

A System Biology Approach to Explore Host-Pathogen Interaction Under Phytochemical Cross Linkages

Muhammad Junaid Yousaf (✉ junaidyousaf44@gmail.com)

Abdul Wali Khan University Mardan

Anwar Hussain

Abdul Wali Khan University Mardan

Amjad Iqbal

Abdul Wali Khan University Mardan

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Abstract

Phyto-signalling molecules are minute, but tangible that has rigorous roles in any plant-pathogen interaction. Certainly, most of the pathogen alters their biosynthesis, transport, degradation and cellular signalling responses to pave their virulence. Therefore, the gene expressions of such molecules with their correlated defense mechanisms were analysed in *Arabidopsis thaliana* against *Erysiphe orontii* (a potential biotroph), *Botrytis cinerea* (a potential necrotroph), *Pseudomonas syringae* (a bacterial hemibiotroph), and *Phytophthora infestans* (a fungal hemibiotroph) using molecular biology/ system biology techniques. The findings strongly suggested that each pathogen has its own unique infection strategy based on up-regulation and down-regulation of host phyto-signalling genes. Our studies also explored four basic pathogenic infection maps based on cross linking phyto-signalling molecules.

Background

Host (plant) - pathogen interaction is the multifaceted process that includes the binding of protein receptors to elicit a response by altering a wide range of host physiological aspects (1). The elicited response is manipulated by the small, but tangible molecules in host, commonly known phyto-signalling molecules (2). The phytopathogens are divided into three basic categories (biotroph, necrotroph and hemibiotroph) on the basis of their biosynthesis, transport, degradation, cell signal perception, repression and response in host plant (3). Biotroph is a plant pathogen that infects, yet fetches nutrients out of the host without killing it (4). This pathogen is adapted to cause low damage to the host, but requires high pathogenic strategic network to drive food from it (5). Due to its advanced pathogenic strategy, there is usually limited range of hosts available for it (6). Biotroph usually lacks in host cell wall degrading enzymes, but they can produce secondary metabolites, which act as virulence (7). Plant defense signalling in biotrophy involves the biosynthesis of phyto-signalling molecules and correlated expression of genes, which undergo cell death or apoptosis (8). Necrotroph is another group of plant pathogen that kills the host and then lives on it saprophytically to drive nutrients out of it (9). Necrotrophic pathogens are not specialized in their virulence as they only tend to kill their host (10). Plants resists the cell death caused by necrotrophs and detoxify their toxins by specialised defense mechanisms (11). Beside the biotrophs and necrotrophs, hemibiotroph is a class of pathogens, which first adjust and then kill the host to drive energy out of them (12). Initially, they establish haustoria in the host tissues and then produce host degrading enzyme to destruct host tissues (13). Plant defense system against hemibiotroph is quite challenging (14). The integrated role of phyto-signalling molecules with co-expression of the genes for these pathogens is still unknown (15). In fact, the host defense response defines the category of pathogen (16). Along with this, there is the correlated defense response initiated by the host to overcome the virulence of the pathogen (17). Among the phyto-signaling molecules, IAA appeared to be the most prevalent phyto-hormones because of variety in its biosynthetic pathways (18). These pathways need to be exploited in relation to plant-pathogen interactions. In the past few decades, this field has been explored through mathematical and bioinformatics models to understand various facets of the interactions (19).

The aim of this study was to interlink the current understanding on the biotroph, hemibiotroph and necrotroph with the host phyto-signalling molecules with correlated co-expression of host genes to elucidate the pathogen infection strategies and host defense strategies against each class. We also intended to explore the manipulating role of phyto-signalling molecules through its biosynthesis, transport, degradation and cell signalling in host-pathogen interaction using system biology approach. In this connection, all the associated contrasting defense and virulence responses were studied to highlight the possible gaps in the field. Therefore, we depicted four infection maps to analyse the conclusive virulence strategy of pathogen and host defense strategies.

Methods And Materials

Gene Expression Analyses of Phyto-signalling molecules

To study the effect pathogenesis on various aspects of phyto-signalling molecules, genes expressional data of their biosynthesis, transports, degradation, cell signalling perception, cell signalling repression and cell signalling response were extracted from online available browser BAR (Bio-Analytical Resource for Plant Biology) (20). *Arabidopsis* (Col-0) leaf tissue was inoculated through syringe infiltration method (20). BAR provided *Arabidopsis eFP* (Electronic Fluorescent Profile) tool, to check the expression of selected significant genes for *B. cinerea*, *P. syringae*, *P. infestans* and *E. orontii* infection in *Arabidopsis* (21). The mode of inoculation was on the host leaf and the data was extracted after the host showed infection symptoms. The value obtained from expression data was in log2 ratio where negative values indicated down-regulation while positive values indicated up-regulation. Using the retrieved gene expression data, heat maps and schematic pathways were drawn revealing the strategy of each pathogen for altering various aspects of phyto-signalling molecules.

Co-expressional analyses of defensive genes

Three principle genes involved in defense responses correlated with phyto-signalling molecules were studied using *Expression Angler 2016* tool with r-cut off range up to -0.23 to 0.99 (22). The data so extracted from *Expression Angler 2016* tool with desired r-cut off range were subjected to yED (23) to produce co-expression networks where different colour nodes represented up-regulation and down-regulation with the reference of the expressional value of principle defense gene and pathogen. Details of each gene were taken from *TAIR* tool (24) and a conclusive co-expression genes networks and infection maps were depicted which elucidated the infection strategy and infection level for each pathogen.

Results

IAA biosynthesis under pathogenic attack

Expression data of IAA biosynthetic genes revealed two major IAA biosynthetic pathways in *Arabidopsis*, predominated by *TAA1* and *CYP79B3* genes. Due to the down-regulation of *TAA1* under the all selected pathogens (Figure 1), it was designated as control pathway. On the other hand, expression of *CYP79B3*

was up-regulated under pathogenic attack hence, this pathway was referred to as pathogenic pathways (Figure 2). *B. cinerea* attack induced high level of *CYP71A13* transcript in *Arabidopsis* (Figure 1a-c), modulating IAA biosynthesis pathways to enhance camalexin production on the expense of IAA (Figure 2c). By up-regulating *NIT3*, *E. orontii* and *P. syringae* enhanced IAA production (Figure 1d & 2a). Moreover, IAA biosynthesis was also manipulated in *P. infestans* infection shifting the balance toward GL biosynthesis by up-regulating expression *SUR2* genes (Figure 1b & 2b).

IAA transport under Pathogenic attack

Under pathogen attack, the host expressed and repressed differential genes. Under *B. cinerea* attack, the host expressed *PIN2*, *PIN5*, *PIN8* genes, while repressed *PIN1*, *PIN3*, *PIN4*, *PIN6*, *PIN7*, *AUX1*, *LAX2*, and *LAX3* genes an indication of acropetal IAA transport (Figure 3a, 4a). *E. orontii* induced an exactly opposite response in *Arabisopsis* by up-regulating expression of *PIN3*, *PIN6*, *PIN1*, *PIN4* and *LAX2*, accompanied by repression of *PIN2*, *PIN4*, *PIN7*, *PIN5*, *PIN8*, *LAX2*, and *AUX1* which means multidirectional IAA transport (Figure 3d, 4d). *P. infestans* infection up-regulated *PIN3* expression while repressing all other transport genes restricting IAA transport (Figure 3c, 4c). The *P. syringae* infected *Arabidopsis* seemed to possess basipetal IAA transport as indicated by up-regulated expression of *PIN5*, *PIN8*, *LAX2*, and *PIN2* and a shutdown of the remaining genes

(Figure 3b, 4b).

