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Prachi Singh
Department of Mycology & Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Utter Pradesh, India

Pitambara
Department of Agricultural Biotechnology, Anand Agricultural University, Anand - 388110

Rahul Singh Rajput
Department of Mycology & Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Utter Pradesh, India

Devanshu Dev
Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar-263145, U.S. Nagar, Uttarakhand, India

Anupam Maharshi
Department of Mycology & Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Utter Pradesh, India

Correspondence
Prachi Singh
Department of Mycology & Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Utter Pradesh, India

Proteomics Approaches to Study Host Pathogen Interaction

Prachi Singh, Pitambara, Rahul Singh Rajput, Devanshu Dev and Anupam Maharshi

Abstract
Proteomics is the branch of functional genomics which deals with the study of proteins along with analysis that have genetic read out i.e. mRNA analysis & genomic analysis. Genomic studies integrated with protein studies confirm the functionality of a particular gene. Advances in mass spectrometry, nucleotide sequencing information combined with computational algorithm, chip-based approaches and genetic approaches lead to the proteomics as an emerging field of functional genomics. Different approaches in proteomics include protein extraction and then separation of proteins either by Gel based method (2DE, DIGE) or non-Gel based method (ITAC, ITRAQ, SILAC, Mud PIT) and then quantification and identification using mass spectrometry and database comparison. Plant serves as a host for wide range of pathogen. On the basis of maintaining effective defence response towards pathogen there are two types of plant-pathogen interaction namely compatible and incompatible interaction. In both type of interaction several defence related and biotic stress response proteins are induced but their extent to provide defence is different. Proteomics help in studying plant–virus, plant-fungus, plant-bacteria and plant-nematode interaction in details to identify proteins produced in both compatible and incompatible interaction and to sort out the protein with differential expression.

Keywords: Bacteria, Fungi, Interaction, Proteomics, Nematode and Virus

Introduction
Proteomics deals with the extensive study of expression levels, post translational modifications and interaction of protein with other molecules to have deeper insight of cellular processes at the protein level. Proteomics can be broadly divided into two areas first protein expression mapping and second one is protein interaction mapping. Other areas of proteomics research work are post translational modification, protein-protein interaction, structural proteomics, functional proteomics, proteome mining. Protein-protein interaction mapping involves determining, proteome on wide scale, the interaction patterns for each of encoded proteins of a cell or organism (Wasinger *et al.*, 1995) [52]. Creation of a protein-protein interaction map of the cell would be of great value for understanding biology of the cell. Protein expression mapping encompasses the quantitative study of changes in protein expression of the cells or tissues using 2D electrophoresis followed by mass spectrometry. The protein spots on 2D gels can be quickly identified by in-gel proteolysis and peptide mass fingerprinting using mass spectrometry (Bantscheff *et al.*, 2007) [5]. The partial sequence information can be quickly produced from spots on 2D gels with recent development in tandem mass spectrometry using nano-electrospray methods. It has made possible generation of databases of protein expression profiles for various cells and tissues (Rasmussen *et al.*, 1996) [46]. Besides this rapid advancement has been made in identification of post-translation modifications of proteins (Oda *et al*., 2001) [40]. The protein expression mapping aims to compare large number of proteins expressed in cells or tissues under different environmental conditions and different stages of disease progression.

Need of Proteomics
According to recent studies conducted it was found that number of proteins from one gene in bacteria was one or two, in Yeast it was three and it was found to be three or more in human, this is so because there is poor co-relation between mRNA and protein expression level. M RNA goes for post–transcriptional control like alternate splicing, polyadenylation and editing. It can also be subjected to regulation at level of protein translation. Protein can also be regulated by proteolysis, compartmentalization. Proteins, not genes are responsible for phenotype of cells. It is impossible to study mechanism of disease, ageing and effect of environment on genome basis. So, genome information integrated with protein studies confirm

the existence of particular gene (Thurston *et al*., 2005) [50].

