# Chapter 4
# Signal Transduction and Regulatory Networks in Plant-Pathogen Interaction: A Proteomics Perspective

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## Introduction

Plant diseases are amongst the major limiting factors of agricultural crop production worldwide. Depending upon the time of infection and severity of the disease, they can cause average yield losses of about 10–90 %. Plant disease directly or indirectly affects the life of human, as it may cause famine, mass migration and even death. For example, Irish potato famine of 1845–1846 killed hundreds of thousands of people. This event initiated a large scale migration. Within decade that follows the population of Ireland dropped from 8 million to 4 million (Ristaino 2002). Plant diseases significantly influence world economy, as crop plants make up large proportion of the world's economy, and in many countries constitute main sustenance for humans. According to one estimate, plant diseases could cost the US alone $33 billion per year (Maor and Shirasu 2005). To meet the ever increasing food demands of the rapidly increasing population, crop production will need to increase by 50 % by 2025 (Khush 2001). Currently, worldwide crop losses due to diseases are estimated to exceed $140 billion (Shani et al. 2006). Although application of fungicides and pesticides has helped in controlling plant diseases, chemical control is economically costly as well as environmentally undesirable. Keeping in view of the global food scarcity, there is, hence, an urgent need to develop crop plants with increased biotic stress tolerance so as to meet the global

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food demands. A detailed study of the molecular interactions between crops plants and their pathogens would, therefore, be of primary importance for devising new strategies based on plants self defense mechanisms to develop crops with increased disease tolerance for sustainable agricultural production.

When a plant and a pathogen come into contact, close communications occur between the two organisms (Hammond-Kosack and Jones 2000). Pathogen activities focus on colonization of the host and utilization of its resources, while plants are adapted to detect the presence of pathogens and to respond accordingly with antimicrobial defenses and other stress responses. During the long process of host-pathogen coevolution, plants have developed various elaborate mechanisms to ward off pathogen attack. Whereas some of these defense mechanisms are preformed and provide physical and chemical barriers to hinder pathogen infection, others are induced only after pathogen attack (Yang et al. 1997). Intercellular detection of the pathogen activates the first line of defense, termed innate or basal resistance, which involves recognition of evolutionarily conserved and essential features of pathogens such as bacterial lipopolysaccharides (LPS) or fungal chitin. These Extracellular signals are perceived by host cells through plasma membrane (PM) receptors that transduce the signals to an intracellular signal transduction cascade. It ends in the activation of transcription of the appropriate set of genes, which results into alteration or modification of cellular metabolism, accumulation of barrier forming substances (thickening of cell walls) and production of anti microbial compounds. In most cases, the transduction of signal relies on post translational modifications of the signaling proteins and the generation of so called secondary messenger molecules.

Among all post-translational modifications, phosphorylation has been studied most intensively (Pawson and Scott 2005), which can lead to changes in conformation, protein–protein interaction and protein activity. In eukaryotic cells, protein phosphorylation occurs predominantly on serine, threonine and tyrosine residues, but has also been described to occur on aspartate and histidine residues. The regulatory mechanisms of plant–pathogen interaction are extremely complex and dynamic, and the ongoing interactions between the pathogen and the plant are difficult to monitor with more traditional genetic and biochemical methods. The two approaches that are most promising for understanding the full network of the responses are microarray and proteomic analyses. Both permit a global analysis of cellular regulation while the microarray is restricted to the analysis of gene expression. The proteomics follows the accumulation and modification of proteins directly responsible for final cellular responses. Recent advancement in liquid chromatography–tandem mass spectrometry (LC–MS/MS) has greatly improved the throughput and sensitivity of protein measurements. In order to efficiently describe the status of phosphorylated molecules, a variety of enrichment strategies for phosphorylated peptides have been developed. The most commonly used are based on affinity purification of phosphoryl-group containing peptides and include immobilized metal affinity chromatography (IMAC), strong cation exchange chromatography (SCX) and metal oxide affinity chromatography (MOAC) (Mithoe and Menke 2011).

Over the last few years, significant progress has been made in understanding the signaling processes involved in plant-pathogen interactions. In this chapter, we

focus on signaling pathways involved in plant defense against pathogens and the role of proteomics technology in understanding the underlying mechanisms.

## Plant Signal Transduction in Plant Defense Against Pathogens

Plants have integrated signaling system that mediate the perception and responses to the hormones, nutrients, environmental and stresses that govern plant growth and development. Interactions between plants and pathogens induce a series of plant defense responses (Hammond-Kosack and Jones 1996). The rate at which the plant cell mobilise its defenses often determines whether it survives or succumbs to the attack. Therefore, highly sensitive perception systems for either pathogen derived (exogenous) or plant-derived (endogenous) elicitors are the key to successful plant pathogen defense. The sensing of stress signals and their transduction into appropriate responses is crucial for the adaptation and survival of plants. Plant receptors are instrumental for signal recognition and initiation of an intracellular signal transduction cascade mediating activation of multifaceted defense reactions, both in host and non-host incompatible plant pathogen interactions.

The current knowledge of plant signal transduction pathways has come from the identification of the sensors and receptors that perceive the signal, the transcription factors and target genes that coordinate the response (Hammond-Kosack and Jones 1996). The activation of defense responses in plants is initiated by host recognition of pathogen-encoded molecules called elicitors (e.g., microbial proteins, small peptides, and oligosaccharides, etc.). The term 'elicitor', originally coined for compounds that induce accumulation of antimicrobial phytoalexins in plants, is now commonly applied to agents stimulating any type of defense response (Ebel and Scheel 1997). Elicitors of diverse chemical nature and from a variety of different plant pathogenic microbes have been characterized and shown to trigger defense responses in intact plants or cultured plant cells. These elicitors include (poly) peptides, glycoproteins, lipids and oligosaccharides. Binding of the elicitor ligand to its receptor initiates a signal transduction cascade that may involve protein phosphorylation, ion fluxes, reactive oxygen species (ROS), nitric oxide (NO) and other signaling events.

### *Ion Fluxes and Reactive Oxygen Species*

The earliest reactions of plant cells include changes in plasma membrane permeability leading to calcium and proton influx and potassium and chloride efflux (McDowell and Dangl 2000). Various fungal and bacterial elicitors have been reported to trigger fluxes of $H^+$, $K^+$, $Cl^-$, and $Ca^{2+}$ across the plasma membrane (Atkinson et al. 1996; Mathieu et al. 1991; Bach et al. 1993; Kuchitsu et al. 1993; Popham et al. 1995). In suspension cells of parsley, a transient influx of $Ca^{2+}$ and $H^+$ and an efflux of $K^+$ and $C1^-$ are initiated within two to five minutes after the addition of a fungal oligopeptide

elicitor (Hahlbrock et al. 1995). Ion fluxes subsequently induce extracellular production of reactive oxygen intermediates, such as superoxide ($O^{2-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl free radical ($OH^-$), known as oxidative burst, catalyzed by a plasma membrane-located NADPH oxidase and/or apoplastic-localized peroxidases (Somssich and Hahlbrock 1998). Oxidative burst is a central component of plants defense machinery (Alvarez et al. 1998). The generation of ROS is likely dependent on the activation of a plasma membrane NADPH oxidase similar to that present in mammalian phagocytes. Using the mammalian system as a model, homologues of the large gp91-phox protein of NADPH oxidase have been cloned from several plant species including *Arabidopsis* and rice (Desikan et al. 1998; Torres et al. 1998). Eight such sequences have been identified in the *Arabidopsis* genome (The Arabidopsis Genome Initiative 2000).

Interestingly, plant homologues contain calcium-binding EF-hand regions, suggesting that calcium may be important in the regulation of their activity (Desikan et al. 1997). Immunological evidence points to the presence of the NADPH oxidase cytoplasmic peptides too, in several species of plants including *Arabidopsis* (Desikan et al. 1996), tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*), soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) (Dwyer et al. 1996; Tenhaken et al. 1995; Xing et al. 1997).

Cell wall peroxidases have also been reported to be involved in ROS generation following pathogen challenge (Bolwell et al. 1995). For example, in French bean (*Phaseolus vulgaris*) ROS production in response to a cell wall elicitor from *Colletotrichum lindemuthianum* was dependent on an exocellular peroxidase, and *Arabidopsis* plants expressing an antisense peroxidase from French bean exhibit enhanced disease susceptibility (Bolwell 1999). Therefore, it can be seen that there are several potential sources of ROS in plant tissues, and future research should aim to elucidate the role of distinct sources of ROS not only in plant defense, but also in response to a variety of abiotic stresses.