Gene expression of defensive phyto-signalling molecules

Gene expression data of *ACS2*, *ICS1* and *LOX3* (signalling genes for ethylene, salicylic acid, jasmonic acid) with their correlated defense genes such as *PDF1.2*, *PR-1* and *VSP2* respectively, showed that *LOX3* expression was high in *B. cinerea* infection as compared to *ACS2* and *ICS1*. Therefore, the expression of *VSP2* was high. Interestingly, the expression of *PR-1* was also high despite of low expression of its correlated signalling gene. As opposed, *E. orontii* repressed *LOX3* while expressed *ICS1* and *ACS2* gene. This response was accompanied by up-regulated expression of *PR-1* and *PDF1.2* and down-regulation of *VSP2*. *P. infestans* infection increased the expression of *ACS2*, followed by up-regulation of *PDF1.2*. Interestingly, leaves challenged with *P. syringae* had induced expression of *ACS1* and *VSP2* genes (Figure 5).

Co-expression of cross linking phyto-signalling molecules

Co-expression of high expressed *VSP2* included genes encoding glycosyl hydrolase beta amylase, and cysteine lyase that can be involved in the biosynthesis of cellulose, starch and ethylene precursors. Similarly, arginine amidohydrolase, polygalacturonase inhibiting protein, myrosinase-binding protein and phytosulfokinase were also up-regulated. Moreover, the up-regulated genes including the defense signalling genes *BGL1*, *BAM5*, and *JAR2* were also the most prominent. The down-regulated genes were those coding for cysteine synthase c1, cyclic nucleotide-gated channel 12 and phosphoinositide

phosphatase protein. Genes involved in ions transport such as nitrate transport, and nodulin MtN21-like transporter family were found to be down-regulated (Figure 6a).

Co-expression with upregulated *PR-1* comprised genes related to chitin catabolism, glutaredoxin production, and zinc transport. Thaumatin-like protein, vacuolar sorting receptor, glutathione s-transferase production and phosphofructokinase and ADP/ATP carriers were up-regulated for cell signalling purpose. Enzymatic processes such farnesol dehydrogenase and Pumilio (APUM) proteins were also enhanced. Down-regulated genes in high *PR-1* expressing *Arabidopsis* were found to be coding for thylakoidal ascorbate peroxidase, uracil phosphoribosyl transferase and salicylic acid-binding proteins. Some biosynthetic processes in the infection were down-regulated such as starch synthase for normal amylopectin synthesis, lysophosphatidylcholine acyltransferase (Figure 6b).

Genes co-expressed with Up-regulated *PDF1.2* included hevein-like pathogenesis-related protein, cytochrome p450 for secondary metabolites and UDP-glucosyl transferase for quercetin 3-O-glucosyltransferase activity. Cell defense proteins such as Transporter k⁺, glutamate-cysteine ligase, and octadecanoid-responsive *Arabidopsis* AP2/ERF were also up-regulated. The o-mtase family 3 protein and transcription-coupled nucleotide-excision repair genes were also co-expressed. Contrary to these genes, TAT protein binding, homeobox protein and ATP synthesis coupled electron transport proteins coding genes were not expressed in *Arabidopsis* with induced expression of *PDF1.2*. The biosynthetic processes such as cellulose synthase, RNA polymerase beta' subunit-1 and trichome birefringence protein were also down-regulated (Figure 6c).

Phyto-signalling make-up at pathogenic attack

Infection Map 1st

Infection with the biotrophic pathogen, *B. cinerea* modulated host physiology by inducing the expression of genes involved in biosynthesis of ABA, ET, and JA and degradation of auxins, GA, BR and SA. More notably, the CKs biosynthesis and degradation genes were simultaneously repressed. Expression of genes involved in signal perception (a first step of signalling mechanism that occurs at the cell receptor level) of GAs, CKs, ET and JA was high. Contrary to this, expression of genes responsible for auxins and SA signalling were repressed. The cell signal response (a last step of signalling mechanism, when cell produces protein) was only high for ET, JA and CKs (Figure 7a).

Infection Map 2nd

E. orontii infected leaves were characterized by enhanced expression of genes coding for GAs biosynthesis enzymes and repression of GAs degrading gene (Figure 7b). Genes involved in the biosynthesis and degradation of the rest of the phytohormones showed exact opposite trend. An exception was JA, where the biosynthesis as well as degrading genes were down-regulated. Expression of cell signal perception was very low for SA and GA, while very high for auxins, JA and ET. Interestingly, cell

signalling repression to all hormones were very low, while cell signalling response was only low for auxins and SA.

Infection Map 3rd

In *P. infestans* exposed *Arabidopsis thaliana*, auxins, SA, ET, ABA, GAs and CKs biosynthesis and degradation genes were up and down regulated respectively. Interestingly, only auxins cell signalling perception genes was found to have low transcripts while those for the rest of phytohormones had high expression. where cell signalling repression of only SA and ABA was high. Moreover, cell signalling response of all were high except ABA (Figure 7c).

Infection Map 4th

Under *P. syringae* infection, expression of auxins biosynthesis genes was low as compared to its degradation genes. while the rest had high degradation. Genes involved in cell signal perception for all phytohormones was high except JA and ET while cell signal repression genes had high expression only for ABA and SA and high for the rest. Cell signalling response was found to be high only for GAs and CKs (Figure 7d).

Discussion

IAA biosynthesis and transport varies under various pathogenic attack

The role of IAA in pathogenesis in plants already revealed that high IAA level in host promotes the advents of biotroph virulence whereas demotes the necrotroph virulence (25). Our results evaluated from gene expression of principle IAA biosynthetic gene predicted that the host increased or decreased IAA biosynthesis upon encountering a biotroph (*E. orontii*) or necrotroph respectively. This is evident as *B. cinerea* had deviated the IAA biosynthesis to camalexin production in the infected host as predicted from gene expression data, signifying the necrotroph virulence. Therefore, it can generally be assumed the total level of IAA is deviated to camalexin production under necrotroph virulence. Camalexin is a defensive secondary metabolite produced in plant during a pathogen attack (26). Similarly, up-regulated expression of genes that could re-direct the flow of IAA gravitropically from leaf to root, and from the cytosol into the mitochondria, might further minimize the level of IAA in host cell during the *B. cinerea* infection. Thus, IAA transport and biosynthesis were manipulated to exclude it from infection site under necrotroph virulence. However, this strategy has a potential disadvantage as camalexin can restrict the pathogen. Repression of IAA biosynthesis increases susceptibility of the host to necrotrophic fungi. This is because IAA undergoes cell proliferation and resist cell death which is opposite to necrotrophic strategy. Thus in this context, *B. cinerea* has diverted IAA biosynthesis to camalexin to increase its virulence. This also contributes to reduce IAA level in host, thereby accelerating cell death. BcLTF1 is a pathogen factor of *B. cinerea* which aids in its virulence by accelerating cell death (27). We concluded that the same factor may be involved in reducing IAA level to camalexin to accelerate cell death.

