

Metabolomics and its role in plant pathology

La métabolomique et son rôle en phytopathologie

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Résumé de l'article

La métabolomique est l'une des sciences omiques les plus éminentes et les plus récentes. Elle constitue un outil puissant pour étudier les changements métaboliques qui se produisent dans un organisme. Les plantes produisent un large éventail de métabolites et l'étude de ces métabolites peut répondre à un certain nombre de questions que se posent les chercheurs. La modification des métabolites est le caractère déterminant dans une plante génétiquement modifiée ou dans les interactions de la plante avec les ravageurs, les pathogènes et l'environnement. Les interactions entre les plantes et les pathogènes sont parmi les mécanismes les plus complexes sur le plan biochimique et constituent un grand défi pour les phytopathologistes; la métabolomique joue non seulement un rôle important dans le décryptage de ces interactions complexes, mais l'étude de certains changements métaboliques liés à la défense peut être utilisée de plusieurs façons pour protéger la plante contre les pathogènes nuisibles. La science de la métabolomique utilise un certain nombre de techniques pour étudier une grande variété de métabolites. Cette revue donne un aperçu des différentes techniques utilisées en métabolomique et comment certaines de ces techniques ont été utilisées avec succès dans le domaine de la phytopathologie.

Metabolomics and its role in plant pathology

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Metabolomics is one of the most eminent and newly emerging omic sciences. It is a powerful tool to study metabolic changes that occur in an organism. Plants produce a wide range of metabolites and the study of these metabolites can answer a number of questions that arise in the minds of researchers. Change in the metabolites is the most important feature in a genetically modified plant or plant interactions with pests, pathogens and the environment. Plant pathogen interactions are amongst the most biochemically complex mechanisms and pose a great challenge in front of plant pathologists; metabolomics not only play a great role in deciphering these complex interactions but also the study of certain defence-related metabolic changes can be utilized in a number of ways to protect the plant from the harmful pathogens. The science of metabolomics utilizes a number of techniques to study the wide variety of metabolites. This review will give a brief about the various techniques used in metabolomics and how some of these techniques have been successfully utilized in the field of plant pathology.

Keywords: metabolites, metabolomics, pathogens, plants.

[La métabolomique et son rôle en phytopathologie]

La métabolomique est l'une des sciences omiques les plus éminentes et les plus récentes. Elle constitue un outil puissant pour étudier les changements métaboliques qui se produisent dans un organisme. Les plantes produisent un large éventail de métabolites et l'étude de ces métabolites peut répondre à un certain nombre de questions que se posent les chercheurs. La modification des métabolites est le caractère déterminant dans une plante génétiquement modifiée ou dans les interactions de la plante avec les ravageurs, les pathogènes et l'environnement. Les interactions entre les plantes et les pathogènes sont parmi les mécanismes les plus complexes sur le plan biochimique et constituent un grand défi pour les phytopathologistes; la métabolomique joue non seulement un rôle important dans le décryptage de ces interactions complexes, mais l'étude de certains changements métaboliques liés à la défense peut être utilisée de plusieurs façons pour protéger la plante contre les pathogènes nuisibles. La science de la métabolomique utilise un certain nombre de techniques pour étudier une grande variété de métabolites. Cette revue donne un aperçu des différentes techniques utilisées en métabolomique et comment certaines de ces techniques ont été utilisées avec succès dans le domaine de la phytopathologie.

Mots-clés : métabolites, métabolomique, pathogènes, plantes.

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INTRODUCTION

Plant pathogen interactions are highly complex in nature, as pathogens and plants try to counter each other. Plants in their defence to pathogens produce a wide range of secondary metabolites. Plants carry out a web of different bio-synthetic pathways for the production of these metabolites. Since metabolites are the final products of all the cellular processes, the levels of these metabolites can give an idea regarding the ultimate response of biological systems to any kind of genetic or environmental changes (Fiehn 2002). Metabolomics is a newly emerging omic science which facilitates us to do the quantitative and qualitative analysis of these metabolites. This can help us to get acquainted with the actual biological interactions taking place in a particular biological system. Metabolites are highly dynamic that is they keep on changing with time and space and the versatility of these metabolites in terms of structure and function makes their analysis a bit difficult task (Stitt and Fernie 2003). Study of the complete metabolome through a single technique is impossible as all the metabolites differ in certain properties, therefore various metabolomic techniques are utilized like mass spectrometry (MS) based methods including GC-MS (gas chromatography-MS), LC-MS (liquid chromatography-MS), CE-MS (capillary electrophoresis-MS), and FI-ICR-MS (Fourier transform ion cyclotron resonance-MS), and non-destructive NMR (nuclear magnetic resonance spectroscopy) (Khakimov *et al.* 2014; Okazaki and Saito 2012). Looking into the complexities of the study, this review aims at providing a brief about the different techniques that can be used to study the metabolic changes that occur during plant pathogen interactions, also will focus on some examples of how these techniques are being utilized by the researchers in deciphering the changes in metabolic profile of plants.

