Indian Phytopath. 69 (4s) : 1-9 (2016)

REVIEW ARTICLE

The Post Genomic Era: Novel approaches for studying plant diseases and their management

M. ANANDARAJ[1]* AND P. UMADEVI[2]
ICAR-Indian Institute of Spices Research, Kozhikode 673 012, Kerala, India

ABSTRACT: Plant pathologists the world over are racing with plant pathogens to deploy resistance genes but pathogens continue to cause about 15% crops loss. Hence protecting crops from devastating pathogens is a continuous challenge to plant pathologists. Understanding host pathogen interactions and the molecular arms race between pathogens and their host plant has been the task of plant pathologists. The advancements in sequencing techniques, computational biology have helped to a greater understanding of the molecular events during pathogenesis. The science of omics - transcriptomics, proteomics, ionomics and metabolomics in the post genomic era have provided opportunities to understand the interaction well. In this review transcriptomics and proteomics in understanding the mechanisms of resistance in *Piper colubrinum* and *P. nigrum* to *P. capsici* and *Curcuma amada* and *Zingiber officinale* to *Ralstonia soanacearum* are discussed. Proteomic of temperature stress on expression of Piper yellow mottle virus infection and a management strategy in the field are discussed.

Key words: Comparative genomics, Proteomics, *Phytophthora capsici*, Piper yellow mottle virus, Transcriptomics

Plant diseases and their management need complete knowledge on the interaction between the host and pathogen. The sequential steps on immunity, resistance by the plant and effectors and other molecules from the pathogen to overcome the resistance exerted by the plants upon infection needs to be brought out to pinpoint the time and method for management of the pathogen in natural condition. Towards achieving this, scientific community has developed many tools since early 1900s.The tools of each era also affect the rigor of the validation tests and the knowledge on the subject. The first was the era to deal with disease physiology (early 1900s to mid 1980), the second era was of molecular genetics focused on one or a few genes (1980 to 2000) mainly on bacterial pathogens, with meager work for pathogenic fungi, oomycetes and nematodes. The third is the genomics era began in 2000 with the complete genome of the bacterial pathogen *Xylella fastidiosa* (Simpson *et al.,* 2000). The post genomic era is an important era running with the translational research on the knowledge obtained from the genomic era. The newer tools *viz.,* next generation sequencing platforms, transcriptomics, proteomics, ionomics and metabolomics makes this new era as “omics” era. This review brings out the importance of these tools towards elucidation of host pathogen interaction with some of the real time case studies attempted in black pepper - *Phytophthora* and ginger- *Ralstonia* pathosystems using the omics (post genomic era tools) platform under the National outreach Project on *Phytophthora, Furarium* and *Ralstonia* (www.phytofura.net.in).

Post genomic era- The step to translational research

The post genomic era has next generation sequencing platforms as the core technological support. “Generation” refers to the chemistry and technology used by the sequencing process, the term next-generation denotes any of the high-throughput methods which were developed after Sanger sequencing method of sequencing. With these advancement of technologies and less cost, resequencing of any organism became easy. As reference genome sequences become available cataloguing sequence variations and understanding their biological consequences have become major research goals, for this re sequencing became a major technique as only a small subset of the genome is sequenced, such as the exome, a set of genes or a region of interest with precise information and implications. Towards focused genomics, the comparative genomics, turned to be major option for the researchers as it provides windows for finding out how pathogens have evolved both common and divergent virulence strategies to infect related plant species. In general, the next generation sequencing helps in understanding the molecular mechanisms underpinning pathogenesis and resistance, in developing novel markers for rapid pathogen detection and diagnosis, in getting further insights into pathogen populations, and in developing translational research strategies to improve disease resistance in crops.

Comparative genomics

Comparative genomics relies on the *de novo* detection of shared virulence strategies of many isolates and many different organisms in one analysis which provides an exciting possibility of uncovering new insights into the pathogenesis-related processes. Unbiased comparative genomic analysis also provides insights into the co-evolution of virulence, niche specialization functions and the mechanisms of plant defense. There are many striking examples on the implication of comparative genomics in the science of pathology. A locus-specific horizontal gene transfer event emerged from the sequencing of the wheat pathogen

*Corresponding author: arajiisr@gmail.com

Phaeosphaeria nodorum genome, in which a gene encoding a host specific protein toxin (ToxA) was identified by homology to a known toxin from another wheat pathogen *Pyrenophora tritici repentis.* Functional validation revealed that ToxA was necessary for virulence in both pathogens (Friesen *et al.,* 2006). It was proposed that transfer of ToxA from *P. nodorum* to *P. tritici-repentis* resulted in the emergence of the tan spot disease of wheat caused by *P. tritici-repentis* in the 1930s. Genome analysis of the tomato vascular wilt pathogen *F. oxysporum* f. sp. *lycopersici* revealed the presence of several supernumerary chromosomes. Similarly, it was recently suggested that the ability to synthesize auxin in two genera of fungi namely, *Fusarium* and *Colletotrichum* has probably resulted from horizontal transfer of auxin biosynthetic genes from bacteria (Tsavkelova *et al.,* 2012). The *de novo* detection of shared virulence strategies without a prior information on the roles of shared genes in pathogen virulence offers an exciting possibility of uncovering new insights into the pathogenesis-related processes.

Fusarium crown and root rots (*Fusarium pseudograminearum*) are important diseases of wheat and barley world-wide. Comparative genomic analyses showed that the *F. pseudograminearum* genome encodes proteins that are present in fungal pathogens of cereals but absent in non-cereal pathogens. These cereal pathogen specific genes were also found in bacteria associated with plants. Two horizontally acquired genes with no previously known role in fungal pathogenesis were studied using gene knockout methods and found to affect virulence of *F. pseudograminearum* on the cereal hosts which revealed the role of genes specific to pathogens of related hosts and the importance of horizontal gene transfer in the evolution of plant infecting fungi (Gardiner *et al.,* 2012). Comparative genomics /association genomics has provided enormous information on effectors. Using this technique it was found that Ave1 (Fungal effector) had very high homologies to plant proteins, suggesting that a cross-kingdom gene transfer event from plant to fungi.