Upon encountering biotrophic *E. orontii*, the host IAA production is elevated as a result of up-regulated expression of *NIT* family genes. The pathogen also manipulated gene expression of host to impose multidirectional transport of IAA which strongly suggested that IAA level through biosynthesis and transport was high at the infection site to facilitate the (Bahari, 2018 #52) necrotroph are involved in this differential response of host.

P. infestans is a fungal hemibiotroph responsible for infecting several members of solanaceae and brassicaceae including *Arabidopsis* (28). The genes expression pattern under *P. infestans* infection suggest that this pathogen also used an anomaly IAA pathway to discourage IAA biosynthesis by diverting it to GL production. Thus, it can be deduced that IAA might also toxic for hemibiotroph fungus at earlier stages of infection (such as when they are inoculated to the host plant which is specified at 24th hour of the treatment) (28). By contrast, bacterial hemibiotroph, *P. syringae* alters host auxin biology in order to promote its virulence in host (29). Analysis of the gene expression data in this regard suggested that *P. syringae* maintained IAA biosynthesis by up-regulating *NIT3* to keep IAA high at the infection site. This alteration in auxin biology in host causes two main advantages to *P. syringae*, IAA increases the leaking of nutrients to the apoplast and also detoxifies the antimicrobial compounds. Therefore, *P. syringae* like *E. orontii*, repressed *CYP71A13* to discourage the production of camalexin in host. Interestingly, *P. syringae* is also known to produce its own IAA which might also be contributing to the endogenous IAA pool (30). This concludes the importance of IAA for the virulence of the bacterial pathogen. Regarding IAA transport, both followed the same strategy to increase IAA level into the infection site.

In short, the obtained conclusion from results deposed that production of IAA was not in favour of necrotrophs and fungal hemibiotrophs as IAA proliferates the infecting cell which was against the lethal strategies of fungal hemibiotroph and necrotrophs. Therefore *B. cinerea* and *P. infestans* detained the production of IAA to camalexin and GL respectively. These two group of compounds kill the infected cell, rendering it for their dinner (31). In case of biotrophs, IAA was in favour of their lethal strategy as biotrophs keep the cell alive and devour on its metabolites. Therefore *E. orontii* and *P. syringae* induced the production of IAA, keeping the cell off other secondary metabolites such camalexin and GL.

Phyto-signalling molecules are tactically manipulated under host-pathogen interactions

Each pathogen has portrayed its own manipulation over hormonal set-up of the host. Phyto-signalling molecules which also termed as phytohormones are largely involved in regulation of host-pathogen trade-offs in contrast with growth and development (32). When model of hormonal manipulation and plant defense signalling of each class was compared with our predictions based gene expression data, some interesting facts and various gaps in the field were discovered (Figure 8). As already discussed, IAA is considered to be toxic for necrotroph and beneficial for biotroph (33). *B. cinerea* (a necrotroph) has completely adopted the virulent way for auxin manipulation as it down regulated auxin biosynthesis genes and un-regulated its degradation genes accompanied by repression of genes involved in signal

perception. On another hand, under *E. orontii* (a biotroph) infection, the host adopted defensive strategy as auxin biosynthesis was low while its degradation was high. This clearly indicated the importance of auxin biosynthesis and signalling for necrotrophic virulence rather than biotrophic virulence.

Apposing to the fact, the high auxin signal perception creates a gap despite of low cell signalling response. Our prediction regarding high auxins cell signal perception is that either *E. orontii* has produced auxins or auxin analogues up-regulating the transcription of perception proteins, needs to be investigated. Most of the hemi-biotrophs manipulate auxin in early stages for proliferating infection site while produce its own toxins to kill the cell in later stage for completion of its developmental stages (34). Regarding the above fact, genes involved in auxin cell signal perception for *P. syringae* infection was high despite of repression of its biosynthesis and up-regulation of its degradation genes. More surprisingly, in fungal hemibiotroph infection (*P. infestans*), we predicted high auxin degradation and cell signal response with low biosynthesis and perception. Taken together, these finding suggest that auxin cell response genes are necessary for either increasing hemi-biotroph virulence or the subsequent host defense which need to be investigated in future research.

Gibberellins were also found to have a role in plant-pathogen interaction (35). Interestingly, the host adopted gibberellins regulation for both *E. orontii* and *B. cinerea* as defensive hormone but at the same time, cell signal repression and cell signal perception of gibberellins at *B. cinerea* infection produced a research gap for future inspection. Our scope is that through unknown factors, *B. cinerea* reduced the gibberellins cell signal response to favour its necrotrophy whereas, the host decreased the gibberellins signal perception to reduce biotrophy of *E. orontii*. In case of hemitrophs, it was elucidated that gibberellins biosynthesis upregulation was used against *P. infestans* while in favour of *P. syringae*. Therefore, gibberellins decreased virulence of fungal hemibiotroph while increased virulence for *P. syringae*. Role of ABA for the host-pathogen interaction in case of our selected model pathogens produced a vast gap (36). Biosynthesis and degradation of ABA was high at the same time during *B. cinerea* virulence and high cellular response for *E. orontii* attack despite of low signalling needs to be properly investigated. Moreover, ABA shielded the host cell against *P. infestans* attack. However, the host cellular response was low, revealing *P. syringae* grip over ABA cell signalling.

Like other hormones, plant's cytokinin is also instrumental signalling molecule in host-pathogen interaction (37). Regarding biosynthesis and degradation, cytokinin increased virulence for *B. cinerea* where the cell signalling responses favoured host defense strategy. Interestingly, *E. orontii* infection opposed the strategy followed by *B. cinerea* as the pathogen followed cell signalling response to its favour while biosynthesis and degradation was utilized by the host defense against its virulence. *P. syringae* increased cytokinins degradation and at the same time the host increased its biosynthesis, indicating a pitch battle between the two. The cell signalling response in this regard was completely low which needs to investigated in wet lab. As opposed to *P. syringae*, *P. infestans* increased the cell signalling response while decreasing its biosynthesis and degradation. Salicylic acid (SA) depicted a typical system of host defense and pathogen virulence responses (38) as it favoured virulence in *B. cinerea* and *P. infestans* attack but decreased *P. syringae* and *E. orontii* attack. As opposed to SA, JA

produced defense response in *B. cinerea* while in *E. orontii* attack, the host strived hard to produce an appropriate response against the virulence. In this case, the inhibition of JA biosynthesis and degradation at same time needs to be investigated under *E. orontii* attack. Interestingly, under *P. infestans* and *P. syringae* attack, JA favoured virulence. This determined the toxicity of JA under hemibiotroph attack on host.

B. cinerea utilized ethylene biosynthesis and signalling for its virulence whereas *E. orontii* used its biosynthesis and degradation for its virulence while the host utilized its signalling for defense response. It is now necessary to develop a proper understanding on the signalling defense of ethylene under *E. orontii* attack. Similarly, in case of hemibiotrophs, *P. infestans* used its ethylene biosynthesis for virulence while the signalling was utilized by the host for defense. *P. syringae* could not produce an appropriate virulence at level of ethylene signalling and biosynthesis. Brassinosteroids appeared to an emerging hormone in host-pathogen interaction as most of its part is clear in question. Our studies put forth few assumptions regarding BR signalling and biosynthesis as BR solely used as defensive hormone against the *B. cinerea* while *E. orontii* used it for its own virulence. Similarly, *P. infestans* used only its degradation for virulence while the remaining aspects needs to be investigated. More lucidly, BR was utilized by *P. syringae* for its virulence.