METABOLOMICS

Omic sciences are gaining a lot of popularity nowadays and the advancements in the technologies and informatics used to generate and process large sets of biological data are promoting a critical shift in the study of biological sciences. Omic sciences mainly aim at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological sample in a non-targeted and unbiased manner (Horgan and Kenny 2011). The integration of all these techniques can more appropriately be termed as “system biology”. Omic science not only aids us in better understanding of normal physiological processes but also can be efficiently utilized to study the disease processes through screening, diagnosis and prognosis as well as to perceive the etiology of diseases (Horgan and Kenny 2011). Metabolomics is a branch of omic sciences and it can be defined as the study of global metabolite profiles in a system (cell, tissue or organism) under a given set of conditions (Goodacre *et al.* 2004). Metabolomics is said to have many theoretical advantages over the other omic technologies. The metabolome is considered as the final output of transcription of a gene and, thus, metabolic changes are amplified compared to the changes in the transcriptome and the proteome (Urbanczyk *et al.* 2003). Moreover as the downstream product, the metabolome is more closely associated to the phenotype of the biological system (Burt and Nandal 2016). Though amongst all the omic sciences metabolome forms the smallest domain (~5000 metabolites), but due to the presence of a wide variety of metabolites it is physically and chemically more complex compared to other omics.

METABOLIC CHANGES DURING PLANT PATHOGEN INTERACTIONS

Plants unlike animals do not have an immune system but it's not that they cannot defend themselves against the attack of harmful organisms or adverse environmental conditions. They utilize a unique network of metabolic processes which leads to the production of certain miraculous compounds (plant defence proteins and enzymes): secondary metabolites. These secondary metabolites are not directly involved in growth, development and reproduction of an organism (as are primary metabolites) but are known to play certain other specific roles (Agostini-Costa *et al.* 2012). The huge plant community is known to produce more than 100 000 secondary metabolites which are categorized under certain taxonomic groups (Oksman-Caldentey and Inzé 2004). They can be either preformed metabolites also referred as phytoanticipins which get converted into toxic molecules upon pathogen perception or phytoalexins that are produced after the perception of pathogens (Arbona and Gómez-Cadenas 2016). As in mulberry plant infection with fungal pathogens *Fusarium solani* or *Stigmella mori* led to the identification of a diphenylpropane derivative named morusin which on accumulation is inhibitory to fungal and bacterial growth (Gottstein and Gross 1992).

These secondary metabolites are produced by different synthetic pathways and on the basis of that are broadly classified into three: terpenes, phenolic compounds and nitrogen-containing compounds (Fang *et al.* 2011). The study of these metabolites can be utilized as a tool to decipher plant pathogen interaction and also to differentiate the type of compounds formed in resistant and susceptible reactions. Pushpa *et al.* (2014) conducted a non-targeted metabolic study of resistant and susceptible potato cultivars (F06037 and Shepody respectively) against *Phytophthora infestans* (US-8 genotype) using LC-MS. It was observed that hydroxycinnamic acid amides (HCAAs) of the shunt phenylpropanoid pathway are increased in resistant cultivars after inoculation with pathogens. Deposition of HCAAs in the host cell wall inhibits pathogen colonization. Various flavonoids have been extensively studied in plant defence: pisatin in pea (Perrin and Bottomley 1961), medicarpin in alfalfa (He and Dixon 2000), glyceollins in soybean (Ebel *et al.* 1976) and many others. They are not only antifungal but also antibacterial thus providing resistance against a wide range of pathogens (Piasecka *et al.* 2015). Plant metabolites are not only altered by fungal and bacterial pathogens but viral diseases also interfere with plant metabolism. In order to facilitate its own dispersal Cucumber Mosaic Virus (CMV) leads to emission of certain volatile compounds from the infected squash to attract the aphid vectors (Mauck *et al.* 2014). Similarly, tobacco which is a relatively poor host for *Bemisia tabaci* (Gennadius) on infection with Tomato yellow leaf curl virus (TYLCV) shows some alteration in synthesis of volatile terpenoid and increases host suitability (Luan *et al.* 2013). All these alterations in and through metabolites during plant pathogen interactions increase our curiosity on what the particular compound is involved and what are the techniques that we can use to identify them, so the basic steps for the study of metabolites are as follows.