There are many examples on the identification of effectors based on their similarity to known proteins or the presence of known domains or motifs. This includes similarities to effectors (usually Avr proteins) from other pathogens, to enzymes or to the presence of eukaryote-like domains and motifs. The evolution of effectors is a trade-off between escaping detection by the plants and optimizing the virulence function for the successful establishment of disease in the plants by the pathogen. Hence, the long-term fitness of a pathogen must rely mainly on the continuous emergence of novel effectors to capture new host targets which makes the effector repertoire for the particular organism. In light of this view, genome-wide analyses of plant pathogenic fungi had shown a high degree of positive selection in genes encoding secreted proteins compared with genes encoding non'-secreted proteins (Hacquard, 2013). The next-generation sequencing /targeted sequencing technologies will provide the genome sequences of multiple strains of many plant pathogen species, towards accelerating effector discovery.

By sensing the importance of comparative genomics attempts made, one on comparative genomics of two *Phytophthora* isolates infecting black pepper and ten *Ralstonia* isolates from different host under different geographical regions of India under the project PhytoFuRa (www.phytofura.net.in).

Comparative genomics on *Phytophthora capsici* infecting black pepper

The comparative analysis of two virulent isolates (05-06) and (98-93) infecting black pepper with the reference genome from Joint genome initiative showed the difference in their RXLR and CRN effector gene composition which attributed to the virulence of the isolates. (Table1). Other effectors present in secretory proteins were also showed the significance of the presence of effectors in the virulence factor of the isolates. Though both are highly virulent, on a susceptible host strain 98-93 is more aggressive and produce larger lesions with water soaked margins. Compared to reference isolate both possess some unique genes (Fig. 1).

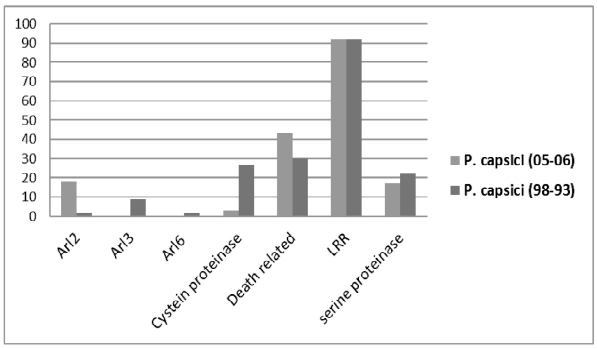

Fig. 1. Effectors in two highly virulent strains of *P. capsici*

Table 1. Comparative genomic analysis of two *Phytophthora* strains infecting black pepper

Organism	Genome Size (Mb)	No. of genes	No. of SNPs	No. of secretory proteins	Total secretome	RXLR effectors	CRN effectors
P. capsici (98-93)	46.1	13,068	37839	2966	2966	26	28
P. capsici (05-06)	63.8	19,356	6316	2864	2864	164	196
P. capsici (JGI)	64	19,805	Ref. genome	1402	1402	357	99

Table 2. Source of *Ralstonia* strains and the host plants

Strain & Platform used	Host	Phylotype (Geographic region)	Biovar	Institute
RS-SIK,RS-MEP (Illumina)	Ginger	I (Asia)	3	ICAR-IISR, Kozhikode
UTT-25 (Illumina)	Tomato	I (Asia)	3	ICAR-IARI, New Delhi
RS48 (Roche)	Potato	I (Asia)	3	ICAR-CPRI, Shimla
RS25 (Roche)	Potato	I (Asia)	4	
RS2 (Roche)	Potato	IIB (America)	2	
RS75 (Roche)	Potato	IV (Indonesia)	2T	
RS9, RS10 (Illumina)	Brinjal Chilli	I (Asia) I (Asia)	3 3	ICAR-CCARI, Goa
RS-IIHR (Ion Torrent)	Brinjal	I (Asia)	3	IIHR, Bengaluru

Comparative genomics of Ralstonia solanacearum in Indian subcontinent

To gain insights on the mechanism that confers pathogenicity for the particular *Ralstonia* isolates an attempt was made on the comparative genomics of ten strains which differs in their host specificity (Anandaraj *et al.,* 2015) (Table 2). The analysis identified 1463 gene families as conserved in all ten isolates and set of unique genes present in each strain showing that the strains are much diverse among themselves (Table 3, Fig. 2).

Transcriptomics

The transcriptomics platform provides the entire information on genes that are expressed from particular tissue at given point of time. This technology is attractive technology as the data provides insights into the orchestration of global changes in plant host physiology, metabolism and the activation of different components of the defense pathways. Expression of genes during both pathogen triggered immunity (PTI) and effector triggered (ETI) stages of host response and on the particular stage of infection and interaction of pathogen with the host are obtained along with the expressed effectors from the pathogen side. Cooke *et al.* (2012) demonstrated differences in the biotrophic phase that correlated with pathogen aggressiveness among *P. infestans* genotypes against potato. Studies also indicated that the mechanisms by which potato and tomato respond to *P. infestans* and the factors that are associated with the various stages of its infection (Rietman *et al.,* 2012). Understanding transcriptional changes in both the host and the pathogen is invaluable as it would pinpoint the time of management of the pathogen in the field condition and also to develop effective crop protection strategies. Foot-rot caused by the soil-borne oomycetous pathogen, *Phytophtora capsici*, is a major constraint for the black pepper production in India (Anandaraj *et al.,* 1989; Anandaraj, 2000). In light of this Transcriptomics of *Piper nigrum* and its wild relative *P. colubrinum* response to *P. capsici* to understand the mechanism of interaction (Johnson *et al.,* 2012) was attempted.