Superoxide anion and $H_2O_2$ generated during the oxidative burst play multiple roles in plant defense responses. During a hypersensitive response (HR), a highly localized accumulation of $H_2O_2$ was found in the lettuce cell walls adjacent to invading bacteria (Bestwick et al. 1997). In addition, constitutive expression of an $H_2O_2$ generating glucose oxidase in the transgenic potato was shown to confer enhanced resistance to the bacterial pathogen, *Erwinia carotovora* pv. *carotovora* and the fungal pathogen, *Phytophthora infestans* (Wu et al. 1995). $H_2O_2$ was also demonstrated to have direct antimicrobial activity (Peng and Kuc 1992) and to contribute to cell wall reinforcement by stimulating lignification and crosslinking of cell wall hydroxyproline-rich glycoproteins (Bradley et al. 1992; Brisson et al. 1994). Furthermore, superoxide anion and $H_2O_2$ may also act as secondary messengers to induce plant defense-related genes (Levine et al. 1994; Green and Fluhr 1995) and hypersensitive host cell death (Doke 1983a; Doke 1983b; Doke and Ohashi 1988; Levine et al. 1994). Elicitor-stimulated superoxide anion from the oxidative burst was also shown to be essential in triggering defense gene activation and phytoalexin synthesis in parsley (Jabs et al. 1997).

## *Cellular Generation of Nitric Oxide*

Nitric Oxide (NO) like ROS, is an important signaling molecule that is rapidly generated after recognition of pathogens (Perchepied et al. 2010). It is a small gaseous radical with broad spectrum of regulatory functions in lateral root development, germination, leaf expansion, stomatal closure, flowering, hormonal signaling, defense against biotic and abiotic stresses and cell death (Leitner et al. 2009). The sources of NO synthesis in plants include nitrate reductase (NR) dependent NO formation, oxidation of arginine to citrulline by NO Synthase (NOS) like activity, and a non-enzymatic NO generation system in the apoplast (Leitner et al. 2009). Although a number of studies had demonstrated NOS-like activity in plants, no gene or protein that has a sequence similar to known mammalian-type NOS has been establish in plants (Leitner et al. 2009). Guo et al. (2003) reported a NOS-like enzyme activity from *Arabidopsis thaliana* (At NOS1) with a sequence similar to a protein that has been implicated in NO synthesis in the snail *Helix pomatia*. Recently, the only postulated plant NOS (AtNOA1/RIF1) has been shown to have no NOS activity (Moreau et al. 2008). Instead, it is the chloroplast-targeted GTPase essential for proper ribosome assembly (Moreau et al. 2008). Mutation in this gene leads to reduced NO accumulation, probably because of its rapid reaction with the elevated amounts of ROS observed in the Atnoa1 mutant (Moreau et al. 2008). *Arabidopsis* mutant *noa*1, however, is still useful for its phenotype, which shows reduced levels of NO in plant growth, fertility, hormonal signaling, salt tolerance, and plant-pathogen responses. Knocking out or down NOA1 expression provides a powerful tool to analyze NO function (Asai et al. 2008). So, the identification and characterization of NO-producing enzymes in plants, other than NR, still remains a challenging tasks for plant biologists.

Recently, Perchepied et al. (2010) reported that NO production in *Arabidopsis* leaf was significantly reduced by the mammalian NO synthase (NOS) inhibitor L-NAME (37% inhibition). They further demonstrated that like ROS, NO is an early-induced signal during the interaction between *Arabidopsis* and *S. sclerotiorum*. In order to genetically determine the role of the signaling molecules during the interaction, mutants altered in their production were tested for their response to *S. sclerotiorum* and found that NO synthesis was strongly reduced in *noa1* mutant (83% inhibition) and also in *nia1 nia2* mutant (62% inhibition). Therefore, these results demonstrate that NO might have an important role in disease resistance to *S. sclerotiorum*. To further analyze the role of ROS and NO in the activation of defenses to *S. sclerotiorum*, Perchepied et al. (2010) also demonstrated that expression of *PR1, PDF1-2*, and *ABI1* was found to be abolished or strongly reduced compared with the wild type in double mutant *nia1 nia2*, whereas *VSP1* expression was delayed by 24 h. Similarly, in the *rboh-D rboh-F* double mutant, *PR1* expression was abolished, *PDF1-2* and *VSP1* expression was delayed, and *ABI1* expression was upregulated. These results suggest a differential regulatory effect of NO and ROS on the different defense pathways (Perchepied et al. 2010).

## *G Proteins*

G Proteins are one of the most important components of signaling system. There are two types of G proteins, monomeric and heterotrimeric. The heterotrimeric G proteins contain α, β and γ subunits, and the monomeric small G proteins appear to be similar to free α subunits, operating without the βγ heterodimer. The Gα subunit has both GTP-binding and GTPase activity and acts as a molecular switch for signal transduction. G proteins have been implicated in plant defense, however much remains to be explored especially in case of Arabidopsis and rice. The genome of the model plant *Arabidopsis thaliana* contains one prototypical Gα (GPA1), one Gβ (AGB1), and two identified Gγ (AGG1 and AGG2) subunits (Jones and Assmann 2004) and one RGS protein, AtRGS1 (Chen et al. 2003). Two Gα subunits (PGA1 and PGA2) are reported from pea (Marsh and Kaufman 1999).

A variety of evidence suggest that heterotrimeric GTP-binding proteins are involved in transferring elicitor signals from the receptor to calcium channels that activate downstream reactions, such as the oxidative burst and phytoalexin accumulation (Gelli et al. 1997; Xing et al. 1997). Llorente et al. (2005) reported that the ERECTA receptor-like kinase and G proteins are required for resistance to the necrotrophic fungus *Plectosphaerella cucumerina* in *Arabidopsis*. In cultured soybean cells, mastoparan, a G protein-activating peptide, was found to stimulate calcium influx, increases in cytosolic calcium levels and production of reactive oxygen species in the absence of elicitor (Chandra and Low 1997). Ectopic expression of the cholera toxin A1 subunit inhibiting GTPase activity of G proteins in tobacco plants resulted in high salicylate levels, constitutive expression of PR proteins and enhanced pathogen resistance (Beffa et al. 1995). Recently, Trusov et al. (2006) found in *Arabidopsis* that Gβ deficient mutants are more susceptible to infection with *A. brassicicola* and *F. oxysporum* when compared to wild type (Col-0), while Gα-deficient mutants are less susceptible to the disease than wild type. They also reported that the Gβγ subunit is an integral component and a positive regulator of the JA-signaling cascade.

## *Phospholipid Derived Molecules*

Phospholipids are emerging as important secondary messengers in plant defense signaling. Recent research has begun to reveal the signals produced by the enzymes phospholipase C (PLC), phospholipase D (PLD) and phospholipase A2 ($PLA_2$), and their putative downstream targets (Laxalt and Munnik 2002). Upon perception of the invading pathogen, several phospholipid hydrolyzing enzymes are activated that contribute to the establishment of an appropriate defense response. These include activation of G proteins based signaling leading to the production of oxylipins and jasmonates, as well as the potent second messenger, phosphatidic acid (PA) (Canonne et al. 2011).

Three PAT-PLA genes (AAF98368, AAF98369, AAF98370) found in tobacco, were strongly induced in response to microbial infections or elicitors before the accumulation of jasmonic acid in the infiltrated zone, but poorly induced in response to wounding (Dhondt et al. 2002), indicating that further studies are required. Additionally, PLA2 activity was found to be involved in the wound-activated cascade leading to the production of aldehydes by generating C20 fatty acids in diatoms (Pohnert 2002). PLA activation has been reported in elicitor-treated cultured parsley cells. Virus infection also activates PLA (Ryu 2004). The role of PLA in cell elongation, auxin signaling, shoot gravitropism, pollen maturation, anther dehiscence and flower opening are well established, but its role in plant defense is still need to be revealed.