BASIC STEPS IN METABOLOMICS

(Courant *et al.* 2014)

Sample preparation

Metabolites differ in their physicochemical properties thus sample collection and preparation will depend on the kind of metabolite to be studied and the method used for processing and detecting those metabolites. In some cases preliminary quenching is needed to stabilize the sample which stops the metabolic reactions (Álvarez-Sánchez *et al.* 2010). It is recommended to store sample at -80 °C and sample aliquoting should be done at the time of collection to avoid repetitive freezing and thawing of samples (Dunn *et al.* 2011).

Metabolomic profiles generation

Due to great diversity in the metabolites there is no single technique for the separation and quantification for all the metabolites. A number of techniques are used for metabolites with different properties. GC-MS is mostly used for volatile compounds while analysis of polar or ionic metabolites may be achieved with LC-MS. CE-MS can also be used for polar or ionic compounds (Barbas *et al.* 2011).

Data processing

Metabolomic studies produce a huge amount of raw data. It is really difficult to handle such complex datasets manually and thus specific software tools and algorithms are required to convert this complex raw data to comprehensive extracted data that can easily be processed using statistical tools (Courant *et al.* 2014).

Data analysis

Metabolomics generate high dimensionality data. In metabolomic fingerprinting, the number of variables measured per subject vastly exceeds the number of subjects to be considered under that study (Guo *et al.* 2010). Univariate methods such as the classical Student's t-test can be used to identify candidate compounds showing significant differences in levels between two subgroups of samples. Multivariate techniques are also used to reduce the complexity of the datasets and to derive the analytical information of biological importance (Brown *et al.* 2005; Trygg *et al.* 2007).

TECHNIQUES USED IN METABOLOMICS

Metabolomics deal with two types of analysis: targeted and non-targeted analysis of both endogenous as well as exogenous metabolites (< 1500 Da). Metabolites may include peptides, amino acids, nucleic acids, organic acids, carbohydrates, vitamins, alkaloids, polyphenols and other inorganic compounds (Sumner *et al.* 2003). These metabolites can also act as biomarkers (Arakaki *et al.* 2008). Metabolomics has been applied to define metabolites related to prognosis or diagnosis of diseases and could provide greater pathophysiological understanding of disease (Zhang *et al.* 2012). Metabolome being a very complex entity cannot be analyzed by a single analytical approach thus a combination of various novel techniques such as Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC), Capillary Electrophoresis (CE) is needed for the separation of the metabolites based on different properties of the compounds (Hegeman 2010). These

separation techniques need to be coupled to detection techniques such as Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy. Here is a brief description about the principle on which these different techniques are based and their application in the field of plant pathology:

Liquid Chromatography Mass Spectrometry (LC-MS)

The advance form of liquid chromatography includes HPLC and UPLC. In HPLC and UPLC, liquid is the mobile phase. Both the technique work on the same principle that is the molecules passing through the stationary phase moves in different speeds depending on their chemical structure. Though complex biological mixtures cannot be separated through LC columns (Tolstikov and Fiehn 2002) but it allows good mass library reproducibility (Huhman and Sumner 2002). HPLC separations are widely used for analysis of labile and non-volatile polar and non-polar compounds in their native form. UPLC utilizes porous particles with diameters smaller than 2 mm leading to increased surface area and thus provide a better separation. UPLC has certain advantages over HPLC as it allows analysis of more number of samples in a particular time, has high efficacy and peak capacity compared to that of HPLC columns (Zhang *et al.* 2012).

