-pathogenic fungus *Fusarium oxysporum* and tomato [62]. The composition of the xylem sap proteome of infected tomato plants was investigated and compared with that of healthy plants. Two-dimensional gel separation and MS identified 33 different proteins. Sixteen tomato proteins were found in the xylem sap for the first time. Amongst these proteins were peroxidases, chitinases, polygalacturonase and a subtilisin-like protease. It should be noted that these induced proteins are involved in cell wall, cell structure and antioxidant protection.

Plant–nematode interactions

Plants are continuously attacked by phytonematodes, which cause severe damage in susceptible agricultural crops, resulting in extensive economic losses worldwide [63]. Some of the most harmful plant-parasitic nematodes include the obligate sedentary endoparasites *Meloidogyne* spp., *Heterodera* spp. and *Globodera* spp. [63]. These organisms invade plant roots as juvenile larvae (J2) and, after three moults, develop into adult forms that reproduce in repeated cycles. This leads to severe modifications in the root system, which cause significant reductions in nutrient and water uptake and plant death [64].

In recent years, several nematode expressed sequence tag (EST) libraries have been constructed, mainly to identify parasitic nematode-specific genes, and approximately 100 000 ESTs have been sequenced from *Meloidogyne*, *Globodera* and *Heterodera* species (http://www.nematode.net). Despite the large number of ESTs, only a few of these genes are known to be involved in parasitism, although many of the transcripts are differentially expressed during parasitic stages [65–68]. Proteomic approaches have also contributed to the identification of candidates for the phytonematode parasitome, although to a lesser extent [69–71]. Some of these identified nematode proteins are highlighted in Table 6, and are involved in feeding site and cell wall degradation.

Despite the few proteomic studies, 2DE allied to MS is a powerful and rapid strategy to generate peptide sequence tags that can be linked to ESTs *in silico*. These peptides can be further used to design primers in order to obtain full-length gene sequences, contributing to parasitic genome projects [72]. In spite of the large amount of experimental and *in silico* evidence, few studies have aimed to determine the real importance of these sequences in plant–nematode interactions. In addition, EST libraries obtained by the micro-aspiration of cytoplasmic material from the oesophageal glands of

Meloidogyne incognita and *Heterodera glycines* reveal that the majority of the genes expressed in these salivary glands encode proteins with unknown function (*Ml. incognita*, 89%; *H. glycines*, 72%) [66,67].

Considering the other side of the plant–nematode interaction, some plants have evolved protective mechanisms to prevent nematode attraction, penetration, migration, feeding site formation, nourishment by digestion, reproduction and survival. Several resistance genes have been isolated in various plants [73]; however, studies on the proteome of the plant–nematode interaction are at an early stage. In a recent study, three proteins expressed in response to nematode infection have been reported using the proteomic approach, including a chitinase and a PR protein in *Coffea canephora* and a quinone reductase 2 in *Gossipium hirsutum* [74].

Understanding plant–pathogen interactions in the light of proteomic studies

In this review, we have presented the recent proteomic studies performed to better understand plant–virus, plant–bacterium, plant–fungus and plant–nematode interactions. Taken together, the data available reveal that several proteins are commonly expressed in diverse pathosystems (Fig. 1).

In the case of pathogens, several of the proteins involved in pathogenicity are secretion proteins, which were observed in bacteria, fungi and nematodes, and were mainly identified by secretomic studies. These proteins include proteases, cellulases and pectate lyases, which are important CWDEs, crucial for host plant colonization (Fig. 1). These results clearly show the importance of secretomic studies when searching for pathogenicity proteins. In addition to these well-known enzymes, other proteins, such as SODs and oxidases, have also been reported in the different pathogens, and are associated with protection against the oxidative stress response by the plant on infection.

A similar scenario was observed with regard to defence-related proteins in plants. The most reported defence-related proteins are PR proteins, including thaumatins, glucanases, peroxidases and chitinases, observed in several pathosystems described here (Fig. 1). The involvement of these proteins in plant defence has been well established; however, their direct role in resistance enhancement still needs to be demonstrated. The general biotic stress response represents another class of regulated proteins, which include GST, SOD and heat shock proteins, also commonly identified in several plant–pathogen proteomic studies described in this review (Fig. 1).