The involvement of PLC in stress signaling has been indicated in a number of studies. The genes encoding PI-PLC were found to be induced to a significant extent under environmental stresses (Tuteja and Sopory 2008). Van der Luit et al. (2000) reported that tomato cells responded to general elicitors, such as xylanase, flagellin or chitotetraose, by rapidly (i.e. within minutes) and transiently producing phosphatidic acid (PA). Role of PLC in Avr-induced disease resistance has also been implicated. In this case PLC/diacylglycerol kinase (PLC/DGK) mediated production of PA was found to be involved in disease resistance signaling (Andersson et al. 2006).

PLD activity has been associated to a variety of stress responses in plants. Activation of PLD activity during plant defense was first described in rice, after infection by *Xanthomonas oryzae* (Young et al. 1996). In *Arabidopsis*, expression of the α, β and γ class of *PLD* genes is induced after infiltration by both virulent and avirulent strains of *P. syringae* (de Torres Zabela et al. 2002). The effects of PLD activation during plant-pathogen interactions are varied. Indeed, PA has been shown to induce ROS production1 and activate defense-related or ethylene-responsive genes PLDs also participate in salicylic acid-dependent signaling (Canonne et al. 2011). Five different tomato PLDs have been cloned and only one of them, PLDβ1, is specifically upregulated in response to xylanase. Silencing of this gene in tomato cell suspensions resulted in the loss of the xylanase-PLD response, indicating that PLDβ1 generates PA in response to xylanase treatments (Laxalt and Munnik 2002).

## *Protein Kinase*

Protein phosphorylation plays an important role in plant responses to pathogen attack. Signaling systems which involve phosphorylation, and can lead directly to altered gene expression pattern in cells, are the MAPK (Miotgen Activated Protein Kinase) pathways (Hancock et al. 2002). The MAPK cascades are highly conserved modules in all eukaryotes (Pitzschke et al. 2009). In plants, MAPK pathways are

involved in the regulation of development, growth and programmed cell death in responses to a diversity of environmental stimuli including cold, heat, reactive oxygen species, UV, drought and pathogen attack (Colcombet and Hirt 2008). These cascades are minimally composed of a MAPKKK (MAPK kinase kinase), a MAPKK (MAPK kinase) and a MAPK. The *Arabidopsis* genome contains about 110 genes coding for putative MAPK pathway components: 20 MAPKs, 10 MAPKKs and more than 80 MAPKKKs (MAPK Group 2002). In plants, pathogen challenge along with cold, drought and phytohormones may lead to the activation of MAPK cascades, resulting in the modulation of nuclear gene expression (Hirt 1997). Exogenous hydrogen peroxide can lead to the activation of MAP kinases (Kovtun et al. 2000; Samuel et al. 2000). In *Arabidopsis* suspension cultures and leaves, hydrogen peroxide treatment activates AtMPK6 (Desikan et al. 2001). One of the potential targets of NO in cells is also the MAPK cascade. MAPK activation by NO has been reported in *Arabidopsis* (Clarke et al. 2000) and tobacco (Durner and Klessig 2000). Activation of such MAPK cascades is likely to lead to alteration in gene expression profiles. In *Arabidopsis*, MPK3, MPK4 and MPK6 are all activated by bacterial and fungal elicitors (Desikan et al. 2001; Nuhse et al. 2000). The flagellin derived peptide, flg22 triggers a rapid and strong activation of MPK3, MPK4 and MPK6 (Droillard et al. 2004). MPK4 andMPK6 are also activated by harpin proteins, which are encoded by hrp (hypersensitive response and pathogenicity) genes in many plant pathogenic bacteria. This activation is followed by the induction of pathogenesis-related (PR) genes (Desikan et al. 2001), encoding for proteins with antimicrobial activities. Similarly, various NLPs (necrosis and ethylene-inducing peptide1-like proteins) trigger MAPK activation and induce defence responses (Qutob et al. 2006).

## *Salicylic Acid, Jasmonic Acid and Ethylene*

Most of the inducible, defense-related genes are regulated by signal pathways involving one or more of the three regulators jasmonate, ethylene and salicylic acid (Sticher et al. 1997; Reymond and Farmer 1998; Ananieva and Ananiev 1999).

SA levels increase in plant tissue following pathogen infection, and exogenous application of SA results in enhanced resistance to a broad range of pathogens (Kunkel and Brooks 2002). Genetic studies have shown that SA is required for the rapid activation of defense responses that are mediated by several resistance genes, for the induction of local defenses that contain the growth of virulent pathogens, and for the establishment of systemic acquired resistance (SAR) (Ryals et al. 1996). Several studies have also demonstrated that when SA accumulation is prevented, resistance is compromised. Transgenic tobacco and *Arabidopsis* plants unable to accumulate SA because of the expression of the *Pseudomonas putida nahG* gene encoding salicylate hydroxylase, exhibit poor induction of *PR* genes after pathogen infection and fail to develop SAR (Gaffney et al. 1993; Delaney et al. 1994). The signal transduction pathway downstream of SA leads to the expression

of a number of PR genes, such as PR-1 and β-1,3-glucanase (Ryals et al. 1996). Activation of R-gene-mediated defense signaling induces SA synthesis and downstream defense responses. Significantly, the application of SA activates the expression of R genes of the toll-interleukin-2 receptor (TIR)–nucleotide-binding site (NBS)–leucine-rich repeat (LRR) type (Shirano et al. 2002). Similarly, SA activates expression of RPW8, which confers resistance to the powdery mildew pathogen (Xiao et al. 2003).

SA also activates expression of the EDS1 gene, which is required for SA accumulation and resistance conferred by these R-gene-activated pathways (Feys et al. 2001). *Arabidopsis* mutants that are impaired in SA responsiveness, such as npr1 (nonexpressor of PR) or are defective in pathogen-induced SA accumulation, such as eds1 (enhanced disease susceptibility 1), eds5 (enhanced disease susceptibility 5), sid2 (isochorishmate synthase) and pad4 (phytoalexin deficient 4), exhibit enhanced susceptibility to pathogen infection and show impaired PR gene expression (Venugopal et al. 2009).

Jasmonates are produced from the major plant plasma membrane lipid, linolenic acid via the octadecanoid biosynthetic pathway (Yang et al. 1997). First indications for a role of jasmonates in the regulation of gene expression were obtained by Parthier and co-workers who observed the accumulation of jasmonate inducible proteins (JIPs) in senescing barley leaves (Weidhase et al. 1987; Mueller-Uri et al. 1988). The rapid accumulation of jasmonate has been observed in many cultured plant cells in response to various elicitor treatments (Ebel and Scheel 1997; Gundlach et al. 1992). In suspension-cultured rice cells, an *N*-acetylchitohepatose elicitor induces the synthesis of the phytoalexin, momilactone A, which is preceded by transient accumulation of jasmonate (Nojiri et al. 1996). *A. thaliana* mutants that are impaired in JA production (e.g. *fatty acid desaturase fad3/fad7/fad8* triple mutants) or perception (e.g. *coronatine insensitive1* [*coi1*] and *jasmonic acid resistant1* [*jar1*]) exhibit enhanced susceptibility to a variety of pathogens, including the fungal pathogens *Alternaria brassicicola*, *Botrytis cinerea*, and *Pythium sp*., and the bacterial pathogen *Erwinia carotovora* (Thomma et al. 1998; Stintzi et al. 2001; Vijayan et al. 1998; Staswick et al. 1998; Norman-Setterblad et al. 2000). Perchepied et al. (2010) demonstrated that the JA-insensitive *coi1-1 arabidopsis* mutant was highly susceptible to *S. sclerotiorum*, thus indicating that JA is a major signal for activation of defenses against this fungus. In the same study, however, *jar1-1,* a jasmonate-resistant mutant shown to exhibit enhanced sensitivity to the fungal necrotroph *Pythium irregulare*, was not affected for responsiveness to *S. sclerotiorum*. Wounded tissue rapidly activates JA biosynthesis, and increased JA triggers the SKP1/Cullin/F-box E3 ubiquitin ligase complex containing the F-box subunit CORONATINE INSENSITIVE1 (SCFCOI1) to degrade the repressors of JA signaling – the JASMONATE-ZIM (JAZ) family proteins – by the ubiquitin/26S-proteasome pathway (Chini et al. 2007; Yoshida et al. 2009). In addition to their local synthesis and action, JAs also move systemically via vascular strands to transmit wound signals to distal tissues (Thorpe et al. 2007).