Approaches used in Proteomics

Different approaches used for proteomic studies includes protein extraction and separation of protein either by gel based or non gel based technique. Gel based proteomic approaches include separation of proteins by two dimensional gel electrophoresis (2-DE) and differential gel electrophoresis (DIGE). Non Gel based technique include isobaric technique for relative and absolute quantification (ITRAQ), isotope coded affinity tag (ICAT), stable isotope labelling by amino acid in cell culture (SILAC), multidimensional protein identification technique (Mud PIT). Separation is followed by identification or quantification by mass spectrometry or tandem mass spectrometry (Quirino *et al.*, 2010) [44]. Finally database comparison is done to identify protein and its function.

Under gel-based method, two-dimensional gel electrophoresis is the most commonly used technique. This method employs separation of proteins based on molecular mass, isoelectric point, solubility and relative abundance (Joshi and patil, 2017) [26]. First step is separation of protein in a pH gradient based on their isoelectric point (PI) using isoelectric focusing (IEF). Proteins migrate to the position in the pH gradient equivalent to PI. Second step in 2DE is to separate protein based on their molecular weight using SDS-PAGE (Gorg *et al.*, 2004) [17]. Followed by individual proteins visualization by Coomassie or Silver Staining or by autoradiography (Merril *et al.*, 1981 and Oakley *et al.*, 1980) [34] [39]. 2DE separates proteins based on independent physical characteristics, a powerful means to resolve complex mixture of proteins. One of the major disadvantage of this technique is its lower sensitivity and limited reproducibility because of which only abundant protein can be identified (Bunai and Yamane, 2005) [8]. Two-Dimensional fluorescence difference gel electrophoresis (2D-DIGE) is another gel-based method to separate proteins on same gel by labelling, two protein samples with different fluorescent dyes and compared directly based on spot intensity (Dunn, 1993) [14].

ICAT (Isotope Coded Affinity Tag) is non gel-based method of protein separation which is used to compare two samples. It contains tag which has two group one is biotin group for affinity purification which contain isotope coded linker, it creates heavy or light versions of tag while second is thiol group. Used in identification of integral membrane protein (Gygi *et al.*, 1999) [19].

ITRAQ (Isobaric Tagging for Relative and Absolute Quantification), involves N- terminus and side chain amine specific isobaric labelled tags. It allows relative and absolute quantification of peptide from different sources simultaneously in a given sample (Ross *et al.*, 2004) [47].

SILAC (Stable Isotope Labelling by amino acid in Cell culture), cell population are grown in either N14 or N15 containing medium. Protein lysates are fractionated and separated by two-dimensional electrophoresis. Presence of N15 results in shift and creates two peaks for each peptide, ratio of intensities of peaks is indicative of expression levels of proteins (Ong *et al.*, 2002 and Oda *et al.*, 1999) [42] [41]. SILAC is most precise method for quantitative MS as it helps in relative assessment of small changes in protein (Blagoev *et al.*, 2004) [6].

Mud PIT (Multidimensional Protein Identification Technique) is another non gel-based method widely used in protein separation by chromatographic technique and is directly coupled with tandem mass spectrometry (McDonald *et al.*, 2002; Wolters *et al.*, 2001 and Washburn *et al.*, 2001) [31] [55] [51].

Protein quantification is done by mass spectrometric analysis. Differentially expressed gel spots based on statistical analysis are excised and processed for identification by mass spectrometry analysis (Link *et al.*, 1999) [30]. In-gel digestion, a fragmentation process involving protein digestion with an enzyme, while protein is attached to matrix, cleaving it at specific sites. Trypsin cleaves the carboxyl side of lysine and arginine of peptide chain, except when followed by proline. The peptide products acquired is introduced into mass analyzer for identification of protein either through peptide mass fingerprinting or tandem MS analysis. In Peptide Mass Fingerprinting, mass spectrometer is used to measure the absolute masses of peptides originating from unknown protein (Clauser *et al.*, 1999) [10]. Comparison of absolute masses are done by bioinformatics, computer programs such as MASCOT, Phenyx, OMSSA. These programmes translate genome into proteins and then cleaves proteins into peptides with help of known sequence of protein or genome of organism in database followed by theoretical calculation of the absolute masses of the peptides from each protein and comparison of peptide masses of unknown target proteins to theoretical peptide masses of each protein deposited in database. Protein Mass Fingerprinting will work only if protein sequence is present in database utilized (Quirino *et al.*, 2010) [44].