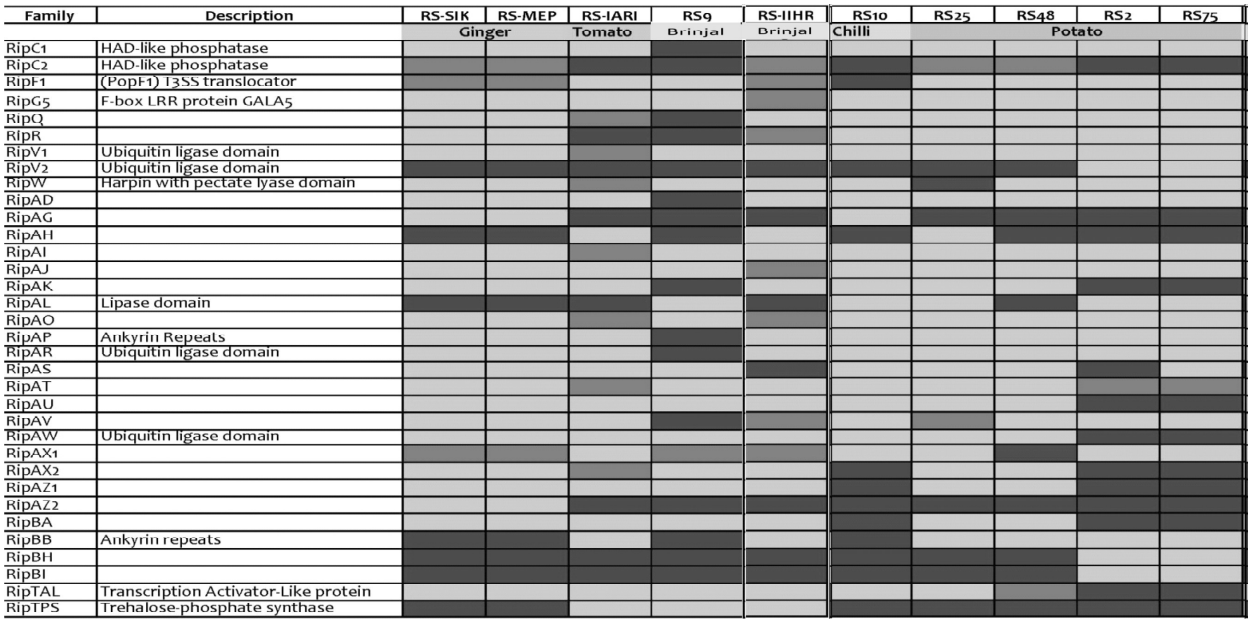

Fig. 2. Identified Effectors on host specificity

Table 3. T3 effectors in the *Ralstonia* isolates from varying host range and from different regions of India

Common in all	AWR3, RipB, RipD, RipG6, RipG7, RipI, RipAA, RipAB, RipAC, RipAE, RipAJ, RipAM, RipAN, RipAY (14/99 effectors
Present in selected Isolates	Rs9 – RipP2 Rs10 – RipP1, RipP2, RipP3, RipAG Rs2 – RipF2, RipS7, RipV2, RipBH, RipBI Rs25 – RipP1, RipAH Rs75 – RipF2, RipS7, RipV2, RipBH, RipBI Rs-MEP – RipAG, RipAZ2 Rs-SIK – RipAG, RipAZ2 Rs-IARI - RipAH Rs-IIHR – RipP3, RipAH
Missing in all	RipG8, RipK, RipO2, RipAF2, RipBE, RipBG (6/99 effectors)
Missing in selected	Rs9 - RipC1, RipE1, RipE2, RipQ, RipR, RipS2, RipU, RipAD, RipAP, RipAQ, RipAR, RipAV, Rs10 - RipA2, RipF1, RipH3, RipAZ1 Rs2 – RipAS, RipH3, RipL, RipAF1, RipAU, RipAW, RipAZ1, RipTAL Rs48 – RipE2 Rs75 – RipH3, RipL, RipAF1, RipAU, RipAW, RipAZ1, RipTAL Rs-MEP – RipN Rs-SIK – RipN Rs-IARI - RipR

Source : Phyto Fu Ra 2016.

Piper colubrinum-P. capsici interactive dual transcriptome

It was found that many genes were up regulated during pathogen infection *viz.,* Osmotin, beta1,3-glucanase, Thaumatin like protein, Defensin, Peroxidase, PAL, Lipoxygenase, Catechol oxidase, Cinnamoyl CoA reductase, Cinnamoyl CoA isomerase, Polyphenol oxidase, EDS1, Allene oxide synthase etc. A number of transcription factors (MYB, MYC and WRKY) also showed up regulation during the host pathogen interaction. The pathogen specific genes which were upregulated were glycoside hydrolase, pectate lyase, NPP1 and RXLR. The expression of some important genes and transcription factors were validated with realtime PCR studies. The upregulation of these genes at early stages with down regulation in later stages of infection provides the information that these genes are important for the early colonization, interaction in the plants (Vijesh kumar *et al.*, 2015).

Expression analysis of defense-related proteins in *P. colubrinum*

Real time PCR analysis was done for defense-related genes viz., peroxidase, lipoxygenase, superoxide dismutase, chalcone synthase, chalcone isomerase, catechol oxidase, phenyalanine ammonia lyase (PAL), catalase, cinnamoyl coA reductase, polyphenol oxidase, EDS1, serine threonine kinase, chitinase II, senescence associated protein, allene oxide synthase and PR proteins like PR-1 and PR-14 in *Piper colubrinum* challenge inoculated with *Phytophthora capsici* (Fig. 3).

The PR proteins (osmotin, b- 1,3 glucanase, defensin & thaumatin like proteins) expression dynamics in *P. colubrinum* on the infection of highly virulent strain 05-06 and less virulent strain (98-93) was observed as upregulation in the plants inoculated with virulent isolate while subdued expression in plants infected with less virulent isolate (Vijesh kumar *et al.,* 2016)

In planta expression and docking studies of a glucanase inhibitor gene from *Phytophthora capsici* and beta-1-3-glucanse gene from *Piper colubrinum* were done. Molecular modelling and docking studies were done to find domains and key residues of the glucanase inhibitor protein under interaction with beta glucanase gene.