Alterations in the metabolites of three South African sorghum cultivars; namely Amazi Mhlophe, NS 5511 (bitter abbreviated B) and NS 5655 (sweet, abbreviated S) responding to *Colletotrichum sublineolum*, were investigated by Tugizimana *et al.* (2019) using LC-MS based untargeted metabolomics and accumulation of a wide range of antifungal phenolic compounds were observed, particularly apigeninidin, luteolinidin, 3-deoxyanthocynidin phytoalexins and other related conjugates were detected. Yogendra *et al.* (2014) did a non-targeted metabolomic study of the resistant related metabolites by comparing metabolomics of potato late blight resistant (AC04 and AC09) and susceptible potato genotypes (Criolla Colombia). Through LC-MS, it was observed that defence-related compounds such as flavonoids phenylpropanoids and alkaloid chemical groups were highly induced in resistant genotypes compared to susceptible ones. Deposition of HCAs, alkaloids and amides flavonoids leads to the thickening of the cell wall leading to resistance against pathogen penetration.

Chalcone synthase is a key enzyme of flavonoid synthesis pathway which leads to accumulation of cell wall-bound phenolic compounds resulting in resistance in *Arabidopsis* plants against bacterial pathogen *Pseudomonas syringae* (Soylu 2006).

Chen *et al.* (2018) conducted untargeted metabolomics of *Arabidopsis* through LC-MS. The focus of the study was to observe the role of metabolite N-hydroxy-pipecolic acid in inducing systemic disease resistance in *Arabidopsis* against bacterial pathogen, *P. syringae* pathovar *tomato*. The study demonstrated that Flavin-Dependent Monooxygenase 1 (FMO1), which plays a key role in inducing systemic acquired resistance (SAR), can synthesize N-OH-Pip from pipecolic acid present in plant, and *fmo1* mutants when applied exogenously with N-OH-Pip move systemically in *Arabidopsis* plant and can overcome the SAR-deficiency of the mutants. Several plant metabolites including Pipecolic acid (Pip) (Návarová *et al.* 2012) are reported to be involved in signal amplification and long-distance communication during SAR (Dempsey and Klessig 2012; Shah and Zeier 2013; Shah *et al.* 2014).

Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS is mainly used for volatile compounds which vaporize without decomposition. Mobile phase is mainly an inert or unreactive gas such as nitrogen or helium. Mostly helium gas is used as carrier gas. Compounds which are volatile and thermally stable such as short chain alcohols, acid, esters, and hydrocarbons are mainly analyzed using this technique.

GC can also be used for analysis of many other compounds only after following derivatization which includes alkylation and silylation (Begley *et al.* 2009; Broeckling *et al.* 2005). Polar compounds like carbohydrates, carboxylic acids and free amino acids can be analyzed using GC-MS as derivatives of methoxime/trimethylsilyl (Roessner *et al.* 2001) whereas no further processing is needed for volatiles (Beck *et al.* 2014). The sample metabolites differ in their retention times and thus can be automatically identified by comparing and matching the retention time from mass spectra of chromatograms through pure chemical standards (Gross 2004).

Studies of plant metabolites can also help to differentiate pathogenic infections and thus may be a great tool for detection and diagnosis. A study was conducted by Lui *et al.* (2005) to discriminate three fungal diseases of potato through metabolite profiling using GC-MS. Potato tubers were inoculated with three pathogens: *P. infestans*, *Botrytis cinerea* and *Pythium ultimum*. Tubers inoculated with *Botrytis* produced two specific volatiles: 2-2-propenyl-1,3-dioxolane and 3, 5-heptadiyn-2-one and those inoculated with *Pythium* produced three metabolites: 2-butanone, 2-methyl-1-butanol and 2-methyl-2-butanal. However ethoxyethene was observed in tubers inoculated with *Phytophthora* inoculated tubers.

Several plant defence compounds have been analyzed using GC-MS techniques; various phenolic compounds were mainly found in potato (Wagner *et al.* 2003; Yang and Bernards 2007), soybean leaves (Benkeblia *et al.* 2007), maize (Röhlig *et al.* 2009) and tobacco (Dauwe *et al.* 2007).

Capillary Electrophoresis Mass Spectrometry (CE-MS)

In capillary electrophoresis, analytes are separated on the basis of their ionic mobility or partitioning into an alternate phase through non-covalent interactions. CE-MS is an effective promising separation technique providing high-analyte resolution, for charged metabolites and provides information mainly on polar or ionic compounds (Barbas *et al.* 2011). It applies separations with high resolutions, sensitive mass determination and specific chemical standards to identify and quantify thousands of metabolites on the basis of their charge over both negative and positive ionization modes (Sato *et al.* 2004). It is really applicable for the analysis of water-soluble polar metabolites.