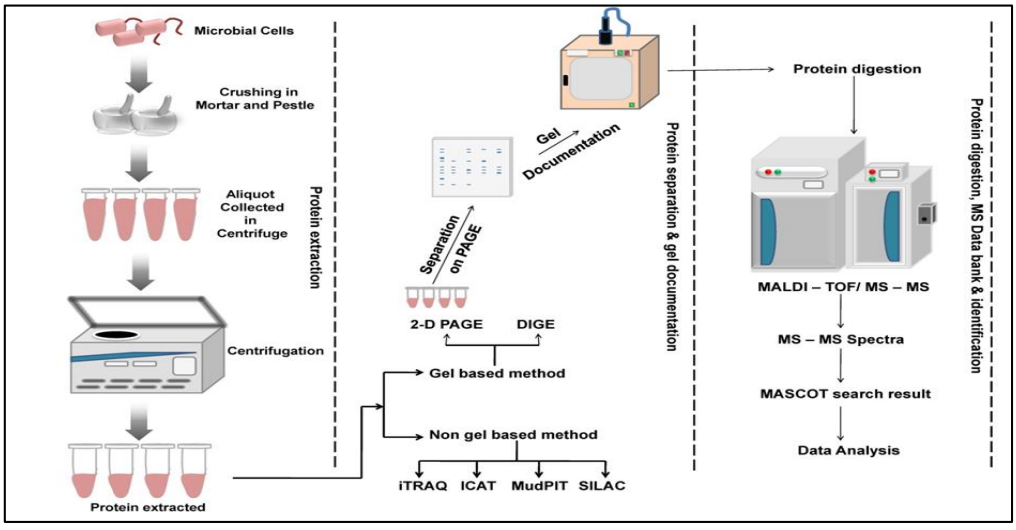

Fig 1: Overall schematic representation of Proteomics approaches.

Plant Pathogen interaction

A plant become diseased in most cases when it is attacked by pathogen or when affected by abiotic agent. For plant disease to occur, it follows disease triangle in which plant and pathogens must come in contact and must interact under favourable environment condition. Plant serves as host to vast number of pathogens such as bacteria, fungi, nematodes, virus etc. (Dangl and Jones, 2001) [12]. In plant-pathogen interaction, plant possess receptor that can activate basal resistance, mediated by pathogen associated molecular patterns (PAMPs) or cell wall degrading enzymes (CWDEs) which may lead to compatible or incompatible interaction (Jones and Dangl, 2006) [23]. Various defense related and biotic stress responsive proteins are induced in both the interactions. Suppression of plant defenses by pathogen effectors leads to susceptibility in host plants. The resistance (R) proteins expression in some host plants, guard them against pathogenic attack and activate specific resistance mechanism, referred to as hypersensitive response (HR). Proteomic studies of plant-pathogen interactions have revealed several pathogen and plant proteins expressed in different systems. Plant pathogen interaction is of two types, in compatible interaction plants are incapable of maintaining effective anti-infectious defence response allowing the pathogen to complete their life cycle. Incompatible interaction plant triggers a series of complex defence responses against pathogenic interaction to forestall pathogen growth (Mehta *et al*., 2008) [33].

Steps in Plant-pathogen interaction

There are three steps in plant-pathogen interaction these are perception, signalling and response. When pathogen comes in contact with host plants it secretes some elicitors compounds which is perceived by host plant and transfers the signal to host nucleus. Two types of defense responses occurs during host pathogen interaction. The first one is local response which begins with recognition when plant sense pathogen via elicitor/effectors produced by them. This type of recognition is mediated by plant resistance proteins and results in much more reliable, high impact defence response leading to rapid ion fluxes across plasma membrane, cell wall reinforcement, MAP kinase activation, active production of Reactive Oxygen Species and culminating in Programmed cell death known as hypersensitive response (Heath, 2000; Jones 2000 and Greenberg and Yao, 2004) [20] [24] [18]. Second type of defense response during plant pathogen interaction is induced, systemic or delayed defense response in which plant not only restricts pathogen infection locally, but also induces signals that enhance defense response to pathogen in distal systemic tissue, a phenomenon known as systemic acquired resistance (SAR) (Schneider *et al*., 1996) [49]. SAR plays a crucial role in developing resistance to disease as well as to recover from disease damage. Activation of Systemic Acquired Resistance require endogenous Salicylic acid (SA) and elevates the level of ethylene, jasmonic acid (JA), Nitric oxide (NO) induces production of Pathogenesis Related proteins (PR) which in turn activates many downstream process (Ryals *et al.,* 1996) [48].