In silico mining of micro RNA and RGAs from black pepper transcriptome

Micro RNAs are known to regulate the plant innate immune receptors. The NBS-LRR gene receptors are the major targets of micro RNA in plant defense (Li *et al.,* 2011). *P. colubrinum* miRNA has been selected to search corresponding targets in *P. capsici* (genome & de-novo trinity transcripts) to identify miRNA interaction between *P. colubrinum* and *P. capsici*. Number of micro RNAs expressed during pathogen interaction were identified (Unpublished data).

The degenerate primer based gene identification results in identification of pseudogenes in many cases. To avoid this transcriptome data based gene mining is of valuable alternative as it is from the same genotype. In light of this, attempts were made to find R gene analogs from *P. nigrum* and *P. colubrinum* transcriptomes. *In-silico* mining

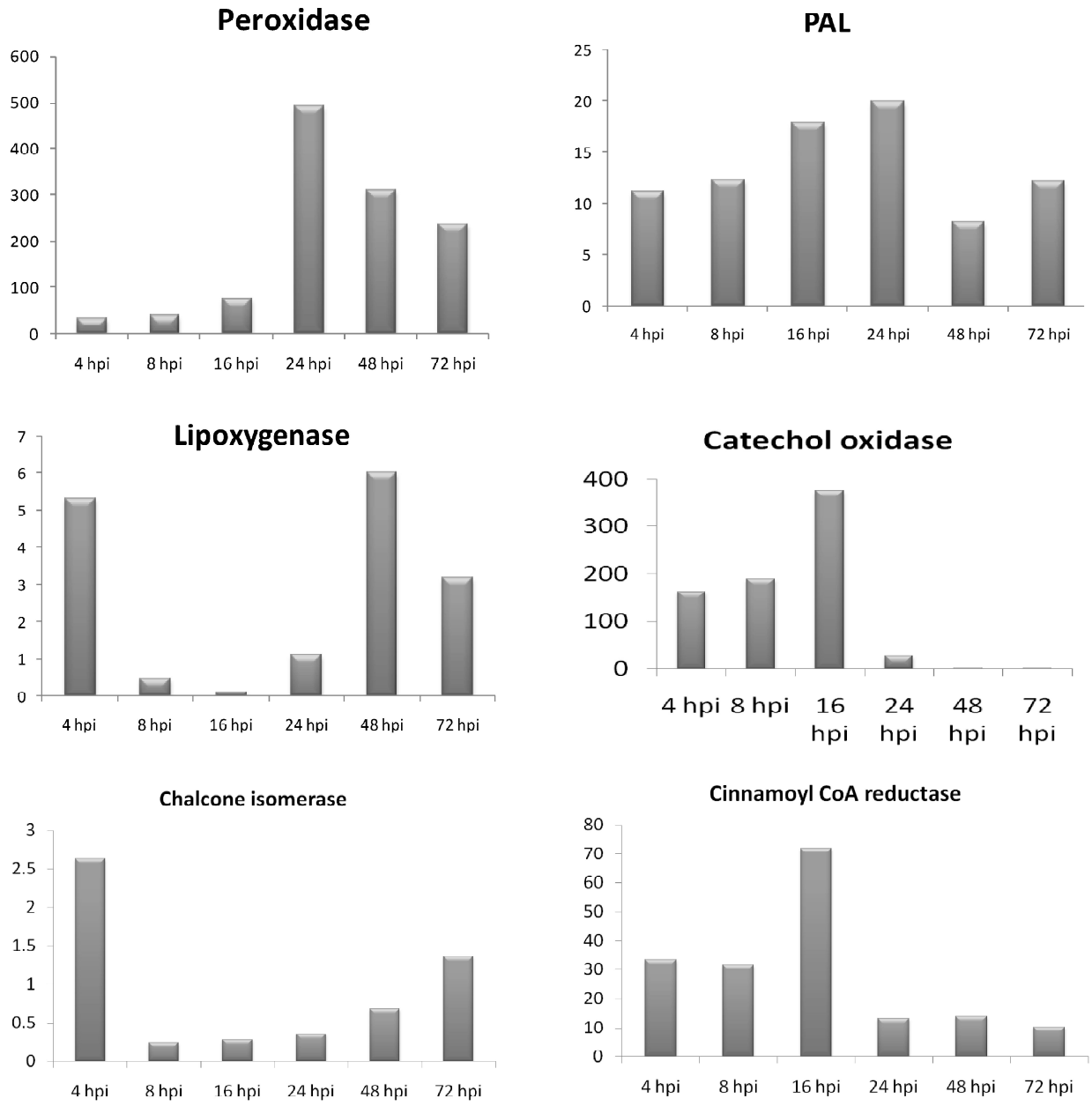

Fig. 3. Relative expression of genes *viz*., peroxidase, PAL, lipoxygenase, catechol oxidase, chalcone isomerase and cinnamoyl CoA reductase in black pepper inoculated with *Phytophthora capsici*.

of R gene analog in *Piper colubrinum* and validation through real time expression analysis showed the involvement of RGA in defense against *P. capsici*. Around 1289 candidate R gene homologues were mined from *P. colubrinum* transcriptome by reverse alignment using amino acid sequences of 42 known R genes. The sequences were functionally annotated and clustered to 91 clusters by h-cd-hit on CD-HIT suite using multiple CD-HIT runs. The expression pattern variability of NBS LRR *P. colubrinum* RGAs (PcRGAs) in *P. colubrinum* was analyzed by relative quantification of mRNA transcript in comparison with un-inoculated control. Basal level expressions of NBS-LRR PcRGAs were observed in *P. colubrinum*. In challenge inoculated *P. colubrinum* leaves, there was marginally high expression of PcRGA1 (at 0.5 and 1 hpi), PcRGA2 (at 8 hpi), PcRGA3 (at 1, 8 and 48 hpi) compared to uninoculated control. In the case of PcRGA1, maximum fold change recorded was at 8 hpi (3.36) and after 8 hpi expression of PcRGA1 is downregulated. Significant upregulation of PcRGA5 was observed at 0.5 hpi (7.36 fold) which was downregulated after 24 hpi, while the expression of PcRGA8 was downregulated at all time points post inoculation (Suraby *et al.,* 2015).