Ethylene (ET) plays a critical role in the activation of plant defenses against different biotic stresses through its participation in a complex signaling network that

includes jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) (Adie et al. 2007). ET has been implicated in both local and systemic defense responses to *A. brassicicola* through its regulation of GLIP1. This secreted lipase has antifungal properties and is induced by ET but not by SA or JA (Oh et al. 2005). It is believed that crosstalk between ethylene and JA pathways enables plants to optimize their defense strategies more efficiently and economically (Baldwin 1998). It has also been reported that ethylene and jasmonate pathways converge in the transcriptional activation of ETHYLENE RESPONSE FACTOR1 (ERF1), which encodes a transcription factor that regulates the expression of pathogen response genes that prevent disease progression. The expression of *ERF1* can be activated rapidly by ethylene or jasmonate and can be activated synergistically by both hormones (Lorenzo et al. 2003). In another study with ET-insensitive (Tetr) tobacco plants, it was reported that ET is essential for the onset of SA-dependent SAR that is triggered upon infection by tobacco mosaic virus (Verberne et al. 2003). Moreover, ET was shown to enhance the response of *Arabidopsis* to SA, resulting in a potentiated expression of the SA-responsive marker gene *PR-1* (Lawton et al. 1994; De Vos et al. 2006). This synergistic effect of ET on SA-induced *PR-1* expression was blocked in the ET-insensitive mutant *ein2* (De Vos et al. 2006), which indicates that the modulation of the SA pathway by ET is EIN2 dependent and thus functions through the ET signaling pathway. Therefore, the SA, JA and ET response pathways serve as the backbone of the induced defense signaling network in plants.

## Plant-Pathogen Interactions and Proteomics

The common approach utilized for proteomics based experiments comprises two-dimensional gel electrophoresis protein profile followed by MS analysis of differential expressed spots (MALDI-TOF or MS-MS) and identification by DNA, EST or protein database searching using specific algorithms (i.e. MASCOT, phenyx and OMSAA). In brief, the workflow of a standard proteomics experiment includes all or most of the following steps: experimental design, sampling, sample preparation, protein extraction/fractionation/purification, labeling/modification, separation, MS analysis, protein identification, and statistical analysis of data and validation. The most appropriate protocol to be used depends on and must be optimized for the biological system and type of tissue/cells, as well as the objectives of the research (descriptive, comparative, Post Translational Modifications, interactions, targeted Proteomics) (Jorrin-Novo et al. 2009). There are number of technical advances available and constantly evolving particularly for sample preparation, gel free proteomics, protein identification and data analysis, but this will not be the focus of this chapter. Those interested in further reading can refer to the reviews (Ong et al. 2003; Chen and Harmon 2006; Domon and Aebersold 2006; Rossignol et al. 2006; Everberg et al. 2008; Carpentier et al. 2008; Chen 2008; Jorrin-Novo et al. 2009). Herein, we address the challenges in proteomics and phosphoproteomics studies of plant pathogen interactions.

## *Comparative Proteomics*

The aim of most of the proteomics studies was to compare the plant response to infection by the pathogen and to identify and characterize common and specific changes in protein expression patterns. Geddes et al. (2008), using two-dimensional Electrophoresis (2-DE) coupled with LC-MS/MS, identified differentially expressed proteins in *Fusarium* head blight-resistant and *Fusarium* head blight-susceptible barley genotypes under infected and uninfected conditions. In this study, approximately 600 protein spots were resolved in the pH range of 4–7 in the 2-DE gels. A total of 16 different acidic proteins associated with resistance mechanisms against *Fusarium* head blight were identified, out of which 12 proteins were associated with oxidative burst response and 4 proteins were associated with PR-Proteins. Takemoto et al. (1997) reported that chitinase (PR3) and osmotin (PR5) were associated with the actin cytoskeleton that is involved in cytoplasmic aggregation in the early stages of the hypersensitive response (HR) between *Phytophthora infestans* and potato. *Fusarium* head blight, caused mainly by *F. graminearum*, is one of the most destructive diseases of wheat. The interaction between *F. graminearum and wheat* has been investigated by Zhou and his coworkers in 2006. They found that 33 plant proteins were expressed in response to *F. graminearum* in wheat spikes. These proteins were divided into two groups, each related to defense response or metabolism. The authors suggested that several of these proteins were directly involved in mounting the plant defense against infection by protecting against the oxidative burst inside the plant cell. Such a burst can be caused in plant cells by invading fungus.

Proteomics analysis was carried out to study the compatible and incompatible interactions between rice and bacteria, *Xanthomonas oryzae* pv. *oryzae (Xoo)* (Mahmood et al. 2006). In this study, four different defense-related proteins were identified, namely thaumatin-like protein (PR5), PBZ, SOD, and peroxiredoxin. Overexpression of PR5 in transgenic rice plants enhanced the resistance of rice to *Rhizoctonia solani*, the causal organism of sheath blight of rice (Datta et al. 1999). Wei et al. (2009) used isobaric tag- based methodology for relative peptide quantification (iTRAQ) coupled with multidimensional liquid chromatography and tandem mass spectrometry to study the response of rice to brown plant hopper (BPH) attack. In this study, three proteins involved in JA biosynthesis were induced in rice in response to infestation by the BPH: cytochrome P450, AOC 4 and alpha-DOX2. Alpha-DOX2 is a dioxygenase that catalyzes the synthesis of 13-hydroperoxylinolenic acid from linolenic acid in JA biosynthesis. Koeduka et al. (2005) reported that alpha-DOX can be induced by blight bacteria infection, and both oxidative and heavy metal stresses, through the jasmonate signaling pathway in the leaves of rice seedling. AOC4 catalyzes the stereospecific cyclization of an unstable allene oxide to (9S, 13S)-12-oxo- (10, 15Z)-phytodienoic acid, and experiments with the JA deficient *Arabidopsis* mutant opr3 indicate that AOC is the preferential target in the regulation of JA biosynthetic capacity (Stenzel et al. 2003). Since the BPH is a phloem-feeding insect, AOC may have a role in systemic defense signaling. Liang et al. (2008), using 2-DE identified 9 proteins related with

defense responses. Out of these two proteins were identifies as methionine adenosyltransferase (MAT) involved in ethylene biosynthesis, and JA-responsive proteins (JR1) MAT catalyzes the synthesis of the ethylene precursor, S-adenosylmethionine (AdoMet) and plays an important role in mediating the cross talk between ethylene and NO signaling pathways (Lindermayr et al. 2006). JA has crucial role in regulating many plant processes including mediation of resistance to pathogens (Creelman and Mullet 1997). JA-responsive (JR) genes, including JR1, have been demonstrated to be induced by wounding (Leon et al. 1998). Oh et al. (2005) started with a proteomic comparison of the proteins secreted by *Arabidopsis* cultured cells in the presence of salicylic acid (SA). Thirteen different proteins that responded to the SA treatment were identified by MALDI-ToF MS. One of them was GDSL LIPASE 1, or GLIP 1, a SA-induced protein. Upon further characterization, it was found to play a role in the defense against the necrotrophic fungus *Alternaria brassicola*. In another study, a proteomics analysis was carried out to understand the molecular mechanism of interaction between *Fusarium graminearum* and *Triticum aestivum*. About 1,380 protein spots were resolved on 2-D gels stained with Sypro Ruby. In total, 41 proteins were detected which are differentially regulated due to *F. graminearum* infection, and were analyzed with LC-MS/MS for their identification. The proteins involved in the antioxidant and jasmonic acid signaling pathways, pathogenesis-related response, amino acid synthesis and nitrogen metabolism were up-regulated, while those related to photosynthesis were less abundant following *F. graminearum* infection (Zhou et al. 2006).