Plant-Pathogen Interaction in relation to Proteomics

Genomic and post genomic studies have made available an enormous amount of information resulting in better understanding of pathogenicity strategies exploited by microbial pathogens and defense mechanism of plants. Proteomic is a fundamental approach to develop an understanding and recognition of the functions of proteins expressed in a specific conditions. Proteomics involves evaluation of protein complement of genome and validation of genome sequence information obtained experimentally with the protein profile of specific cell or tissue under diverse growth and treatments conditions. Proteins expressed during different plant pathogen interaction have been studied by Proteomics approach (Mehta *et al.,* 2008) [33].

Plant- Virus Interaction

For the viral infection to be successful, virus should be first transmitted by vector or mechanical means, followed by its replication in plant cells and movement through plasmodesmata to the neighbouring cells and finally enters vascular tissue of phloem to reach the target host tissues systemically. This process of loading and unloading of phloem virus is repeated several times leading to spread of infection**.** The compatible and incompatible virus interactions, both uses plant host proteins to complete the infection process and influences host proteins in response to infection against pathogen (Whitham *et al.,* 2006; Mehta *et al.,* 2008) [54] [33].

Lee and co-workers (2006) [28] conducted study on *Capsicum annum* cv. Bugang infected by Tobacco Mosaic Virus (TMV). This cultivar was reported to be susceptible to TMV-P1.2 strains and resistance against TMV-P0. Subsequent cultivar analysis by 2DE and MALDI-TOF MS, revealed that some defence related proteins namely, 26S proteosome subunit RPN 7 was present in hot pepper infected by TMV-P0. These defence related proteins where involved in Programme cell death, mRNA binding protein interfere with RNA metabolism, Rab 11 GTPase is responsible for membrane trafficking/recycling and endocytosis/exocytosis. Another study by Delalande and co-workers (2005) [13], on proteomic analysis was performed to study the compatible interaction between *Oryza sativa* (rice) and rice yellow mottle sobemovirus (RYMV). This analysis led to the recognition of a phenylalanine ammonia-lyase, a mitochondrial chaperonin-60 and an adolase C, but their role during RYMV infection of rice remains to be determined.

Plant Fungus interaction

A major challenge to the fungal biology is to explore the function, expression and regulation of protein encoded by sequenced fungal genome. Proteomic studies have revealed that various proteins involved in pathogenicity are up and down-regulated to enhance the infection process of fungi (Murad *et al.,* 2006, 2007) [36] [37]. Rust is one of the most devastating disease worldwide, reducing the annual cereal production. Proteomic analyses of wheat leaf rust pathogen and host have been done to understand the disease development at molecular level. 2DE and MS analysis was used to compare susceptible wheat lines inoculated with a virulent race of *Puccinia triticina* and mock-inoculated wheat. During analysis 22 different proteins were reported during pathogen infection with known and unknown functions (Rampitsch *et al.,* 2006; Webb and Fellers, 2006) [45] [53]. Very few proteomic studies have been done on host pathogen interaction out of it plant fungus interaction is the most studied one. Several proteins involved in various biological processes, such as defense and stress responses, photosynthesis, electron transport, signal transduction and metabolism, have been found. Houterman *et al.,* 2007 [21] studied the xylem sap proteome of tomato plants infected with *Fusarium oxysporum.* Out of 33 different proteins identified by 2DE and MS, 13 was specific to infected plant which

consist of proteins such as chitinases, peroxidases, polygalacturonase and a subtilisin-like protease involved in protection of cell structure and cell wall. Secretome is another approach, generally performed for study of fungal proteins. Exoproteome of phytopathogenic fungus *Sclerotinia sclerotiorum* was extracted from liquid culture, separated by 2DE and annotated by MALDI-MS/MS. Many of the secreted protein identified to be cell wall degrading enzymes was earlier considered to be pathogenicity or virulence factors of *Sclerotinia sclerotiorum* (Yajima and Kav, 2006) [56].