The expression of NBS LRR *Piper nigrum* RGAs in *P. colubrinum* and vice versa was analyzed by relative quantification of mRNA transcripts with mock inoculated control. When expression of 11 PnRGAs was evaluated in *P. colubrinum*, relatively more significant expression of PnRGA1, PnRGA3, PnRGA5, PnRGA7, PnRGA9, PnRGA11 and PnRGA24 was observed in *P. colubrinum*

compared to *P. nigrum*. Expression of PnRGAs *viz.*, PnRGA1, PnRGA3, PnRGA8, PnRGA9 and PnRGA24 was comparatively higher in *P. colubrinum* than in uninoculated control. On the other hand, when the expression of five PcRGAs, when tested on *P. nigrum*, the expression of PcRGA5 was downregulated in black pepper except its slight expression in 04-P24-1 at 1 hpi and 8 hpi.

qRT- PCR based expression pattern of PnRGAs in response to foot rot pathogen, *P. capsici* revealed differential expression pattern among moderately resistant and susceptible genotypes of black pepper. While comparing expression of PnRGAs in IISR Shakthi, 04-P24-1, Subhakara and *P. colubrinum*, PnRGA1 and PnRGA24 were identified as potential candidate genes. Expression of PnRGA24 is significantly higher in *P. colubrinum*, IISR Shakthi and 04-P24-1 compared to susceptible variety Subhakara, with the maximum being in *P. colubrinum.* Expression of PnRGA1 was significantly higher in IISR Shakthi and *P. colubrinum*. However, the expression of PnRGAs was found to be expressed during the early hours of infection and down regulated towards the latter phases of infection. The PnRGAs which are not significantly expressed in *P. nigrum* (PnRGA5, PnRGA7, and PnRGA8) were highly expressed in *P. colubrinum*. However, genetic transformation studies are required to confirm the functionality of R gene. [Phyto FuRa 2016]

Towards understanding the nature of reaction in black pepper against highly virulent and less virulent strains of *P capsici* transcriptome analysis was done in susceptible black pepper variety, Sreekara at three to four leaf stage. Plants were sampled at 8, 24 and 48 hour post inoculation (hpi) with mock inoculated plants at 8, 24 and 48 (hpi) as control. From the analysis 324 annotated and 64 un-annotated transcripts were detected. Of these, 11 genes showed above 500 folds upregulation in 05-06 as compared to 98-49 a less virulent isolate. The results showed the higher expression of glutathione-s-transferase (4571.63 RPKM) and catalase peroxidase (3192.35 RPKM) (Unpublished data).

Ginger-*Ralstonia* transcriptomics

Ginger, an important spice, severely affected by diseases such as bacterial wilt and soft rot. Lack of seed set, a major handicap in sexual recombination. No genomic information available. Crop improvement is based on clonal selection. Mutation breeding, induction of polyploidy and *in vitro* pollination met with little success. No source of natural disease resistance was found. A related species (*Curcuma amada*) mango ginger, is resistant to bacterial wilt caused by *Ralstonia solanacearum.* To understand the molecular mechanism of resistance, attempted Illumina sequencing of the transcriptomes of ginger and mango ginger species with the goal of isolating genes underlying resistance to the bacterial wilt. Over 36,000 and 32,000 genes/ ESTs from *C. amada* and *Z. officinale* were identified. Compared the transcriptomes of ginger and mango ginger following infection by *R. solanacearum* and Identified several candidate genes for resistance to bacterial wilt pathogen in *C. amada* .We also developed interactive database (gTDB). A list of functionally grouped up and down regulated genes indicate that 49 genes show differential gene expression of which 30 genes are up regulated and 19 genes are down regulated (Prasath *et al.,* 2013).

Proteomics

Proteomics is a mature platform for proteome analysis during plant-pathogen interaction. Proteomics allow screening and analysis, at the sub-cellular level, of peptides and proteins resulting from plants, pathogens, and their interactions. They also highlight post-translational modifications to proteins, *e.g.*, glycosylation, phosphorylation or cleavage. Unraveling mechanisms of more complex proteomic interactions that involve useful microbes, *i.e.*, PGPR and symbiotic fungi, which strengthen plant defenses, will generate valuable information on how plants actually function in probiotic application of the useful organisms and thereby provide clues to solving disease problems that reduces major losses in crops every year.

The 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technology combined with new analytical instruments such as nanoflow liquid chromatography coupled to tandem mass spectrometry (*i.e.*, LC-MS/MS) were used to establish proteome maps and explain specific aspects of host-pathogen interactions. Other label free approaches that do not require the use of PAGE have also been developed. Despite the significant progress made to date, currently available proteomic tools still lack in efficient protein extraction method in many plants as plants are known to be rich in phenolics and other secondary metabolites and also lacks required sensitivity to detect peptides that are in low abundance.

Proteomics in black pepper- *P. capsici* interaction

An efficient protein extraction method fr proteomic analysis was developed in black pepper using 2D electrophoresis coupled with nano-LC-LTQ Orbitrap mass spectrometry (Umadevi and Anandaraj, 2015). Label free proteomics strategy was applied to bring out the protein expression abundance and post translational modifications (PTMs) on tolerant (IISR Shakthi) and susceptible (Subhakara) genotypes. The leaf proteins were extracted from plants inoculated with *P. capsici* at 12 and 24 hpi along with control leaf. LTQ-Orbitrap Discoverer platform was used to fingerprint the quantitative expression of proteins during pathogen infection. The peptide peak data obtained was then annotated and relative expression of peptides was analyzed using Hi-3 (Average normalized abundance). In total, 299 proteins were analyzed out of which 84 proteins were found to have above 4 fold to 973 fold increase in expression and 38 of them were found to be