The expression of co-related defensive genes explores the strategy of the host defense

In the previous sections, it now became obvious that every plant hormone has a dual role in host pathogen interactions. Plant hormones secondarily function is to trigger various co-related defensive genes when any category of pathogen approaches in the vicinity of the plant (32). We focused on the three basic defensive molecules ethylene (*ACS2*), salicylic acid (*ICS1*), and jasmonic acid (*LOX3*) and their co-related defensive genes such as *PDF1.2*, *PR-1*, and *VSP2* respectively in regards to host defense. Expression data of *ACS2*, *ICS1*, and *LOX3* concluded that a fungal biotroph (*E. orontii*) upregulated the synthesis of salicylic acid at its infection. This signalling led to the expression of *PR-1*, but the matter of contention was the expression of *PDF1.2*(*log2 value 2.05*) during the infection despite of low expression of *ACS2*. This created a gap in our studies. Our perception regarding the question is the adoption of host a mechanism to eliminate the virulence by the way independent of *ACS2* signalling which needed to be clarified. *PR-1* and *PDF1.2* expression products are highly involved in the cell early senescence to decrease biotrophy (39).

Co-expression of *PR-1* included predominate genes cadmium ion and cytokinin responsive such as *CHI*, and *PCR2*. Other gene products necessary for defense were phosphofructokinase and ADP/ATP carriers, farnesol dehydrogenase expression, 2-oxoglutarate-dependent dioxygenase and Pumilio (APUM) proteins containing PUF domain for protein transport. Production of farnesol dehydrogenase and PUM domain are not considered to have a rigorous role in host defense against a biotroph (40). Down regulated gene included thylakoidal ascorbate peroxidase, uracil phosphoribosyl transferase which degraded

chloroplast. This strengthened the common perception why photosynthetic activity sustained under biotrophy.

B. cinerea had also found to have role in ethylene signalling because of the expression of its defensive gene at an intermediate level. Up-regulated expression of ACS1 and its correlated PDF1.2 genes in the presence of *P. infestans* indicated that infection strategy of this pathogen is different from both biotroph and necrotroph. Similarly, the high expression of ACS1 and PDF1.2 under infiltrating *P. syringae* infection meant that ethylene signalling has significant role in overcoming the *P. syringae* virulence.

Expression of ABA responsive such as *BGL1*, *BAM5*, *JAR2* was strongly correlated with the *VSP2*. The perception developed from key up-regulated genes during *B. cinerea* infection, is the encoding of glycosyl hydrolase which degraded the cellulose, hemicellulose and even neuraminidases of certain viruses, but they becomes virulent for host in case of *B. cinerea* (41). Similarly, Beta amylase involved in cytosolic starch catabolism; cysteine lyase for generation of precursor of ethylene biosynthesis as ethylene causes senescence, favouring pathogenesis of *B. cinerea*. Other encoded products of up-regulated genes are for plant own defense strategy such as arginine amidohydrolase, polygalacturonase inhibiting protein, myrosinase-binding protein and phytosulfokinase which all were ABA responsive elements. The down-regulated products of genes, involving in virulence included cysteine synthase c1 for cyanide detoxification, cyclic nucleotide-gated channel 12 for regulation of membrane potential, and Phosphoinositide phosphatase family protein necessary for vacuolar organization. Ions transport was also down-regulated to the infected leaves such as nitrate transport, and nodulin MtN21-like transporter family protein for amino import and export across the membrane which thought to be involved in cell to cell signalling during defense (42).

Previously it was reported that *P. infestans* is a fungal hemibiotroph, therefore, *PDF1.2* co-expression was analyzed. This section further explored the role of *P. infestans* as incompatible pathogen due to the facts mentioned. Most of the genes co-expressed are ethylene responsive and cadmium ion as hevein-like protein, cytochrome p450 and UDP-glucosyl transferase. Transporter k+ involved in cell to cell defense system, glutamate-cysteine ligase for cell proliferation, and octadecanoid-responsive *Arabidopsis* AP2/ERF are all proteins for incompatible pathogen (43). The most striking defense system in case of this incompatible pathogen is methylation of cell membranes occur by o-mtase family 3 protein and transcription-coupled nucleotide-excision repair which causes any repair in DNA lesion during infection. The down-regulation of *PDF1.2* showed decrease in TAT protein binding for cell surface signalling; homeobox protein having a role in leucine zinc binding; and ATP synthesis coupled electron transport. Other processes scaffold host defense were also down-regulated at the same fashion such as cellulose synthase; RNA polymerase beta' subunit-1 for transcription of many viable proteins for the host.

Conclusion

Phyto-pathogens manipulate a wide range of phyto-signaling molecules in host plants to pave way to their virulence. Through System Biology approach we found that several aspects of the host-pathogen

interaction are still elusive. At the same time we found that under pathogen attack, modulation of IAA biosynthesis and transport decides fate of the combat between the defending host and attacking pathogen. Beside IAA, the pathogens also influence correlated defense responses at the expense of the host's growth and development. Based on the available data, we suggested infection maps, which depicted the overall strategy of different groups of the pathogens to engage the host plant.

Abbreviations

S. No.	Abbreviations	Full name
1	BAR	(Bio-Analytical Resource for Plant Biology)
2	<i>eFP</i>	(Electronic Fluorescent Profile)
3	IAA	Indole-3-acetic acid
4	GA	Gibberellins
5	CKx	Cytokinin
6	SA	Salicylic acid
7	JA	Jasmonic acid
8	PA	Polyamines
9	ET	Ethylene

Declarations

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

We confirm that all the data generated, and material used in this current study are available within this manuscript and its supplementary materials. Moreover, we included in-silico analyses in this study to link to our in-vitro lab experiments. The in-silico data were taken and analyzed using bioinformatics tool provided on the Bio-Analytic Resource for Plant Biology (BAR; <http://bar.utoronto.ca>).

Conflict of interest/competing interests

We have no competing interests to declare.

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Authors Contribution

MJY performed the in-silico analysis and wrote draft manuscript, AH conceptualize and supervised the study, MH and MI analyzed and compared the primary data with literature and drawn conclusions.