Plant bacterium interaction

Bacteria have diverse secretory pathways which leads to pathogenicity by translocating the bacterial proteins and other molecules into the host plant cell. Five secretion systems are reported in bacteria on the basis of the proteins that form them (Lee and Schneewind, 2001) [29]. Type I secretory pathway are involved in secretion of toxins such as hemolysins, rhizobiocin and found in almost all plant pathogenic bacteria. Main secretion system used by pathogenic bacteria during infection is Type III secretion pathway found mainly in gram negative bacteria (Agrios, 2004; Puhler *et al.,* 2004) [1] [43]. This system promotes bacteria to directly inject proteins called effectors or virulence factors into host cells (Galan and Collmer, 1999; Keen, 1990) [16] [27]. Best studied Type III secretion system effectors are designated Avirulence (Avr) proteins, *Xanthomonas* outer protein (Xop), Hrp outer protein (Hop), Pseudomonas outer proteins (Pop) (Noel *et al.,* 2001; Alfano and Collmer 1997; Arlat *et al.,* 1994) [38] [2] [3]. In addition to Type III, Type II secretion system is also important for bacterial pathogenicity and is involved in the export of various proteins, extracellular enzymes, toxins and virulence factors. Mehta and Rosato, 2001 [32] analyzed that *Xanthomonas axonopodis* pv. *citri* cultivated in presence of host *Citrus sinensis* leaf extract and protein profiles were analyzed and identified by two-dimensional gel electrophoresis (2-DE), differentially expressed proteins including sulphate binding protein, by ammonia terminal sequencing. Miao *et al.,* 2008 [35] reported that in susceptible cultivar of tomato inoculated with bacteria, caffeoyl CoA 3-O-methyltransferase gene was down regulated. Defense-related antioxidants such as pathogenesis-related-9 (PR) and metabolic enzymes were reported in *A. thaliana* in response to *P. syringae* (Jones *et al.,* 2004) [25]. Both of these groups of antioxidant enzymes were considered to have probable significant roles in the regulation of redox conditions within infected tissue. Many new techniques such as hexapeptide ligand libraries (CPLL such as proteominer) had been used to decrease the high abundant proteins for enrichment of low abundant protein. Frohlich *et al.* (2012) [15] applied the CPLL in *A. thaliana* leaf proteins after infection with virulent *P. syringae*. 2-DE showed a decrease in high-abundance proteins and an enrichment of less abundant proteins in leaf samples. Mass spectrometric analyses of leaf extracts led to the identification of 312 bacterial proteins in infected *Arabidopsis* leaves. Accumulation of free linolenic and benzoic acid or reduction in lauric acid was found to be important indicator of an active plant defense response in *G. max*. γ-aminobutyric acid, proline, and glutamine reduction resulted in *G. max* susceptibility after *Bradyrhizobium japonicum* inoculation (Brechenmacher *et al*., 2010) [7]. Transcriptomic and proteomic approaches identified numerous genes and proteins involved in carbon and nitrogen metabolism, plant defense responses, nutrient exchange, and signal transduction that are significantly regulated in *G. max* colonized by *B. japonicum* (Brechenmacher *et al*., 2010) [7].

Plant –Nematode interaction

Some of the most harmful plant parasitic nematodes include obligate sedentary endoparasites *Meloidogyne* spp., *Globodera* spp. and *Heterodera* spp. (Chitwood, 2003) [9]. These nematodes invade plant root as Juvenile larvae (J2) and after 3 moults develop into adult form that reproduce in repeated cycles. This leads to drastic modification in root system, causing consequential reduction in nutrient and water uptake resulting in plant death (Curtis, 2007) [11]. Recently several nematode expressed sequence tag libraries have been constructed, few of these genes known to be involved in parasitism. Proteomics analysis like 2DE allied to MS is strong and quick strategy to generate peptide sequence tags that can be linked to EST in silico These peptides can be used in genome projects by designing primers with aid of peptides, which can be further used to obtain full length gene sequence (Ashton *et al.,* 2001) [4]. Many genes expressed in salivary gland encode proteins with unknown function in *Meleidogyne* (89%) and *Heterodera* (72%). Proteins expressed in plant parasitic nematode species were beta 1,4 endonuclease 2 precursor found in *Heterodera.* Tropomysin in *Meleidogyne incognita*, Myosin Regulatory light chain 2 in *M. Incognita*, ATP synthase beta chain in *M. Incognita*, Translation Initiation factor Eif-4A in *M. incognita* and Enolase in *M. incognita* (Jaubert *et al.,* 2002) [22].