upregulated at 24 hpi. In tolerant genotype the pathogen was suppressed by pattern triggered immunity (PTI) which was triggered by receptor like kinases RLKs, RPP13 (R gene). These were identified as important pattern recognition receptor against *Phytophthora* in black pepper. Salicylic acid (SA) mediated SAR was identified during pathogen infection. PR protein (with antifungal activity) production was found to be more in tolerant black pepper. In susceptible, PTI was found to be weak with alteration in host physiology. Effector triggered immunity (ETI) by *Phytophthora* effector proteins and PRM1 (R gene) with jasmonic acid mediated suppression of SA. Production of PR5 (Thaumatin) protein and PAL production signified the susceptibility. The peptide data was then integrated with the *Piper* transcriptome DB on IISR Shakthi Illumina GLX 2X platform, annotated with Blast2Go and the R gene families in IISR Shakthi were grouped. (Umadevi *et al*., 2015). The antimicrobial peptides expressed during the infection of *P. capsici* in black pepper gives new insights in utilizing the AMPs as next generation molecules against *Phytophthora*. The peptide data from black pepper has been developed as a Piper pep database which includes the important antimicrobial peptide groups from this crop (http://220.227.138.213/piperpep/).

Elucidation of defense pathway in Resistant and Susceptible genotypes

Based on the quantitative expression of proteins in resistant and susceptible genotypes upon infection with *P. capsici* the satellite pathway on defense has been developed (Fig. 4, 5).

Detection of host factors in symptom expression of Virus in black pepper

In black pepper the pepper yellow mottle *badna* virus becomes severe when the plant is exposed to stress especially temperature above 35°C. Proteomic study (Umadevi *et al.,* 2014) revealed that due to temperature stress heat shock proteins are synthesized and some of them help the virus to multiply Table 4. Since the host plant machinery is diverted to manufacture virus

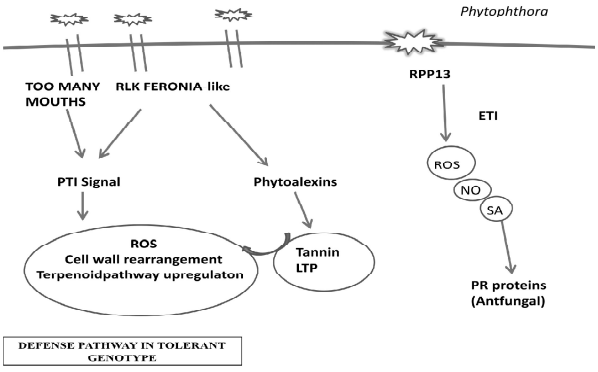

Fig. 4. Defense pathway in IISR Shakthi against *Phytophthora capsici*

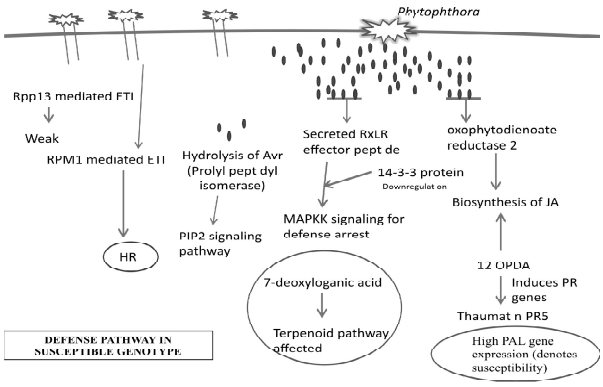

Fig. 5. Defense pathway in Subhakara against *Phytophthora capsici*

requirements and the plant suffers assimilation of essential nutrients such as magnesium. An experiment was undertaken on symptomatic plants to nurse the plants back to normal health by supplementing nutrients with organic manures, PGPR consortia and inorganic form of micronutrients as foliar spray. The experimental plants recovered, produced new leaves were without symptoms and the plants have become productive again. The field trials for two years indicate that in perennial crop like black pepper a simple management strategy would help farmers to regain the health of the vines thereby revive the economy (data unpublished)

Ionomics to study plant pathogen interaction

The ionome is defined as the mineral nutrient and trace element composition of an organism. it has the potential to provide a powerful approach to not only the functional analysis of the genes and gene networks that directly control the ionome, but also to the more extended gene networks that control developmental and physiological processes that affect the ionome indirectly. It is a recent platform with multiple application. Plant nutrients may favor disease susceptibility through plant metabolic changes, thereby creating a favorable environment for disease development. The pathogen infection alters the plant's physiology, mainly mineral nutrient uptake, as-similation, translocation, and utilization. The deficiencies might lead to secondary infections by other pathogens also. Nutrients are the important component in plant-disease interactions. Because of the complex interaction between disease, nutrient and environment complete information is needed on action of a particular nutrient in crop plants to develop a strong defense system. Knowledge on plant nutrition is a priority for disease management since as a rule, plants with an optimal nutritional status have the highest resistance (tolerance) to pests and diseases. Susceptibility increases as nutrient concentrations deviate from this optimum (Spann and Schumann, 2010).

MS data revealed photosystem I protein binding to virus counterpart. Two highest hits from MS data protein similarity showed this protein homology with RNaseH

Table 4. Nano LC-MS identified proteins with accession, molecular weight and the protein score

Protein name	Molecular weight	Accession	Protein Score
Calmodulin	16KDa[3,4]	P93171	72.83
Photosystem I reaction center	25KDa[1]	A5AEB4	437.33
Adenosyl homocysteinase	70KDa[1]	O23255	350.22
17.6 K Da classI heat shock proteins/	17.65KDa[5]	P30693	77.15
Rubisco large subunit	28KDa[5]	A2YVR7	109.64
Superoxide dismutase	25KDa[5]	P33DZ8	193.92
Chaperonin HSP 70 family	70KDa[5]	MORGDO	193.92
Plastocyanin	16 KDa[2]	POO297	67.78
Oxygen evolving enhancer protein/	35KDa[2]	F2XX49	363.144
Monohydro ascorbate reductase	45KDa[2]	A5JPK7	151.26
Chaperonin CPN 60-2	60KDa[2]	Q43298	98.66
2-Cys peroxiredoxin	25 K Da[2]	B6TDA9	142.69

[1], PCR positive before stress; [2], PCR positive after stress; [3], PCR positive (before/after) stress; [4], PCR negative (before/after); and [5], PCR negative after stress