Several metabolites, the phenylpropanoid and shikimate pathways, show rise in their levels after *Rhizoctonia solani* infection (Mutuku and Nose 2012). Plant defence mechanism involves a number of pathways and phenylpropanoid and shikimate pathways form an important part of plant defence (Dixon *et al.* 2002; Tzin and Galili 2010) and they are involved in the synthesis of various secondary metabolites including phenol compounds (Lattanzio *et al.* 2006). Synthesis of phenols plays a key role in providing resistance against infection caused by *R. solani* (Akhtar *et al.* 2011).

Suharti *et al.* (2016) studied the difference in levels of metabolites in *R. solani* infected resistant and susceptible lines of rice (32R and 29S, respectively) through capillary electrophoresis equipped with time of flight mass spectrophotometry (CE/TOF-MS) in positive ion mode. Chlorogenic acid metabolite showed a positive response in 32R and amino acids which showed increase in 29S after inoculation with *R. solani* were: γ -aminobutyric acid, glutamate, glycine, phenylalanine, histidine, tryptophan, tyrosine and serine. Several compounds produced in plants are plant defence response regulators. Mucha *et al.* (2019) did a CE study of systemin (an eighteen amino acid peptide plant hormone) which plays an important role in regulating plant defence response. Systemin peptide was injected into the leaves and stem of tomato plant and its transportation in the plant tissues was traced by CE. Systemin is a signalling compound which plays an important role in systemic defence; about 20 defensive genes (like proteinase inhibitors or polyphenol oxidase genes) are activated and regulated by systemin (Pearce *et al.* 1991; Ryan 2000).

Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI)

The technique involves use of matrix-assisted laser desorption ionization as a mass spectrometry imaging technique in which the sample (tissue section) is moved in two dimensions while the mass spectrum is recorded (Chaurand *et al.* 2006). This method has an advantage as it measures the distribution of several analytes at one time without destroying the sample. This technique provides a means to study the plant pathogen interaction and to discover potential markers of infection. It utilizes a matrix, typically a small organic acid with strong ultraviolet absorbance, mixed with analytes to aid desorption and ionization (Norris and Caprioli 2013). The resulting gas phase analyte ions are detected and displayed in a spectrum according to their mass-to-charge ratios (m/z), which yield specific molecular signatures within complex samples. This label-free technology can be used without *a priori* knowledge of sample composition, allowing for the detection of a variety of analytes, from small molecules to large proteins (Angel and Caprioli 2013).

The technique is a gift of modernization in technology, and can be a great advantage in studying plant pathogen interactions. The technique was successfully utilized by Becker *et al.* (2014) to study the compounds produced by grapevine as a defence response against *Plasmopara viticola*. It was observed from the study that other than resveratrol, more toxic compounds such as pterostilbene and viniferins are produced as defence compounds. The technique plays an important role of detection of pathogenic microbes in soil (Siricord and O'Brien 2008).

Ion exchange chromatography

Ion exchange chromatography is an important technique for separation of ionic compounds. It is mainly of two types: anion exchange and cation exchange. In this technique, analyte molecules are retained on the column through ionic interactions. The ion exchange chromatography matrix consists of positively and negatively charged ions.

Elicitors are known to induce plant defence responses, such as cell wall strengthening, ethylene biosynthesis, reactive oxygen species, induction of hypersensitive response proteins, and expression of pathogenesis-related (Miyata *et al.* 2006; B. Wang *et al.* 2012; J.Y. Wang *et al.* 2004). Some beneficial bacteria, such as the plant growth-promoting rhizobacteria

(PGPR), have the tendency to reduce the activity of pathogenic microorganisms by microbial antagonism through competing for nutrients, secretion of lytic enzymes and production of antibiotics (Handelsman and Stabb 1996; van Loon and Bakker 2003; van Loon and Glick 2004). Furthermore, they can indirectly prevent plant from pathogens by eliciting the plant defence system (Haas and D  fago 2005).