Conclusion

Cellular changes occurs during plant pathogen interaction due to translation of plant and pathogen genome to form new proteins. Although large amount of information is available on transcriptome, proteomic study is needed to validate it. Proteomic studies generally uses 2-D gel electrophoresis followed by mass spectrometric approaches to study plant pathogen interaction. Newer technological approaches such as MudPIT, ITRAQ, SILAC, ICAT and DIGE have made study of plant pathogen interaction easier and reliable. Although various proteins are expressed during plant pathogen interaction, proteins present in ample amount are detected by 2DE and further identified by MS. The novel proteins obtained by proteomic studies need to be further investigated for their cellular function. Protein identification by peptide mass fingerprinting have major constrain that protein can be identified only if genome sequence is known or large amount sequence data is available. A gap between proteomics and bioinformatics need to be bridged by making available complete genome sequence and validating proteomic studies by functional analysis. Rapid advancement in proteomic tools and methodologies have provided newer insight into plant pathogen interaction.

References

1. Agrios GN. Plant Pathology, 5th Ed. Academic Press, San Diego, 2000.
2. Alfano JR, Collmer A. The type III (Hrp) secretion pathway of plant pathogenic bacteria: trafficking harpins, Avr proteins, and death. J. Bacteriol. 1997; 179:5655-5662.
3. Arlat M, Van Gijsegem F, Huet JC, Pernollet JC Boucher CA, PopA1. a protein which induces a hypersensitivity-like response on specific *Petunia* genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*. EMBOJ. 1994; 13:543-553.
4. Ashton PD, Curwen RS, Wilson RA. Linking proteome