Beet necrotic yellow vein virus (BNYVV) is a devastating sugar beet pathogen. Resistance is limited and resistance-breaking isolates are becoming problematic. Larson et al. (2008) studied the differential sugar beet protein expression induced by BNYVV- with multidimensional liquid chromatography. Of more than 1,000 protein peaks detected in root extracts, 7.4 and 11% were affected by BNYVV in the resistant and susceptible genotypes, respectively. Using tandem MALDI-TOF-MS, 65 proteins were identified in this study. Proteomic data suggest involvement of systemic resistance components in Rz1-mediated resistance and phytohormones in symptom development. Several proteins affected by BNYVV are classically associated with plant defense, suggesting inducible resistance may contribute to viral disease suppression. These include pathogenesis-related proteins, such as chitinase, protease, glucanase, peroxidase and defensin. Interestingly, induction of these proteins was not always limited to the resistant genotypes. Some oxidative enzymes, which are also known to contribute to plant defense, appear to have similar timing dependent expression. Polyphenol oxidase, a protein responsible for physical barrier development, and toxic compound and ROS production, is more highly and rapidly expressed in the resistant genotype when compared with expression patterns from the susceptible genotype.

A study on rice proteomics was performed to analyse the protein profile after *Magnaporthe grisea* infection, and was conducted using infected leaf blades fertilized with various levels of nitrogen (Konishi et al. 2001). Rice plants grown with high levels of nitrogen nutrient are more susceptible to infection by the blast fungus (Long et al. 2000). Though, leaf proteins revealed some minor changes

when plants grown under different levels of nitrogen were compared, this study failed to establish any direct correlation between nitrogen application and disease resistance (Rakwal and Agrawal 2003). 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. (2003) using rice suspension cultured cells. In this study, twelve proteins 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. The authors, followed with another proteome study using rice leaves, identified eight proteins newly induced or with increased expression (Kim et al. 2004). The identified proteins belonged to several groups of PR proteins, and included two RLKs, two b-1,3-glucanases (Glu1, Glu2), TLP, peroxidase (POX 22.3), PBZ1 and OsPR-10. Lee et al. (2006) investigated rice sheath leaves after infection with this fungus, *Rhizoctonia solani* and the 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 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, indicating a common pathway for both stress and non-stress plant functions.

Using 2-DE, the root protein profiles of *M. truncatula* were analysed after *Aphanomyces euteiches* pathogen infection (Colditz et al. 2004). 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. Another study focused on *Zea mays* embryos in response to the fungus, *Fusarium verticillioides* (Campo et al. 2004). The proteins identified included PR proteins, antioxidant enzymes and proteins involved in protein biosynthesis, folding and stabilization.

## *Phosphoproteomics*

Protein phosphorylation plays a key role in signal transduction in plant during defense responses. The importance of phosphorylation in plant basal defense responses is exemplified by the FLS22activated MAP kinase cascade. The phosphorylated proteins are primarily involved in the early steps of signal transduction pathways as demonstrated by Lecourieux-Ouaked et al. (2000) in tobacco by using cryptogein, an elicitor of defense reactions. Kinases are implicated in direct interactions with R protein signaling complexes (RPS5/PBS1) and also in the modification of the key effector protein RIN4. Additional kinase activities are necessary for downstream signaling events. Proteomics not only monitors the steady state level of proteins but also co- and post-translational modifications of proteins. These include not only kinases and phosphatases but also their substrates (Xing et al. 2002).

Lecourieux-Ouaked et al. (2000), using 2-DE, tested the in vivo phosphorylation status of proteins after cryptogein, staurosporine (a kinase inhibitor), and calyculin A (a phosphatase inhibitor) treatments in tobacco cells. Out of about 100 phospholabelled polypeptides, 19 showed increased $^{32}P$ incorporation after cryptogein treatment and 12 of these depended upon calcium influx. Staurosporine inhibited the phosphorylation induced by cryptogein whereas calyculin A activated the phosphorylation of 18 polypeptides indicating that the phosphorylation of these proteins were activated by certain protein kinases and inhibited by certain phosphatases. These results demonstrate the power of phosphoproteomics to identify key proteins.

In suspension-cultured cells of *Arabidopsis*, Peck et al. (2000) used $^{32}P$ pulse-label method in conjunction with 2-DE and MS to identify proteins that are rapidly phosphorylated in response to bacterial and fungal elicitors. One of these proteins, AtPhos43 was found to be phosphorylated within minutes after treatment with flagellin. They also found that phosphorylation of AtPhos43 after flagellin treatment was dependent on FLS2, a receptor-like kinase involved in flagellin perception (Gomez-Gomez and Boller 2000). It has also been found that this protein was phosphorylated in response to both bacterial and fungal elicitors, and related proteins are phosphorylated in other monocot and dicot species (Peck 2003; Peck et al. (2000)).

Jones et al. (2006) describe the application of differential mass tags (iTRAQ) to provide relative quantification of phosphorylated peptides during the early stages in plant pathogen interaction the model plant *Arabidopsis thaliana* after challenging with three strains of *Pseudomonas syringae* pv. *tomato* DC3000 (DC3000). The HR induced by the *avrRpm1*/RPM1 interaction was compared with basal resistance through examination of responses to the *hrpA* mutant of DC3000. They identified five proteins which showed reproducible differences between a mock-inoculated control and different bacterial challenges 3 hours post inoculation (hpi), thus identifying proteins. Four of the five proteins, a dehydrin, a putative p23 co-chaperone, heat shock protein 81 and a plastidassociated protein (PAP)/fibrillin, are known to be phosphorylated or have potential phosphorylation sites. One another protein, the large subunit of Rubisco, showed a significant difference between tissue undergoing the hypersensitive response and a basal defence response. This novel study shows the application of iTRAQ to plant–pathogen interactions and the challenge of examining phosphoproteins from intact green leaf tissue, rather than the more commonly used cell culture system.

## Conclusion and Future Perspectives

In the post genomic era, proteomics has emerged as an indispensable tool for understanding signaling mechanisms of plant against pathogen, its potential impact in plant pathology, and the study of plant-pathogen interaction. Previously, a limited number of genes involved in infection process had been identified using conventional molecular genetics and biochemical methods. With the advent of

proteomics technology, a number of proteins involved in plant defenses have been identified. Phosphoproteomics will continue to play a major role in identifying post-translational modifications and, therefore, have an additional benefit of identifying signaling components that may not be revealed by transcriptome analysis alone. Currently, the major challenges for the plant phosphoproteomics are to identify the relevant phosphorylation sites from the vast majority of phosphopeptides. A high throughput technical advancement, therefore, will be an important development that will help to identify these relevant phosphorylation sites in proteins of interest. Another much needed improvement, highly desired in phosphoproteomics study, is the development of improved and novel enrichment strategies for phosphorylated peptides. In future, the integration of proteomics with genomics, transcriptomics, and metabolomics will play a major role in understanding the plant biology and will uncover many unexpected links within the signaling networks in plants. The continued proteomics advances in unrevealing the molecular mechanisms will lead to a better understanding of plant-pathogen interactions, which may ultimately contribute to the development of novel disease tolerant varieties of agriculturally and economically important crops.

## References

Adie B, Chico JM, Rubio-Somoza I, Solano R (2007) Modulation of plant defenses by ethylene. J Plant Growth Regul 26:160–177

Alvarez ME, Pennel RI, Meijer PJ, Ishikawa A, Dixon RA, Lamb C (1998) Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell 92:773–784

Ananieva KI, Ananiev ED (1999) Effect of methyl ester of jasmonic acid and benzylaminopurine on growth and protein profile of excised cotyledons of Cucurbita pepo (zucchini). Biol Plant 42:549–557

Andersson MX, Kourtchenko O, Dangl JL, Mackey D, Elerstrom M (2006) Phopspholipase--dependent signaling during the AvrRpm1 and AvrRpt2—Induced disease resistance response in Arabidopsis thaliana. Plant J 47:947–949

Asai S, Ohta K, Yoshioka H (2008) MAPK signaling regulates nitric oxide and NADPH oxidase-dependent oxidative bursts in Nicotiana benthamiana. Plant Cell 20:1390–1406

Atkinson MM, Midland SL, Sims JJ, Keen NT (1996) Syringolide 1 triggers Ca + influx, K+ efflux, and extracellular alkalization in soybean cells carrying the disease resistance gene Rpg4. Plant Physiol 112:297–302

Bach M, Schnitzler JP, Ulrich H (1993) Elicitor-induced changes in Ca+ influx, K+ efflux, and 4-hydroxybenzoic acid synthesis in protoplasts of Daucus carota. Plant Physiol 103:407–412

Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc Natl Acad Sci USA 95:8113–8118