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Figures

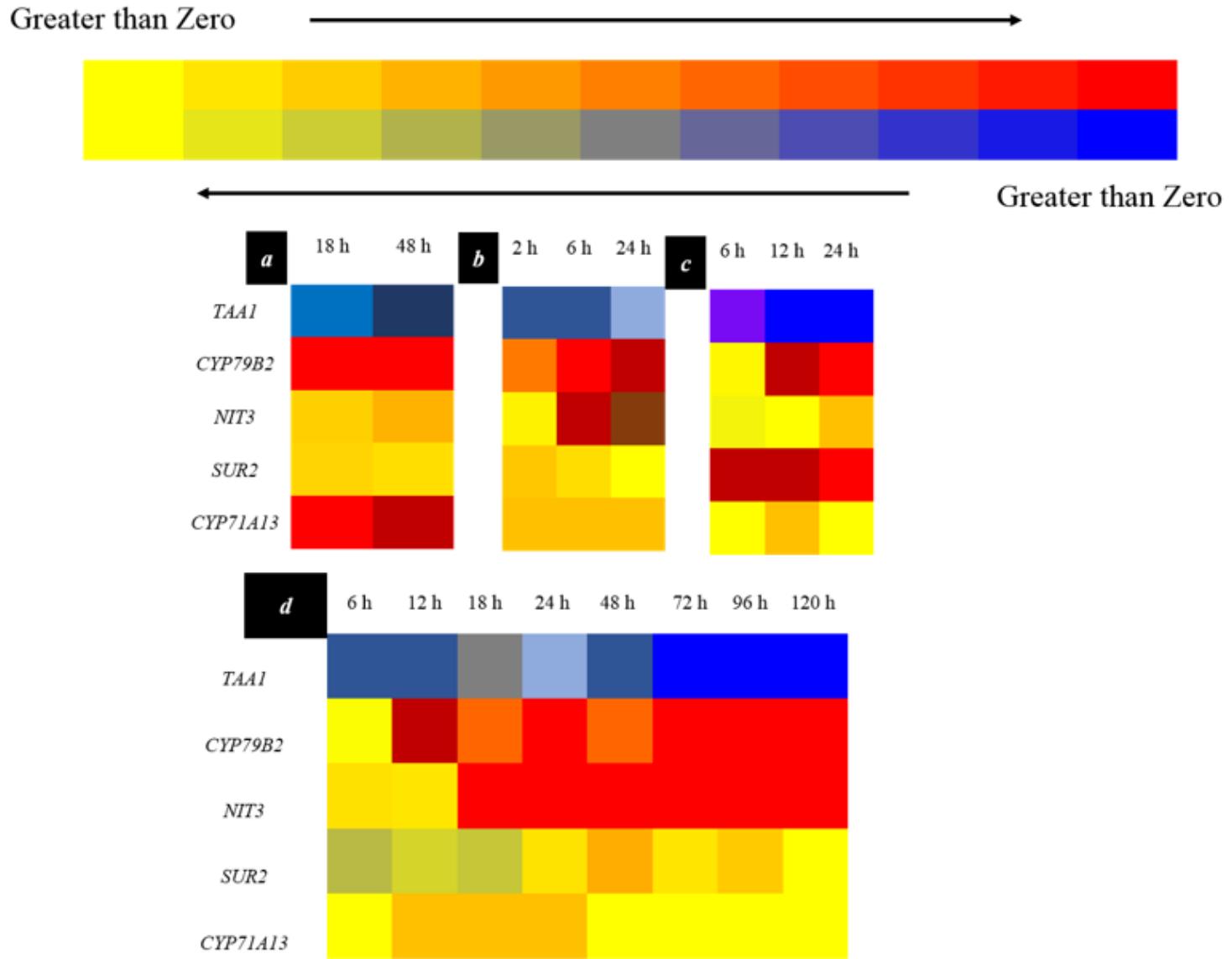


Figure 1

Expression of IAA biosynthesis genes extracted from eFP browser under pathogenic effect as a) *B. cinerea*, b) *P. syringae*, c) *P. infestans*, d) *E. orontii*. The scale indicates the level of gene expression as red shows high expression and blue shows negative expression whereas yellow indicates zero expression of gene.

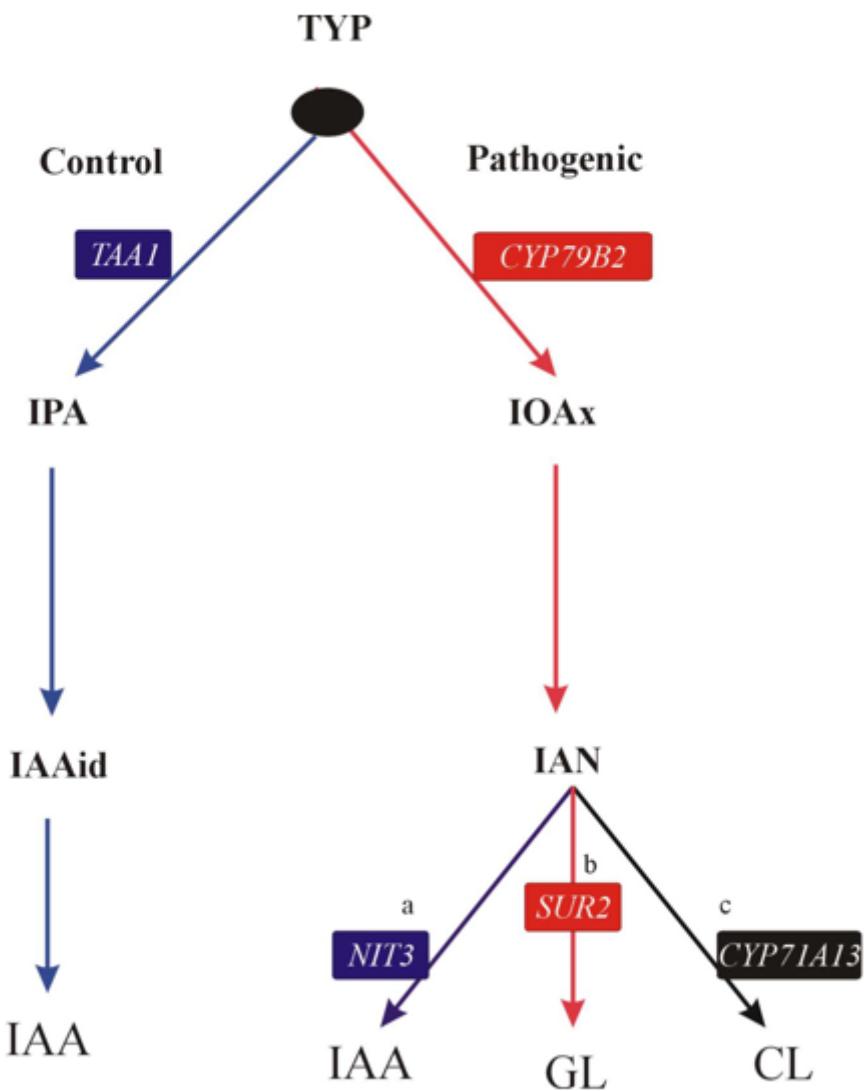


Figure 2

The pathway showed the pathogenic effect on IAA biosynthesis as 1 represents the pathway modulating in *A. thaliana* under the infection of *E. orontii* and virulent half leaf *P. syringae* while 2 is pathway in *A. thaliana* under the stress of *P. infestans* inoculation and 3 occurs in *A. thaliana* under *B. cinerea* and virulent infiltrating *P. syringae*. These pathways elucidated the picture of biotropic and necrotropic strategy on IAA biosynthesis.