and genome: how to identify parasite proteins. Trends Parasitol. 2007; 17:198-202.
5. Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B. Quantitative mass spectrometry in proteomics: a critical review. Analytical and bioanalytical chemistry. 2007; 389(4):1017-1031.
6. Blagoev B, Ong SE, Kratchmarova I, Mann M. Temporal analysis of phosphotyrosine-dependent signaling networks by quantitative proteomics. Nature biotechnology. 2004; 22(9):1139-45.
7. Brechenmacher L, Lei Z, Libault M, Findley S, Sugawara M, Sadowsky MJ, *et al*. Soybean metabolites regulated in root hairs in response to the symbiotic bacterium *Bradyrhizobium japonicum*. Plant Physiol. 2010; 153:1808-1822.
8. Bunai K, Yamane K. Effectiveness and limitation of two-dimensional gel electrophoresis in bacterial membrane protein proteomics and perspectives. Journal of Chromatography B. 2005; 815(1-2):227-236.
9. Chitwood DJ. Research on plant parasitic nematode biology conducted by the United States Department of Agriculture Agricultural Research Service. Pest Manag. Sci. 2003; 59:748-753.
10. Clauser KR, Baker P, Burlingame AL. Role of accurate mass measurement (±10 ppm) in protein identification strategies employing MS or MS/MS and database searching. Anal. Chem. 1999; 71:2871-2882.
11. Curtis RH. Plant parasitic nematode proteins and the host–parasite interaction. Brief Funct Genomic Proteomic. 2007; 6:50-58.
12. Dangl JL, Jones JD. Plant pathogens and integrated defence responses to infection. Nature. 2001; 411(6839):826-833.
13. Delalande F, Carapito C, Brizard JP, Brugidou C, Dorsselaer AV. Multigenic families and proteomics: Extended protein characterization as a tool for paralog gene identification. Proteomics. 2005; 5:450-460.
14. Dunn MJ. Gel Electrophoresis: Proteins, BIOS, Oxford, England, 1993, 41-127.
15. Frohlich A, Gaupels F, Sarioglu H, Holzmeister C, Spannagl M, Lindermayr JC. Looking deep inside: detection of low-abundance proteins in leaf extracts of Arabidopsis and phloem exudates of pumpkin. Plant physiol. 2012; 159:902-914.
16. Galan JE, Collmer A. Type III secretion machines: bacterial devices for protein delivery into host cells. Science. 1999; 284:1322-1328.
17. Gorg A, Weiss W, Dunn MJ. Current two-dimensional electrophoresis technology for proteomics. Proteomics. 2004; 4(12):3665-3685.
18. Greenberg JT, Yao N. The role and regulation of programmed cell death in plant–pathogen interactions. Cellular Microbiology. 2004; 6:201-211.
19. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nature biotechnology. 1999; 17(10):994-999.
20. Heath M. Hypersensitive response-related cell death. Plant Mol. Biol. 2000; 44:321-334.
21. Houterman PM, Speijer D, Dekker HL, De Koster CG, Cornelissen BJC, Rep M. The mixed xylem sap proteome of *Fusarium oxysporum*-infected tomato plants. Mol. Plant Pathol. 2007; 8:215-221.
22. Jaubert S, Ledger TN, Laffaire JB, Piotte C, Abad P, Rosso MN. Direct identification of stylet secreted proteins from root-knot nematodes by a proteomic approach. Mol. Biochem. Parasitol. 2002; 121:205-211.
23. Jones JD, Dangl JL. The plant immune system. Nature. 2006; 444:323-329.
24. Jones A. Does the plant mitochondrion integrate cellular stress and regulate programmed cell death? Trends Plant Science. 2000; 5:225-230.
25. Jones AM, Thomas V, Truman B, Lilley K, Mansfield J, Grant M. Specific changes in the Arabidopsis proteome in response to bacterial challenge: differentiating basal and R-gene mediated resistance. Phytochemistry. 2004; 65:1805-1816.
26. Joshi, Patil. Proteomics. In Patwardhan B, Chaguturu R, (Ed.), Innovative Approaches in Drug Discovery. Academic Press, United State, 2017, 273-294.
27. Keen NT. Gene for gene complementarity in plant–pathogen interactions. Annu. Rev. Genet. 1990; 24:447-463.
28. Lee BJ, Kwon SJ, Kim SK, Kim KJ, Park CJ, Kim YJ, Park OK, Paek KH. Functional study of hot pepper 26S proteasome subunit RPN7 induced by tobacco mosaic virus from nuclear proteome analysis. Biochem. Biophys. Res. Commun. 2006; 351:405-411.
29. Lee VT, Schneewind Olaf. Protein secretion and the pathogenesis of bacterial infections. Genes & development. 2001; 15:1725-52.
30. Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, Morris DR, Garvik BM, Yates JR. Direct analysis of protein complexes using mass spectrometry. Nature biotechnology. 1999; 17(7):676-82.
31. McDonald WH, Ohi R, Miyamoto DT, Mitchison TJ, Yates III JR. Comparison of three directly coupled HPLC MS/MS strategies for identification of proteins from complex mixtures: single-dimension LC-MS/MS, 2-phase MudPIT, and 3-phase MudPIT. International Journal of Mass Spectrometry. 2002; 219(1):245-251.
32. Mehta A, Rosato YB. Differentially expressed proteins in the interaction of *Xanthomonas axonopodis* pv. *citri* with leaf extract of the host plant. Proteomics. 2001; 1:1111-1118.
33. Mehta A, Brasileiro AC, Souza DS, Romano E, Campos MA, Grossi-de-Sa M, Silva MS, Franco OL, Fragoso RR, Bevitori R, Rocha TL. Plant-pathogen interactions: What is proteomics telling us? FEBSJ. 2008; 275:3731-3746.
34. Merril CR, Goldman D, Sedman SA, Ebert MH. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science. 1981; 211(4489):1437-1438.
35. Miao L, Shou S, Zhu Z, Jiang F, Zai W, Yang Y. Isolation of a novel tomato Caffeoyl CoA 3-O-methyltransferase gene following infection with bacterium *Ralstonia solanacearum*. J. Phytopathol. 2008; 156:588-596.
36. Murad AM, Laumann RA, Lima Tde A, Sarmento RB, Noronha EF, Rocha TL, Valadares-Inglis MC, Franco OL. Screening of entomopathogenic *Metarhizium anisopliae* isolates and proteomic analysis of secretion synthesized in response to cowpea weevil (*Callosobruchus maculatus*) exoskeleton. Comp. Biochem. Physiol. C. Toxicol Pharmacol. 2006; 142:365-370.
37. Murad AM, Laumann RA, Mehta A, Noronha EF, Franco OL Screening and secretomic analysis of entomopathogenic *Beauveria bassiana* isolates in response to cowpea weevil (*Callosobruchus maculatus*)