(ribonuclease H) protein of BSV (Accession Id: ACL37070.1) (a member of the genus *Badnavirus* to which PYMoV also belong) with 70% identity in Blastp. When analyzed, the 16 KDa plastocyanin molecule was found to bind with viral protein. The similarity search for 2 highest hits with 116.97 score from MS analysis showed this protein homology with RNaseH protein of BSV (Accession ID: ACL37070.1) with 70% identity in Blastp. The highest hit search with 98.99 score for the homology of this protein yielded the protein similarity towards ORF III polyprotein (Accession ID: AAE86310.1) of BSV with 50% identity in Blastp. The data on protein profile (Table 4) from PYMoV infected black pepper upon temperature stress shows that the temperature is an important factor for the virus to produce symptom. The results suggested that alteration in hormone synthesis, chloroplast, photosynthesis and heat shock proteins may contribute to symptom expression profile on the protein expression before and after exposure to temperature stress in PCR positive and negative plants has given indication of host proteins with virus derived proteins such as photosystem I protein- RNaseH protein of BSV, plastocyanin-RNaseH protein of BSV, Chaperonin CPN 60-2 protein -ORF III polyprotein. Though these initial results throw light on the involvement of host factors in symptom expression, the analysis on the entire protein profile to pinpoint the specific up-/down- regulation of host factors, interaction of host factors with virus factors for the altered physiology and disease development is needed for the development of defined and more potent management strategies to reduce symptom expression (Umadevi *et al.*, 2016).

The elucidation of whole leaf ionome in black pepper-*Trichoderma* interaction is underway in our lab. The information on the ionome on this interaction would pinpoint indirectly the genes and pathways involved during the priming mechanism of *Trichoderma* in black pepper, which would be possible markers and candidate genes for future breeding programmes.

Metabolomics

In plant pathology, metabolomics has been mainly used to study plant responses to a wide range of biotic or abiotic stresses including resistance of plants to pathogens and also as a powerful tool for functional genomics studies. A non-targeted metabolic profiling of resistant and susceptible potato genotypes against *P. infestans*, using a high-resolution liquid chromatography-hybrid mass spectrometry (LC-MS) capable of detecting both volatile and nonvolatile metabolites was used to elucidate quantitative disease resistance mechanisms. The RR metabolites identified mainly belonged to secondary metabolic pathways such as phenylpropanoid, fatty acid, alkaloid, and terpenoid. A set of RR genes involved in the biosynthesis of HCAAs were identified, sequenced and SNPs identified, and their potential in improving potato resistance to late blight is underway (Pushpa *et al.,* 2013).

CONCLUSION

The recent flurry of technological advancement have increased the understanding the molecular events with global and specific focus. The knowledge generated by the omics technologies are now increasingly used as management strategies directly or indirectly in plant-pathogen interaction of research is concerned. The disease forecasting, elucidation of plant-pathogen-biocontrol (tripartite) interaction and the generation of new molecules based (host driven) management would be the most important areas of future translational research using omics technologies.

REFERENCES

Anandaraj, M. (2000). Diseases of black pepper. In: *Black Pepper* (*Piper nigrum* L.). (Ed: Ravindran, P.N), pp. 239-267, Harwood Academic Publishers, Amsterdam.

Anandaraj, M., Jose, A. and Balakrishnan, R. (1989). Crop loss due to foot rot disease of black pepper. *Indian Phytopath* **42**: 473-476

Anandaraj, M., Singh, D., Eapen, S.J., Gopalakrishnan, C., Reddy, KM., Prameela, T.P., Ramesh, R., Rosana, O.B., Singh, B.P., Sagar, V., Bhai, R.S., Patil, V.V. and Srivastav, V. (2016). Comparative genomics of *Ralstonia solanacearum* strains form India reveals their phyletic profiles and diverse effectomes. *In 6th international conference on Plant, Pathogens and People* Feb 23-27, New Delhi, India.

Cooke, D.E.L., Cano, L.M., Raffaele, S., Bain, R.A., Cooke, L.R., Etherington, G.J., Deahl, K.L., Farrer, R.A., Gilroy, E.M., Goss, E.M., Grunwald, N.J., Hein, I., MacLean, D., McNicol, J.W., Randall, E., Oliva, R.F., Pel, M.A., Shaw, D.S., Squires, J.N., Taylor, M.C. and Vleeshouwers, V.G.A.A. (2012). Genome analyses of an aggressive and invasive lineage of the Irish potato famine pathogen. *PLoS Pathog* **8**: e1002940.

Friesen, T.L., Stukenbrock, E.H., Liu, Z., Meinhardt, S., Ling, H., Faris, J.D., Rasmussen, J.B., Solomon, P.S., McDonald, B.A. and Oliver, R.P. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. *Nat. Genet.* **38**: 953-956.

Gardiner, D.M., McDonald, M.C., Covarelli, L., Solomon, P.S., Rusu, A.G., Marshall, M. and Kazan, K. (2012). Comparative Pathogenomics Reveals Horizontally Acquired Novel Virulence Genes in Fungi Infecting Cereal Hosts. *Plos One* **8**: e1002952.

Hacquard, S., Kracher, B., Maekawa, T., Vernaldi, S., Schulze-Lefert, P. and van Themaat, E.V.R. (2013). Mosaic genome structure of the barley powdery mildew pathogen and conservation of transcriptional programs in divergent hosts. *PNAS* **110**: E2219-2228.

Johnson George, K., Vijesh Kumar I.P. and Anandaraj, M. (2012). Transcriptomics approaches for gene discovery in plants - a case study in *Piper*. *Agrotechnol.* **1**: 2.

Li, F., Pignattaa, D., Bendixa, C., Brunkarda, O.J., Cohna, M.M., Tung, J., Sun, H., Kumar, P. and Baker, B. (2013). Micro RNA regulation of plant innate immune receptors. *PNAS* **109**: 1790-1795.

Phyto FuPa (2016). Annual Report 2015-16.