Beffa R, Szell M, Meuwly P, Pay A, Vogeli-Lange R, Métraux JP, Neuhaus G, Meins F, Nagy J, Nagy F (1995) Cholera toxin elevates pathogen resistance and induces pathogenesis-related gene expression in tobacco. EMBO J 14:5753–5761

Bestwick CS, Brown IR, Bennett MHR, Mansfield JW (1997) Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to Pseudomonas syringae pv. phaseolicola. Plant Cell 9:209–221

Bolwell GP (1999) Role of active oxygen species and NO in plant defence. Curr Opin Plant Biol 2:287–294

Bolwell GP, Butt VS, Davies DR, Zimmerlin A (1995) The origin of the oxidative burst in plants. Free Rad Res 23:517–532

Bradley DJ, Kjellbom P, Lamb CJ (1992) Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. Cell 70:21–30

Brisson LF, Tenhaken R, Lamb C (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell 6:1703–1712

Campo S, Carrascal M, Coca M, Abian J, San SB (2004) The defence response of germinating maize embryos against fungal infection: a proteomics approach. Proteomics 4:383–396

Canonne J, Froidure-Nicolas S, Rivas S (2011) Phospholipases in action during plant defense signaling. Plant Signal Behav 6:13–18

Carpentier SC, Panis B, Vertommen A, Swennen R, Sergeant K, Renaut J, Laukens K, Witters E, Samyn B, Devreese B (2008) Proteome analysis of non model plants: a challenging but powerful approach. Mass Spectrom Rev 27:354–377

Chandra S, Low PS (1997) Measurement of Ca2+ fluxes during elicitation of the oxidative burst in aequorin-transformed tobacco cells. J Biol Chem 272:28274–28280

Chen CH (2008) Review of a current role of mass spectrometry for proteome research. Anal Chim Acta 624:16–36

Chen S, Harmon AC (2006) Advances in plant proteomics. Proteomics 6:5504–5516

Chen JG, Willard FS, Huang J, Liang J, Chasse SA, Jones AM, Siderovski DP (2003) A seven-transmembrane RGS protein that modulates plant cell proliferation. Science 301:1728–1731

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-Casado G, López-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R (2007) The JAZ family of repressors is the missing link in jasmonate signaling. Nature 448:666–671

Clarke A, Desikan R, Hurst RD, Hancock JT, Neill SJ (2000) NO way back: nitric oxide and programmed cell death in Arabidopsis thaliana suspension cultures. Plant J 24:667–677

Colcombet J, Hirt H (2008) Arabidopsis MAPKs: a complex signaling network involved in multiple biological processes. Biochem J 413:217–226

Colditz F, Nyamsuren O, Niehaus K, Eubel H, Braun HP, Krajinski F (2004) Proteomic approach: identification of Medicago truncatula proteins induced in roots after infection with the pathogenic oomycete Aphanomyces euteiches. Plant Mol Biol 55:109–120

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48:355–381

Datta K, Velazhahan R, Oliva N, Ona L, Mew T, Khush GS, Muthukrishnan S, Datta SK (1999) Over-expression of the cloned rice thaumatin-like protein (PR-5) gene in transgenic rice plants enhances environmental friendly resistance to Rhizoctonia solani causing sheath blight disease. Theor Appl Genet 98:1138–1145

de Torres Zabela M, Fernandez-Delmond I, Niittyla T, Sanchez P, Grant M (2002) Differential expression of genes encoding Arabidopsis phospholipases after challenge with virulent or avirulent Pseudomonas isolates. Mol Plant Microbe Interact 15:808–816

De Vos M, Van Zaanen W, Koornneef A, Korzelius JP, Dicke M, Van Loon LC, Pieterse CM (2006) Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiol 142:352–363

Delaney TP, Uknes S, Vernoij B, Friedrich L, Weyman K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J (1994) A central role of salicylic acid in plant disease resistance. Science 266:1247–1250

Desikan R, Hancock JT, Coffey MJ, Neill SJ (1996) Generation of active oxygen in elicited cells of Arabidopsis thaliana is mediated by a NADPH oxidase-like enzyme. FEBS Lett 382:213–217

Desikan R, Neill SJ, Hancock JT (1997) Generation of active oxygen in Arabidopsis thaliana. Phyton 37:65–70

Desikan R, Burnett EC, Hancock JT, Neill SJ (1998) Harpin and hydrogen peroxide induce the expression of a homologue of gp91-phox in Arabidopsis thaliana suspension cultures. J Exp Bot 49:1767–1771

Desikan R, Hancock JT, Ichimura K, Shinozaki K, Neill SJ (2001) Harpin induces activation of the Arabidopsis MAP kinasesATMPK4 and ATMPK6. Plant Physiol 126:1579–1587

Dhondt S, Gouzerh G, Müller A, Legrand M, Heitz T (2002) Spatio-temporal expression of patatin-like lipid acyl hydrolases and accumulation of jasmonates in elicitor-treated tobacco leaves are not affected by endogenous levels of salicylic acid. Plant J 32:749–762

Doke N (1983a) Generation of superoxide anion by potato tuber protoplasts during the hypersensitive response to hyphal wall components of Phytophthora infestans and specific inhibition of the reaction by suppressors of hypersensitivity. Physiol Plant Pathol 23:359–367

Doke N (1983b) Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of Phytophthora infestan and to the hyphal wall components. Physiol Plant Pathol 23:345–357

Doke N, Ohashi Y (1988) Involvement of an O2- generating system in the induction of necrotic lesions on tobacco leaves infected with tobacco mosaic virus. Physiol Mol Plant Pathol 32:163–175

Domon B, Aebersold R (2006) Mass spectrometry and protein analysis. Science 312:212–217

Droillard MJ, Boudsocq M, Barbier-Brygoo H, Lauriere C (2004) Involvement of MPK4 in osmotic stress response pathways in cell suspensions and plantlets of Arabidopsis thaliana: activation by hypoosmolarity and negative role in hyperosmolarity tolerance. FEBS Lett 574:42–48

Durner D, Klessig DF (2000) Differential induction of tobacco MAP kinases by the defense signals nitric oxide, salicylic acid, ethylene, and jasmonic acid. Mol Plant Microbe Interact 13:347–351

Dwyer SC, Legendre L, Low PS, Leto TL (1996) Plant and human neutrophil oxidative burst complexes contain immunologically related proteins. Biochem Biophys Acta 1289:231–237

Ebel J, Scheel D (1997) Signals in host–parasite interactions. In: Carroll GC, Tudzynski P (eds) The mycota, vol V, Plant relationships, Part A. Springer, Berlin, pp 85–105

Everberg H, Gustavasson N, Tjerned F (2008) Enrichment of membrane proteins by partitioning in detergent/polymer aqueous two-phase systems. Met Mol Biol 424:403–412

Feys BJ, Moisan LJ, Newman MA, Parker JE (2001) Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4. EMBO J 20:5400–5411

Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. Science 26:754–756

Geddes J, Eudes F, Laroche A, Selinger B (2008) Differential expression of proteins in response to the interaction between the pathogen Fusarium graminearum and its host, Hordeum vulgare. Proteomics 8:545–554

Gelli A, Higgins VJ, Blumwald E (1997) Activation of plant plasma membrane Ca2+−permeable channels by race-specific fungal elicitors. Plant Physiol 113:269–279

Gomez-Gomez L, Boller T (2000) FLS2: a LRR receptor-like kinase involved in recognition of the flagellin elicitor in Arabidopsis. Mol Cell 5:1003–1011

Green R, Fluhr R (1995) UV-B-induced PR-1 accumulation is mediated by active oxygen species. Plant Cell 7:203–212

Gundlach H, Muller MJ, Kutchan TM, Zenk MH (1992) Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc Natl Acad Sci USA 89:2389–2393

Guo FQ, Okamoto M, Crawford NM (2003) Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science 302:100–103

Hahlbrock K, Scheel D, Logemenn E, Nrnberger T, Papniske M, Reinold S, Sacks WR, Schmelzer E (1995) Oligopeptide elicited defense gene activation in cultured parsley cells. Proc Natl Acad Sci USA 92:4150–4157

Hammond-Kosack KE, Jones JDG (1996) Resistance gene dependent plant defense responses. Plant Cell 8:1773–1791

Hammond-Kosack KE, Jones JDG (2000) Responses to plant pathogens. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiology, Maryland, pp 1102–1156