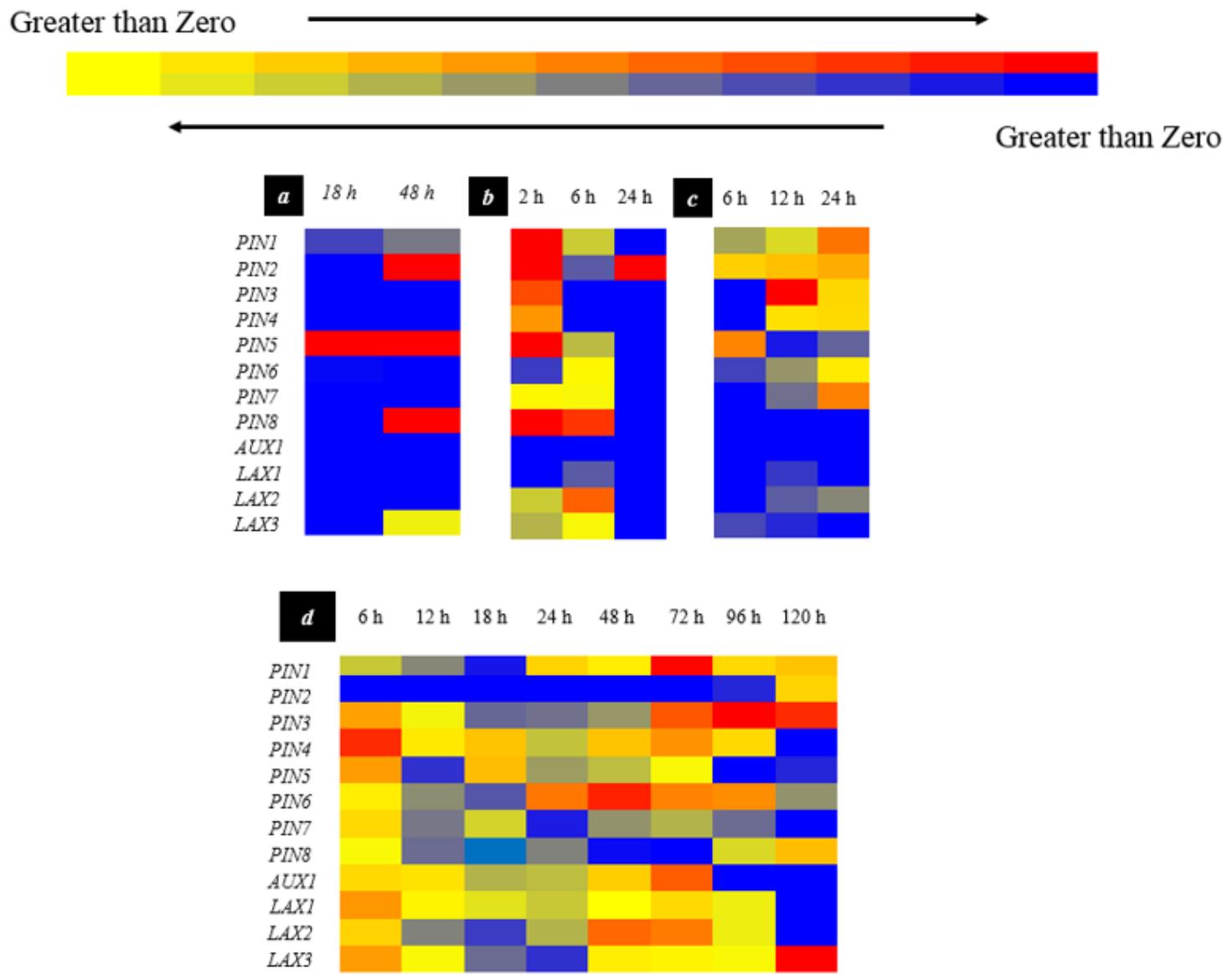


Figure 3

Gene expression of IAA transport genes for different pathway under the pathogenic effect (a for *B. cinerea*, b for *P. syringae*, c for *P. infestans* and d for *E. orontii*) on eFP online available browser. The scale indicates the level of gene expression as red shows high expression and blue shows negative expression whereas yellow indicates zero expression of gene.

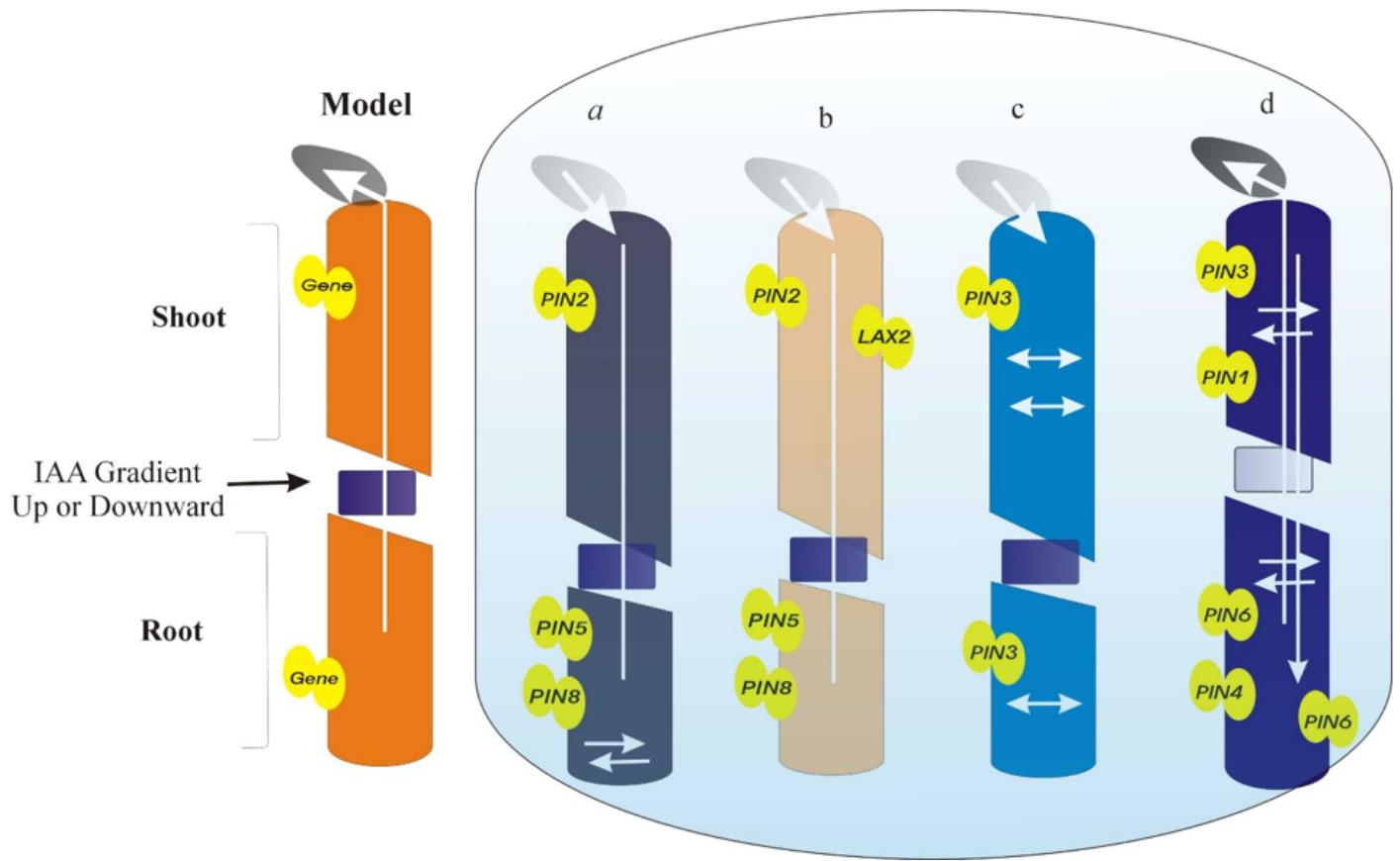


Figure 4

The model shows the pathogenic effect on IAA transport as (a) & (b) are under *B. cinerea* and *P. syringae* attack respectively, which is basipetal IAA transport, (c) is under *P. infestans* attack showing lateral IAA transport while (d) represents multi-directional IAA transport in *E. orontii* attack.