Prasath, D., Karthika, R., Habeeba, N.T., Suraby, E.J., Rosana, O.B., Shaji, A., Eapen, S.J., Despande, U. and Anandaraj, M. (2014). Comparison of the transcriptomes of ginger (*Zingiber officinale* Rosc.) and Mango Ginger (*Curcuma amada* Roxb.) in Response to bacterial wilt infection. *Plos One* **9**: e99731.

Pushpa, D., Yogendra, N.K., Gunnaiah, R., Kushalappa, C.A. and Murphy A. (2014). Identification of Late Blight Resistance-Related Metabolites and Genes in Potato through Non targeted Metabolomics. *Plant Mol Biol Rep* **32:** 584-595

Rietman, H., Bijsterbosch, G., Cano, L.M., Lee, H.R., Vossen, J.H., Jacobsen, E., Visser, R.G., Kamoun, S. and Vleeshouwers, V.G. (2012). Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors. *Mol. Plant-Microbe Interact.* **25**: 910-919.

Simpson, A.J.G., Reinach, F.C., Arruda, P., Abreu, F.A., Acencio, M., Alvarenga, R., Alves, L.M., Araya, J.E., Baia, G.S., Baptista, G.S., Barros, M.H., Bonaccorsi, E.D., Bordin, S., Bove, J.M., Briones, M.R., Bueno, M.R., Camargo, A.A.,Camargo, L.E., Carraro, D.M., Carrer, H., Colauto, N.B., Colombo,C., Costa, F.F., Costa, M.C., Costa-Neto,C.M., Coutinho, L.L., Cristofani, M., Dias-Neto, E., Docena, C., El-Dorry, H., Facincani, A.P., Ferreira, A.J., Ferreria, V.C., Ferro, J.A., Fraga, J.S., Franca, S.C., Franco, M.C., Frohme, M., Furlan, L.R., Garnier, M., Goldman, G.H., Goldman, M.H., Gomes, S.L.,Gruber, A., Ho, P.L., Hoheisel, J.D., Junqueira, M.L., Kemper, E.L., Kitajima, J.P., Krieger, J.E., Kuramae, E.E., Laigret, F., Lambais, M.R., Leite, L.C., Lemos, E.G., Lemos, M.V., Lopes, S.A., Lopes C.R., Machado, J.A., Machado, M.A., Madeira, A.M., Madeira, H.M., Marino, C.L.,Maques, M.V., Martins, E.A., Martins, E.M., Matsukuma, A.Y., Menck, C.F., Miracca, E.C.,Miyaki, C.Y., Monteriro-Vitorello, C.B., Moon, D.H., Nagai, M.A., Nascimento, A.L., Netto, L.E.,Nhani, A, Jr., Nobrega, F.G., Nunes, L.R., Oliveira, M.A., de Oliveira, M.C., de Oliveira, R.C., Palmieri, D.A., Paris, A., Peixoto, B.R., Pereira, G.A., Pereira, H.A., Pesquero, J.B., Quaggio, R.B., Roberto P.G., Rodrigues, V., de M Rosa, A.J., , de Rosa, V.E. Jr., de Sa, R.G., Santelli, R.V., Sawasaki, H.E., da Silva, A.C., da Silva, A.M., da Silva, F.R., da Silva, W.A.Jr., da Silveira, J.F.,Silvestri, M.L., Siqueira, W.J., de Souza, A.A.., Terenzi, M.F., Truffi, D., Tsai, S.M., Tsuhako,M.H., Vallada, H., Van Sluys, M.A., Verjovski-Almeida, S., Vettore, A.L., Zago, M.A., Zatz, M., Meidanis, J. and Setubal, J.C. (2000). The genome sequence of the plant pathogen *Xylella fastidiosa*. *Nature* **406**: 151-157.

Spann, T.M. Schumann, A.W. (2010). Mineral Nutrition Contributes to Plant Disease and Pest Resistance. Series No.HS1181, Horticultural Sciences Department, UF/IFAS Extension, University of Florida, Gainesville, FL.

Suraby, E.J., Nimal babu, K., Prasath, D., Johnson, K.G. and Anandaraj, M. (2015). Expression analysis of Resistance gene analogs in *Piper colubrinum-Phytopthora capsici* incompatible interactions. *Int. J. Innov. Hort.* **4**(2): 105-110.

Tsavkelova, E., Oeser, B., Oren-Young, L., Israeli, M., Sasson, Y., Tudzynski, B. and Sharon, A. (2012).Identification and functional characterization of indole-3-acetamide-mediated IAA biosynthesis in plant-associated *Fusarium* species. *Fungal Genet Biol* **49**: 48-57.

Umadevi, P., Anandaraj, M. and Johnson, K.G. (2015). Towards understanding the black pepper-*Phytophthora* pathosystem using integrated transcriptome and proteome dataset. *In International symposium* On *Phytophthora,* Sep 9th-12th, IIHR, Bangalore.

Umadevi, P. and Anandaraj, M. (2015). An efficient protein extraction method for proteomic analysis of black pepper (*Piper nigrum* L.) and generation of protein map using nano LC-LTQ Orbitrap mass spectrometry. *Plant omics* **8**(6): 500-507.

Umadevi, P., Bhat, A.I., Krishnamurthy, K.S. and Anandaraj, M. (2016). Influence of temperature on symptom expression, detection of host factors in virus affected black pepper (*Piper nigrum* L.). *Ind. J. Expt. Biol*. **54**: 354-360.

Vijesh Kumar, I.P., Johnson, G.K. and Anandaraj, M. (2016). Real-Time Quantitative RT-PCR of some defense response genes in *Piper colubrinum* challenge inoculated with *Phytophthora capsici. Int. J. Agrl. Sci. Res*. **6**: 69-78.

Vijesh Kumar, I.P., Johnson George K., Rosana Babu, O. and Anandaraj, M. (2015). Quantitative RT-PCR analysis of *Phytophthora* specific genes expressed during *Phytophthora capsici-Piper colubrinum* interaction. *Int. J. Bio. Technol. Res.* **5**: 1-8.