Hancock JT, Desikan R, Clarke A, Hurst RD, Neill SJ (2002) Cell signaling following plant/pathogen interactions involves the generation of reactive oxygen and reactive nitrogen species. Plant Physiol Biochem 40:611–617

Hirt H (1997) Multiple roles of MAP kinases in plant signal transduction. Trends Plant Sci 2:11–15

Jabs T, Tschope M, Colling C, Hahlbrock K, Scheel D (1997) Elicitor-stimulated ion fluxes and $O^{2-}$ from the oxidative burst are essential components in triggering defense gene. Proc Natl Acad Sci USA 94(9):4800–4805

Jones AM, Assmann SM (2004) Plants: the latest model system for G-protein research. EMBO Reports 5:572–578

Jones AME, Bennett MH, Mansfield JW, Grant M (2006) Analysis of the defence phosphoproteome of Arabidopsis thaliana using differential mass tagging. Proteomics 6:4155–4165

Jorrin-Novo JV, Maldonado AM, Echevarria-Zomeno S, Valledor L, Castillejo MA, Curto M, Valero J, Sghaier B, Donoso G, Redondo I (2009) Plant proteomics update (2007–2008): second-generation proteomic techniques, an appropriate experimental design, and data analysis to fulfil MIAPE standards, increase plant proteome coverage and expand biological knowledge. J Proteomics 72:285–314

Khush GS (2001) Challenges for meeting the global food and nutrient needs in the new millennium. Proc Nutr Soc 60:15–26

Kim ST, Cho KS, Yu S, Kim SG, Hong JC, Han CD, Bae DW, Nam MH, Kang KY (2003) Proteomic analysis of differentially expressed proteins induced by rice blast fungus and elicitor in suspension-cultured rice cells. Proteomics 3:2368–2378

Kim ST, Kim SG, Hwang DH, Kang SY, Kim HJ, Lee BH, Lee JJ, Kang KY (2004) Proteomic analysis of pathogen-responsive proteins from rice leaves induced by rice blast fungus, Magnaporthe grisea. Proteomics 4:3569–3578

Koeduka T, Matsui K, Hasegawa M, Akakabe Y, Kajiwara T (2005) Rice fatty acid alpha-dioxygenase is induced by pathogen attack and heavy metal stress: activation through jasmonate signaling. J Plant Physiol 162:912–920

Konishi H, Ishiguro K, Komatsu S (2001) A proteomics approach towards understanding blast fungus infection of rice grown under different levels of nitrogen fertilization. Proteomics 1:1162–1171

Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci 97:2940–2945

Kuchitsu K, Kikuyama M, Shibuya N (1993) N-acetylchitooligosaccharides, biotic elicitors for phytoalexin production, induce transient membrane depolarization in suspension-cultured rice cells. Protoplasma 174:79–81

Kunkel BN, Brooks DM (2002) Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 5:325–331

Larson RL, Wintermantel WM, Hill A, Fortis L, Nunez A (2008) Proteome changes in sugar beet in response to Beet necrotic yellow vein virus. Physiol Mol Plant Pathol 72:62–72

Lawton KA, Potter SL, Uknes S, Ryals J (1994) Acquired resistance signal transduction in Arabidopsis is ethylene independent. Plant Cell 6:581–588

Laxalt AM, Munnik T (2002) Phospholipid signaling in plant defence. Curr Opin Plant Biol 5:332–338

Lecourieux-Ouaked F, Pugin A, Lebrun-Garcia A (2000) Phosphoproteins involved in the signal transduction of cryptogein, an elicitor of defense reactions in tobacco. Mol Plant Microbe Interact 13:821–829

Lee J, Bricker TM, Lefevre M, Pinson SRM, Oard JH (2006) Proteomic and genetic approaches to identify defence-related proteins in rice challenged with the fungal pathogen Rhizoctonia solani. Mol Plant Pathol 7:405–416

Leitner M, Vandelle E, Gaupels F, Bellin D, Delledonne M (2009) NO signals in the haze: Nitric oxide signaling in plant defence. Curr Opin Plant Biol 12:451–458

Leon J, Rojo E, Titarenko E, Sanchez-Serrano JJ (1998) Jasmonic acid-dependent and independent wound signal transducyion pathways are differentially regulated by Ca2+/calmodulin in Arabidopsis thaliana. Mol Gen Genet 258:412–419

Levine A, Tenhaken R, Dixon R, Lamb C (1994) H2O2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583–595

Liang Y, Srivastava S, Rahman MH, Strelkov SE, Kav NNV (2008) Proteome changes in leaves of Brassica napus L. as a result of Sclerotinia sclerotiorum challenge. J Agri Food Chem 56:1963–1976

Lindermayr C, Saalbach G, Bahnweg G, Durner J (2006) Differential inhibition of Arabidopsis methionine adenosyltransferases by protein S-nitrosylation. J Biol Chem 281:4285–4291

Llorente F, Alonso-Blanco C, Sanchez-Rodriguez C, Jorda L, Molina A (2005) ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus Plectosphaerella cucumerina. Plant J 43:165–180

Long DH, Lee FN, TeBeest DO (2000) Effect of nitrogen fertilization on disease progress of rice blast on susceptible and resistant cultivars. Plant Dis 84:403–409

Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15:165–178

Mahmood T, Jan A, Kakishima M, Komatsu S (2006) Proteomic analysis of bacterial-blight defense responsive proteins in rice leaf blades. Proteomics 6:6053–6065

Maor R, Shirasu K (2005) The arms race continues: battle strategies between plants and fungal pathogens. Curr Opin Microbiol 8:399–404

MAPK Group (2002) Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trends Plant Sci 7:301–308

Marsh JF, Kaufman LS (1999) Cloning and characterization of PGA1 and PGA2: Two G protein α-subunits from pea that promote growth in the yeast Saccharomyces cerevisiae. Plant J 19:237–247

Mathieu Y, Kurkjian A, Xia H, Guern J, Spiro M, O'Neill M, Albersheim P, Darvill A (1991) Membrane responses induced by oligogalacturonides in suspension-cultured tobacco cells. Plant J 1:333–343

McDowell JM, Dangl JL (2000) Signal transduction in the plant immune response. Trends Biochem Sci 25:79–82

Mithoe SC, Menke FLH (2011) Phosphoproteomics perspective on plant signal transduction and tyrosine phosphorylation. Phytochem 72:997–1006

Moreau M, Lee GI, Wang Y, Crane BR, Klessig DF (2008) AtNOS/AtNOA1 is a functional Arabidopsis thaliana cGTPase and not a nitric-oxide synthase. J Biol Chem 283:32957–32967

Mueller-Uri F, Parthier B, Nover L (1988) Jasmonate-induced alteration of gene expression in barley leaf segments analyzed by in-vivo and in-vitro protein synthesis. Planta 176:241–247

Nojiri H, Sugimori M, Yamane H, Nishimura Y, Yamada A, Shibuya N, Kodama O, Murofushi N, Omori T (1996) Involvement of jasmonic acid in elicitor-induced phytoalexin production in suspension-cultured rice cells. Plant Physiol 110:387–392

Norman-Setterblad C, Vidal S, Palva ET (2000) Interacting signal pathways control defense gene expression in Arabidopsis in response to cell wall-degrading enzymes from Erwinia carotovora. Mol Plant Microbe Interact 13:430–438

Nuhse TS, Peck SC, Hirt H, Boller T (2000) Microbial elicitors induce activation and dual phosphorylation of the Arabidopsis thaliana MAPK 6. J Biol Chem 275:7521–7526

Oh IS, Park AR, Bae MS, Kwon SJ, Kim YS, Lee JE, Kang NY, Lee S, Cheong H, Park OK (2005) Secretome analysis reveals an Arabidopsis lipase involved in defense against Alternaria brassicicola. Plant Cell 17:2832–2847

Ong S, Foster LJ, Mann M (2003) Mass spectrometric-based approaches in quantitative proteomics. Methods 29:124–130

Pawson T, Scott JD (2005) Protein phosphorylation in signaling–50 years and counting. Trends Biochem Sci 30:286–290

Peck SC (2003) Early phosphorylation events in biotic stress. Curr Opin Plant Biol 6:334–338