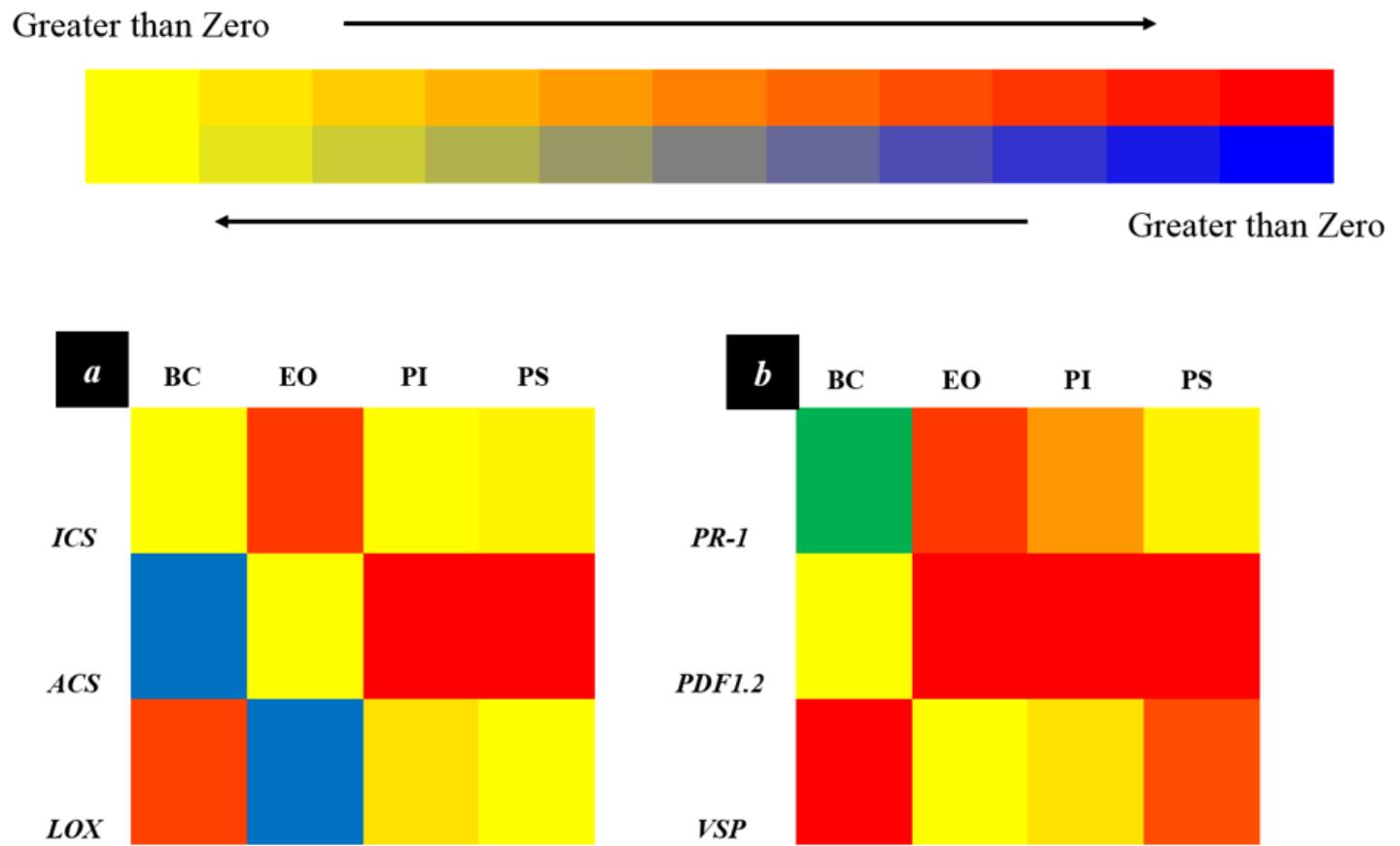


Figure 5

Gene expression of phyto-signaling molecules (a) and correlated defense genes (b) in *Arabidopsis thaliana* challenged with different phytopathogens including *B. cinerea* (BC), *P. syringae*, *P. infestans* (PI) and *E. orontii* on eFP online available browser. Expression is taken relative to mocked treated condition for each pathogen. The scale indicates the level of gene expression as red shows high expression and blue shows negative expression whereas yellow indicates zero expression of gene.

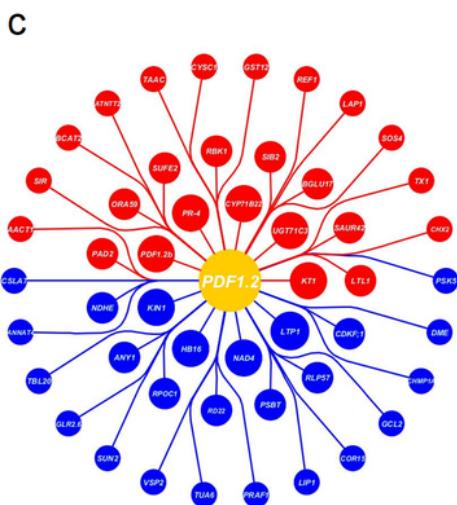
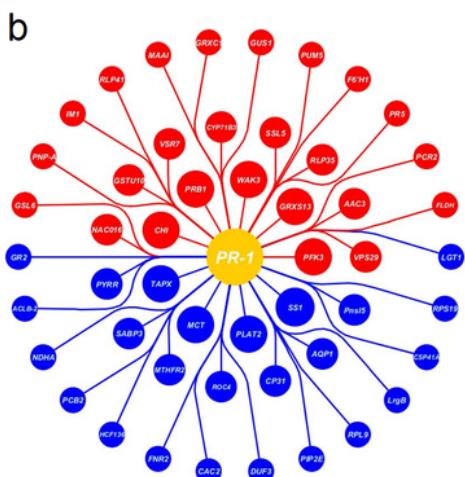
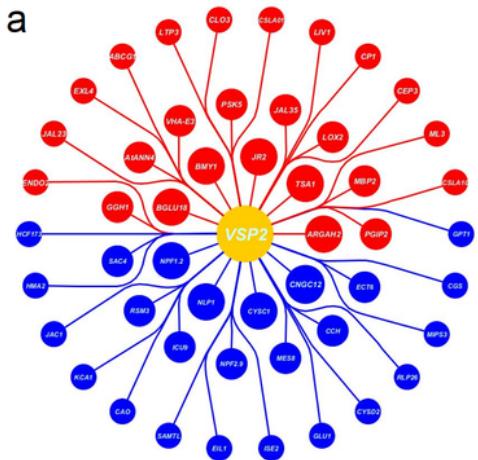


Figure 6

Three principle genes as VSP2 (a), PR-1 (b) PDF1.2 (c) involved in defense responses studied using Expression Angler 2016 tool with r-cut off range up to -0.23 to 0.99. The data so extracted from Expression Angler 2016 tool with desired r-cut off range were subjected to yED to produce co-expression networks where red balls show up-regulation and blue balls shows down-regulation with reference to the principle gene in yellow ball.