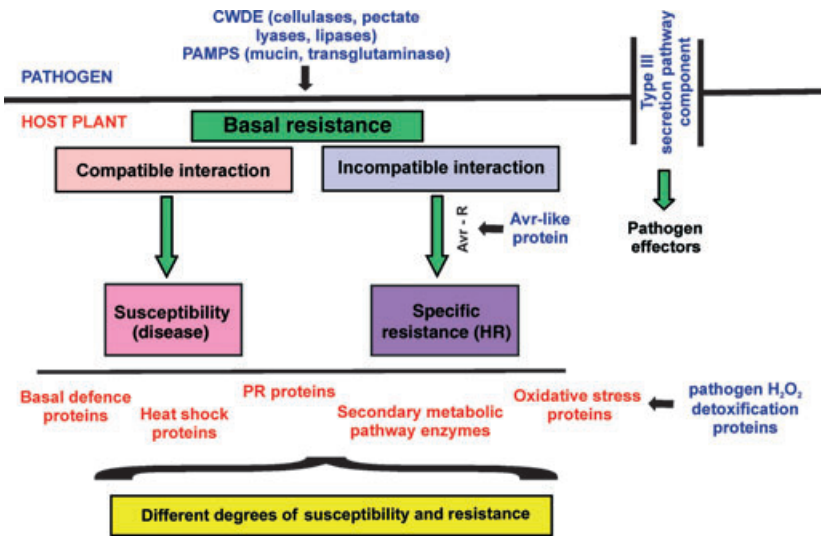

Fig. 1. Overview of plant–pathogen interactions and insights into proteomic studies of the proteins involved in these processes. Plants possess receptors that can activate basal resistance, mediated by pathogen-associated molecular patterns (PAMPs) or cell wall-degrading enzymes (CWDEs), which may result in a compatible or incompatible interaction. In both interactions, several defence-related and biotic stress-responsive proteins are induced. Suppression of plant defences by pathogen effectors leads to susceptibility in host plants. Some host plants express resistance (R) proteins, which guard against this interference and trigger a specific resistance, referred to as the hypersensitive response (HR). Proteomic studies of plant–pathogen interactions have revealed several pathogen and plant proteins expressed in different pathosystems. These proteins, identified using proteomic tools, are highlighted in blue (pathogen) and red (plant) in the different stages of the interaction.

Although several proteins expressed during plant–pathogen interactions have been highlighted, most are well known and are mainly involved in the conflict between the pathogen and the plant to suppress or induce, respectively, the basal plant defence mechanism. The results that emerge from most proteomic analyses are of extreme importance for the validation of the expression of the genes identified by genomic or transcriptomic studies. However, a small amount of novel information has been obtained, and can be explained by the fact that key proteins are expressed in low abundance, and are therefore not detected by current proteomic tools. Indeed, only the most abundant proteins are detected in two-dimensional gels and successfully identified by MS. Another major problem faced in proteomic analyses is protein identification by peptide mass fingerprinting. Unequivocal identification is usually obtained only when the genome sequence or a large amount of sequence data are available in public databases. When analysing poorly studied organisms, identification must be performed by *de novo* sequencing, which requires more sophisticated equipment, not readily available, especially in developing countries. Therefore, a gap appears to exist in the bioinformatics pipeline for the proteomics of organisms with incomplete sequenced genomes. These technical limitations in proteomic studies need to be overcome in order to advance our knowledge on protein expression during plant–pathogen interactions. Nevertheless, proteomic tools are rapidly improving and new methods and equipment are being developed. We believe that future proteomic studies, coupled with functional validation analysis, may provide new insights into disease resistance and pathogenicity.

Another important aspect to be considered when performing proteomic analyses is the follow-up study of the identified proteins, which should be performed in order to correctly assign protein function. The multiple roles of proteins are a significant barrier to progress in the unambiguous identification of proteins involved in processes such as plant–pathogen interactions. Moreover, a frequent result found in proteomic studies is the large amount of proteins obtained with unknown function. It is important to further investigate these proteins, which may present new biological functions and may play important roles in the processes under investigation.

The examples reviewed here demonstrate the complex cellular network that exists in different plant–pathogen interactions. Overall, the use of proteomic studies, allied to functional validation analyses, can provide fascinating contributions to the understanding of complex mechanisms, such as plant–pathogen interactions. The first step in the understanding of disease resistance is currently being met with the identification of the proteins expressed during plant–pathogen interactions. The next step will be to determine which proteins confer pathogenicity and disease resistance, and the mechanisms by which they do so.

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