Peck SC, Nuhse TS, Hess D, Iglesias A, Meins F, Boller T (2000) Directed proteomics identifies a plant specific protein rapidly phosphorylated in response to bacterial and fungal elicitors. Plant Cell 13:1467–1475

Peng M, Kuc J (1992) Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf discs. Phytopathol 82:696–699

Perchepied L, Balague C, Rioum C, Claudel-Renard C, Riviere N, Grezes-Besset B, Roby D (2010) Nitric oxide participates in the complex interplay of defense-related signaling pathways controlling disease resistance to Sclerotinia sclerotiorum in Arabidopsis thaliana. Mol Plant Microbe Interact 23:846–860

Pitzschke A, Schikora A, Hirt H (2009) MAPK cascade signaling networks in plant defence. Curr Opin Plant Biol 12:1–6

Pohnert G (2002) Phospholipase A2 activity triggers the woundactivated chemical defense in the diatom Thalassiosira rotula. Plant Physiol 129:103–111

Popham P, Pike S, Novacky A (1995) The effect of harpin from Erwinia amylovora on the plasmalemma of suspesioncultured tobacco cells. Physiol Mol Plant Pathol 47:39–50

Qutob D, Kemmerling B, Brunner F, Kufner I, Engelhardt S, Gust AA, Luberacki B, Seitz HU, Stahl D, Rauhut T, Glawischnig E, Schweene G, Lacombe B, Watanabe N, Lam E, Schlichting R, Scheel D, Nau K, Dodt G, Hubert G, Gijzen M, Nürnberger T (2006) Phytotoxicity and innate immune responses induced by Nep1-like proteins. Plant Cell 18:3721–3744

Rakwal R, Agrawal GK (2003) Rice proteomics: current status and future perspectives. Electrophoresis 24:3378–3389

Reymond P, Farmer EE (1998) Jasmonate and salicylate as global signals for defense gene expression. Curr Opin Plant Biol 1:404–441

Ristaino JB (2002) Tracking historic migrations of the Irish potato famine pathogen, Phytophthora infestan. Microbes Infect 4:1369–1377

Rossignol M, Peltier JB, Mock HP, Matros A, Maldonado AM, Jorrin JV (2006) Plant proteome analysis: a 2004–2006 update. Proteomics 6:5529–5548

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H, Hunt MD (1996) Systemic acquired resistance. Plant Cell 8:1809–1819

Ryu SB (2004) Phospholipid-derived signaling mediated by phospholipase A in plants. Trends Plant Sci 9:229–235

Samuel MA, Miles GP, Ellis BE (2000) Ozone treatment rapidly activates MAP kinase signaling in plants. Plant J 22:367–376

Shani BB, Suleiman ML, Mohammed US (2006) Performance evaluation of an improved animal drawn ground Metered shrouded disc (gmsd) sprayer. J Agri Engg Tech 14:4–11

Shirano Y, Kachroo P, Shah J, Klessig DF (2002) A gain-of-function mutation in an Arabidopsis toll interleukin 1 receptor-nucleotide binding site-leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. Plant Cell 14:3149–3162

Somssich I, Hahlbrock K (1998) Pathogen defense in plants - a paradigm of biological complexity. Trends Plant Sci 3:86–90

Staswick PE, Yuen GY, Lehman CC (1998) Jasmonate signaling mutants of Arabidopsis are susceptible to the soil fungus Pythium irregulare. Plant J 15:747–754

Stenzel I, Hause B, Miersch O, Kurz T, Maucher H, Weichert H, Ziegler J, Feussner I, Wasternack C (2003) Jasmonate biosynthesis and the allene oxide cyclase family of Arabidopsis thaliana. Plant Mol Biol 51:895–911

Sticher L, Mauch-Mani B, Metraux JP (1997) Systemic acquired resistance. Ann Rev Phytopathol 35:235–270

Stintzi A, Weber H, Reymond P, Browse J, Farmer EE (2001) Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci USA 98:12837–12842

Takemoto D, Furuse K, Doke N, Kawakita K (1997) Identification of chitinase and osmotin-like protein as actin-binding proteins in suspension-cultured potato cells. Plant Cell Physiol 38:441–448

Tenhaken R, Levine A, Brisson LF, Dixon RA, Lamb C (1995) Function of the oxidative burst in hypersensitive disease resistance. Proc Natl Acad Sci USA 92:4158–4163

The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408:796–815

Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF (1998) Separate jasmonate dependent and salicylate-dependent defense pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc Natl Acad Sci USA 95:15107–15111

Thorpe MR, Ferrieri AP, Herth MM, Ferrieri RA (2007) 11Cimaging: methyl jasmonate moves in both phloem and xylem, promotes transport of jasmonate, and of photoassimilate even after proton transport is decoupled. Planta 226:541–551

Torres MA, Onuchi H, Hamada S, Machida C, Hammond-Kosack KE, Jones JDG (1998) Six Arabidopsis thaliana homologues of the human respiratory burst oxidase (gp91phox). Plant J 14:365–370

Trusov Y, Rookes JE, Chakravorty D, Armour D, Schenk PM, Botella JR (2006) Heterotrimeric G proteins facilitate Arabidopsis resistance to necrotrophic pathogens and are involved in jasmonate signaling. Plant Physiol 140:210–220

Tuteja N, Sopory SK (2008) Plant signaling in stress. Plant Signal Behav 3:79–86

Van der Luit AH, Piatti T, Van Doorn A, Musgrave A, Felix G, Boller T, Munnik T (2000) Elicitation of suspension-cultured tomato cells triggers the formation of phosphatidic acid and diacylglycerol pyrophosphate. Plant Physiol 123:1507–1515

Venugopal SC, Jeong RD, Mandal MK, Zhu S, Chandra-Shekara AC, Xia Y, Hersh M, Stromberg AJ, Navarre D, Kachroo A, Kachroo P (2009) Enhanced disease susceptibility 1 and salicylic acid act redundantly to regulate resistance gene-mediated signaling. PLoS Genet 5. doi:10.1371/journal.pgen.1000545

Verberne MC, Hoekstra J, Bol JF, Linthorst HJM (2003) Signaling of systemic acquired resistance in tobacco depends on ethylene perception. Plant J 35:27–32

Vijayan P, Shockey J, Levesque CA, Cook RJ, Browse J (1998) A role for jasmonate in pathogen defense of Arabidopsis. Proc Natl Acad Sci USA 95:7209–7214

Wei Z, Hu W, Lin Q, Cheng X, Tong M, Zhu L, Chen R, He G (2009) Understanding rice plant resistance to the Brown Planthopper (Nilaparvata lugens): A proteomic approach. Proteomics 9:2798–2808

Weidhase RA, Kramell HM, Lehmann J, Liebisch HW, Lerbs W, Parthier B (1987) Methyl jasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. Plant Sci 51:177–186

Wu G, Shortt BJ, Lawrence EB, Levine EB, Fitzsimmons KC, Shah DM (1995) Disease resistance conferred by expression of a gene encoding H2O2-generating glucose oxidase in transgenic potato plants. Plant Cell 7:1357–1368

Xiao S, Brown S, Patrick E, Brearley C, Turner JG (2003) Enhanced transcription of the Arabidopsis disease resistance genes RPW8.1 and RPW8.2 via a salicylic acid-dependent amplification circuit is required for hypersensitive cell death. Plant Cell 15:33–45

Xing T, Higgins VJ, Blumwald E (1997) Race-specific elicitors of Cladosporium fulvum promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells. Plant Cell 9:249–259

Xing T, Ouellet T, Miki BL (2002) Towards genomic and proteomic studies of protein phosphorylation in plant-pathogen interactions. Trends Plant Sci 7:224–230

Yang Y, Shah J, Klessig DF (1997) Signal perception and transduction in plant defense responses. Genes Dev 11:1621–1639

Yoshida Y, Sano R, Wada T, Takabayashi J, Okada K (2009) Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in Arabidopsis. Development 136:1039–1048

Young SA, Wang X, Leach JE (1996) Changes in the plasma membrane distribution of rice phospholipase D during resistant interactions with Xanthomonas oryzae pv.oryzae. Plant Cell 8:1079–1090

Zhou W, Eudes F, Laroche A (2006) Identification of differentially regulated proteins in response to a compatible interaction between the pathogen Fusarium graminearum and its host, Triticum aestivum. Proteomics 6:4599–4609