Protein elicitors from some biocontrol strains have been reported to induce disease resistance, such as fengycins and surfactins from *Bacillus subtilis* (Ongena *et al.* 2007). Shen *et al.* (2019) reported novel protein elicitor (AMEP412) from *B. subtilis* BU412 which leads to hypersensitive response (HR) and SAR in tobacco. Ion-exchange and size exclusion chromatography were used for purification. The study revealed that AMEP412 can trigger a series of defence mechanisms such as the generation of reactive oxygen species. It also induced defence enzymes, including phenylalanine ammonia-lyase, polyphenol oxidase, peroxidase and superoxide dismutase. AMEP412 could stimulate plant systemic resistance against *P. syringae* pv. *tomato* DC3000.

N. Wang *et al.* (2016) reported a novel protein elicitor from *Bacillus amyloliquefaciens* NC6 which was responsible for induction of systemic resistance in tobacco. The elicitor led to the HR necrosis in leaves of tobacco plant. Systemic resistance was observed against a broad range of pathogens, including tobacco mosaic virus (TMV) and *B. cinerea*. The elicitor up regulated many genes including the phenylalanine ammonia lyase (*PAL*), salicylic acid (*SA*)-responsive *PR1a*, *PR1b*, *PR5*, Coronatine insensitive 1 (*COI1*) and jasmonic acid (*JA*)-responsive *PDF1.2* which play a key role in plant defence.

Certain fungi produce some antifungal compounds which can be utilized in a number of ways. The first filamentous fungus to potentially produce an antifungal peptide (AFP) was *Aspergillus giganteus* (Olson and Goerner 1965; Wnendt *et al.* 1994). Rao *et al.* (2015) identified an antifungal protein, AfAFPR9, and purified it from supernatant of culture of *Aspergillus fumigatus* R9. AfAFPR9 showed antifungal effect against plant pathogenic fungi *Fusarium oxysporum*, *Alternaria longipes*, *Colletotrichum gloeosporioides*, *Paecilomyces variotii*, and *Trichoderma viride* at minimum inhibitory concentrations of 0.6, 0.6, 1.2, 1.2, and 2.4 $\mu\text{g disc}^{-1}$, respectively.

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR is a new and non-destructive technique that requires minimal amount of sample preparation and has a high throughput (can process hundreds of samples per day). The principle of NMR utilizes compounds with odd atomic mass numbered nuclei, which tend to act like magnets in a provided external magnetic field through a process known as nuclear spin (Hatada and Kitayama 2004).

Plant pathogens cause metabolic perturbations in plants which can be studied through metabolite profiling of infected plants to gain insights into plant disease response (Abdel-Farid *et al.* 2009; Brechenmacher *et al.* 2010; Choi *et al.* 2004; Dai *et al.* 2010; Krishnan *et al.* 2005; Skirycz *et al.* 2010; Ward *et al.* 2011). Bednarek *et al.* (2005) used wild-type and mutant root cultures of *Arabidopsis thaliana* infected by root rot pathogen *Pythium sylvaticum* and observed how aromatic metabolite profiles differ. 1H-NMR was used to study one heterocyclic, sixteen indolic and three phenylpropanoid compounds. The study revealed a relative increase in levels of indolics upon infection. It also concluded that nature and quantity of phenylpropanoid metabolites differ in roots and leaves. Lima *et al.* (2010) studied the metabolic differences in the healthy and esca disease infected *Vitis vinifera* plants. 1D

and 2D 1H-NMR was used for evaluation and it was observed that various phenolic compounds, methanol, alanine, and γ -aminobutyric acid content were increased in diseased plants which may be due to plant defence activation.

Hong *et al.* (2012) studied the metabolic changes occurring in grapes infected by *B. cinerea*. Metabolite profiling was done using H-NMR and multivariate statistical analysis of berries from botrytized and healthy bunches. It was observed that phenylpropanoids, flavonoid compounds, glycerol, succinate and gluconic acid, all were directly associated with *B. cinerea* growth, and were only detected in *Botrytis* infected berries.

The Table 1 explains how variably these metabolomic techniques have helped us to study the plant pathogen interactions. In case of plant disease complex, they can be utilized to differentiate the diseases, the detection of some important compounds which are produced in the defence mechanism by plants is made easier, also how resistant and susceptible varieties are different can be detected. Studying the soil microbes is difficult but the techniques make it possible for us to identify the microbes that are involved in disease causation in plants. Thus, all these techniques can be considered as the gift of modern technologies to mankind. Metabolomic tools are now being utilized in the field of plant pathology as detectives to find out how does beneficial microbes instigate the defence mechanism in plants, how does the microbiome present in its periphery plays a role in favour or in against the occurrence of a disease, how does certain chemical formulations when applied to plants lead to biochemical changes in the host plant to defend against pathogens, how does a pathogen has an audacity to breach the plant defence mechanisms, how can improved soil health leads to a microbiota which strengthens the plants against pathogens, what are the chemical talks between the microbes inside the plant system or on the surface that greatly decide the fate of the plants.