exoskeleton. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 2007; 145:333-338.
38. Noel L, Thieme F, Nennstiel D, Bonas U. cDNA AFLP analysis unravels a genome-wide hrpG-regulon in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*. Mol. Microbiol. 2001; 41:1271-1281.
39. Oakley BR, Kirsch DR, Morris NR. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. Analytical biochemistry. 1980; 105(1):361-363.
40. Oda Y, Nagasu T, Chait BT. Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome. Nat. Biotechnol. 2001; 19:379-382.
41. Oda Y, Huang K, Cross FR, Cowburn D, Chait BT. Accurate quantitation of protein expression and site-specific phosphorylation. Proceedings of the National Academy of Sciences. 1999; 96(12):6591-6596.
42. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Molecular & cellular proteomics. 2002; 1(5):376-386.
43. Puhler A, Arlat M, Becker A, Göttfert M, Morrissey JP, O'Gara F, What can bacterial genome research teach us about bacteria–plant interactions? Curr. Opin. Plant. Biol. 2004; 7:137-147.
44. Quirino BF, Candido ES, Campos PF, Franco OL, Kruger RH. Proteomic approaches to study plant-pathogen interactions. *Phytochemistry*. 2010; 71:351-362.
45. Rampitsch C, Bykova NV, McCallum B, Beimcik E, Ens W. Analysis of the wheat and *Puccinia triticina* (leaf rust) proteomes during a susceptible host–pathogen interaction. Proteomics. 2006; 6:1897-1907.
46. Rasmussen HH, Orntoft TF, Wolf H, Celis JE. Towards a comprehensive database of proteins from the urine of patients with bladder cancer. J. Urol. 1996; 155:2113-2119.
47. Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. Molecular & cellular proteomics. 2004; 3(12):1154-1169.
48. Ryals J, Neuenschwander U, Willits M, Molina A, Steiner H, Hunt M. Systemic Acquired Resistance. The Plant Cell. 1996; 8(10):1809-1819.
49. Schneider M, Schweizer P, Meuwly P, Métraux JP, Systemic Acquired Resistance in Plants. International Review of cytology. 1996; 168:303-340.
50. Thurston G, Regan S, Rampitsch C, Xing T. Proteomic and phosphoproteomic approaches to understand plant-pathogen interactions. Physiol. Mol. Plant Pathol. 2005; 66:3-11.
51. Washburn MP, Wolters D, Yates III JR. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nature biotechnology. 2001; 19(3):242-247.
52. Wasinger VC, Cordwell SJ, Cerpa-Poljak A, Yan JX, Gooley AA, Wilkins MR, Duncan MW, Harris R, Williams KL, Humphery-Smith I. Progress with gene-product mapping of the Mollicutes: Mycoplasma genitalium. Electrophoresis. 1995; 16(1):1090-1094.
53. Webb CA, Feller JP. Cereal rust fungi genomics and the pursuit of virulence and avirulence factors. FEMS. Microbiol. Lett. 2006; 264:1-7.
54. Whitham SA, Yang C, Goodin MM, Global impact: elucidating plant responses to viral infection. Mol. Plant Microbe. Interact. 2006; 19:1207-1215.
55. Wolters DA, Washburn MP, Yates JR. An automated multidimensional protein identification technology for shotgun proteomics. Analytical chemistry. 2001; 73(23):5683-90.
56. Yajima W, Kav NN. The proteome of the phytopathogenic fungus *Sclerotinia sclerotiorum*. Proteomics. 2006; 6:5995-6007.