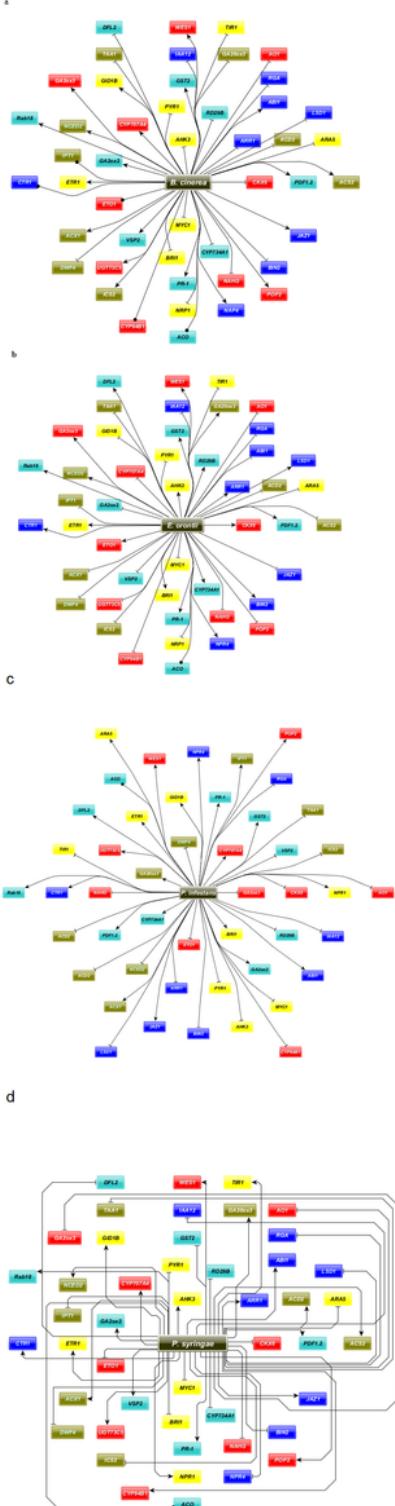
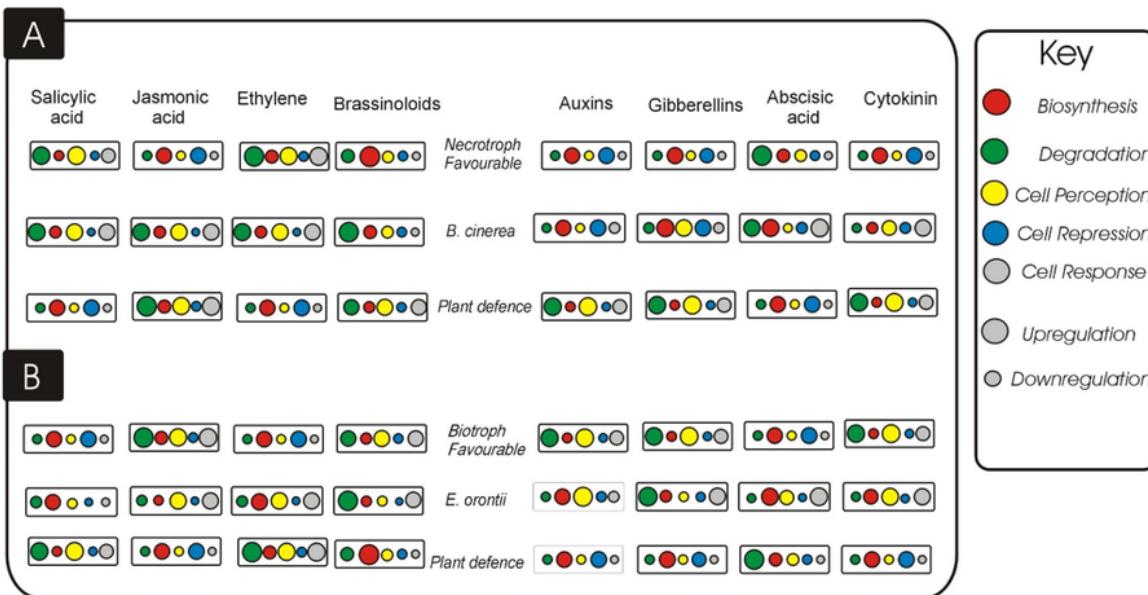


Figure 7

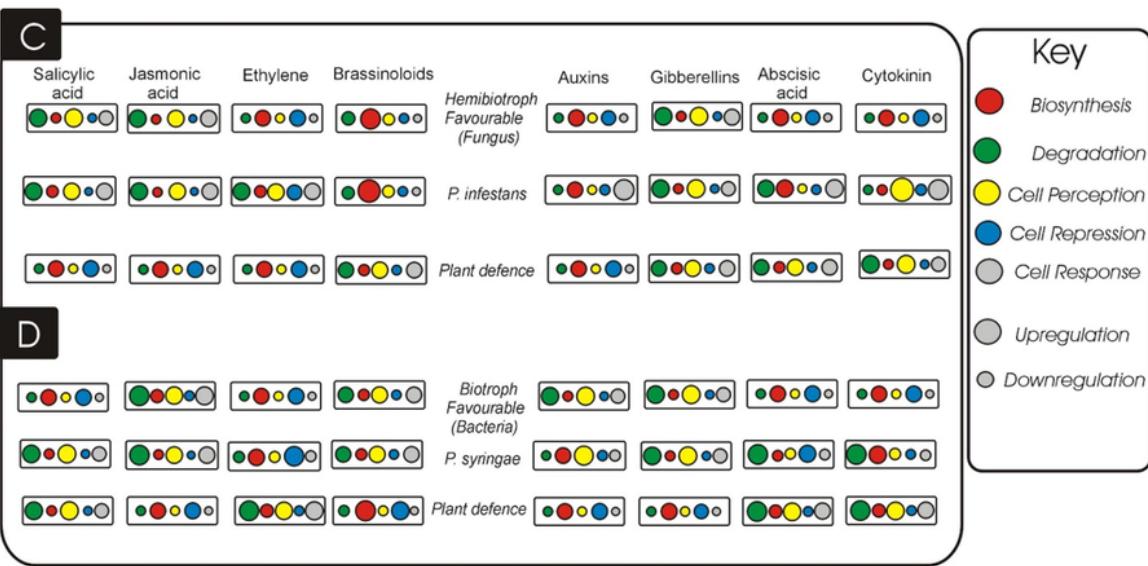
Gene expression network depicted on the basis of data taken from Expression Angler 2016. Red color shows phytohormone degradation gene expression, blue shows phytohormone signal repression, green shows biosynthesis, yellow shows signal perception and grey shows phytohormone response under the infection of a. *B. cinerea*, b. *E. orontii*, c. *P. syringae* and d. *P. infestans*.



Conclusion

B. cinerea virulence = 70 % Host defence = 30 %

E. orontii virulence = 58 % Host defence = 42 %



Conclusion

P. infestans virulence = 57 % Host defence = 43 %

P. syringae virulence = 68 % Host defence = 42 %

Figure 8

Manipulation of host (*A. thaliana*) phytohormones under necrotrophic, biotrophic and hemi-biotrophic pathogen attacks. General susceptible and resistant *Arabidopsis* phenotype under (A) necrotroph in comparison to *B. cinerea* (B) biotroph in comparison to *E. orientii*, (C) fungal hemi-biotroph in comparison to *P. infestans* and (D) bacterial hemi-biotroph in comparison to *P. syringae*