CONCLUSION AND FUTURE PROSPECTS

Metabolomics has greatly helped in exploring various aspects in the field of plant pathology. It has covered a broad range right from exploring certain anti-pathogenic compounds to understanding the plant pathogen interactions and to decipher the plant defence responses against pathogens. It enables us to know novel insights of genetic, biochemical and metabolic network of cellular function (Tugizimana *et al.* 2013). It has also played a role in exploring various compounds from other beneficial microbes to induce resistance in plants against a wide range of pathogens. Since metabolites are more relevant to the plant phenotype advancements in their studies will be very helpful (Niederbacher *et al.* 2015). Different techniques have been developed to enhance the efficiency of identification and quantification of metabolites with large variation in their properties. Since metabolites have a very wide range out of which a few have been explored and there are many more to be explored to answer every new question that arrives in our minds regarding any kind of change occurring in an individual in response to certain stimuli. Development of more comprehensive data analysis software for better interpretation of metabolic studies will make it easier to draw conclusions. Developing a database or metabolite libraries can be of great use for comparing the different compounds which we obtain from plants. Though there are a number of libraries available but strengthening them would be beneficial. A combination of the knowledge in various disciplines can make it easier for us to find the solutions of many unanswered questions.

Table 1. List of some metabolomic techniques and their use in studying plant pathogen interaction

Metabolomic technique used	Study	Reference
Capillary Electrophoresis Mass Spectrometry (CE-MS)	Study of systemin (an eighteen amino acid peptide plant hormone) which plays an important role in regulating plant defence response.	Mucha <i>et al.</i> (2019)
	Difference in levels of metabolites in <i>R. solani</i> infected resistant and susceptible lines of rice.	Suharti <i>et al.</i> (2016)
Gas Chromatography Mass Spectrometry (GC-MS)	Differentiation of selected <i>citrus</i> varieties with different sensitivity to citrus huanglongbing.	Cevallos-Cevallos <i>et al.</i> (2012)
	Discriminate three fungal diseases of potato through metabolite profiling.	Lui <i>et al.</i> (2005)
High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS) and Nuclear Magnetic Resonance Spectroscopy (NMR)	Involvement of acetosyringone in plant-pathogen recognition.	Baker <i>et al.</i> (2005)
High Performance Liquid Chromatography-UV	Increase in hesperidin concentration in <i>Citrus sinensis</i> grafted on <i>C. limonia</i> after <i>Xylella fastidiosa</i> infection.	Soares <i>et al.</i> (2015)
Ion exchange chromatography	<i>Stagonospora nodorum</i> -wheat pathosystem involves multiple proteinaceous host-selective toxins.	Friesen <i>et al.</i> (2007)
	Two phenylalanine ammonia lyase isoforms are involved in the elicitor-induced response of rice to the fungal pathogen <i>Magnaporthe oryzae</i> .	Giberti <i>et al.</i> (2012)
	Reported novel protein elicitor (AMEP412) from <i>B. subtilis</i> BU412 which lead to hypersensitive response (HR) and systemic acquired resistance (SAR) in tobacco.	Shen <i>et al.</i> (2019)
Liquid Chromatography Mass Spectrophotometry (LC-MS)	Non-targeted metabolic study of resistant and susceptible potato cultivars against <i>P. infestans</i> .	Pushpa <i>et al.</i> (2014)
MALDI mass spectrometry	To investigate the in-situ response of grapevine leaves infected by <i>P. viticola</i> .	Becker <i>et al.</i> (2014)
	MALDI-TOF mass spectrometry can be used for detection of pathogenic microorganisms in soil.	Siricord and O'Brien (2008)
Nuclear Magnetic Resonance Spectroscopy (NMR)	Metabolic changes occurring in grapes infected by <i>B. cinerea</i> .	Hong <i>et al.</i> (2012)
	Metabolic response of tomato leaves upon different plant-pathogen interactions.	López-Gresa <i>et al.</i> (2010)

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