Review Article

Basic Concepts of High throughput Metabolomics in Plants

Abstract-

Each cell contains many different metabolite and chemical molecule which are generated during cellular process. All the metabolites present in a cell at a particular time is called metabolome. The study of all the metabolome and the way they are altered in a particular condition is called metabolomics. Metabolome is closely linked with genotype, physiology and environment. So,in a nutshell, metabolomics is the study of substrates and products of metabolism which are influenced by the genetic and environmental factors. In plants, metabolomics has now been frequently developed and studied in biotic and abiotic stress resistance stress resistance studies. High throughput metabolomics deals with all the techniques which are time efficient and can produce effective metabolite profiling. These techniques are chromatography based and chromatography free methods. Chromatographic methods are NMR, GC-MS and LC-MS . Chromatographv free techniques include DI-MS,FI-MS,MALDI and Ambient MS . This paper will give an idea about how metabolomics work in elucidating plant expression, how sample is prepared for metabolite profiling, different techniques of metabolite profiling and various metabolomic databases.

Keywords-Metabolomics, plants, chromatography, metabolite profiling, metabolomic databases

Introduction-

The "metabolome" refers to a plant's or any other organism's whole complement of tiny molecules. It denotes the final phenotype of cells, as determined by gene expression changes and protein function modifications. These, in turn, are influenced by environmental stimuli and genetic mutation (Saito and Matsuda, 2010). The metabolome of a single plant is therefore a highly dynamic component that is constrained both temporally (developmental, seasonal, etc.) and spatially (location, cultivation circumstances, etc). (Fiehn, 2002). Furthermore, the metabolome may be further spatially characterised within a single plant as organs, tissues, and even individual cells with easily distinct chemical profiles (Ebert et al., 2010; Sumner et al., 2011).

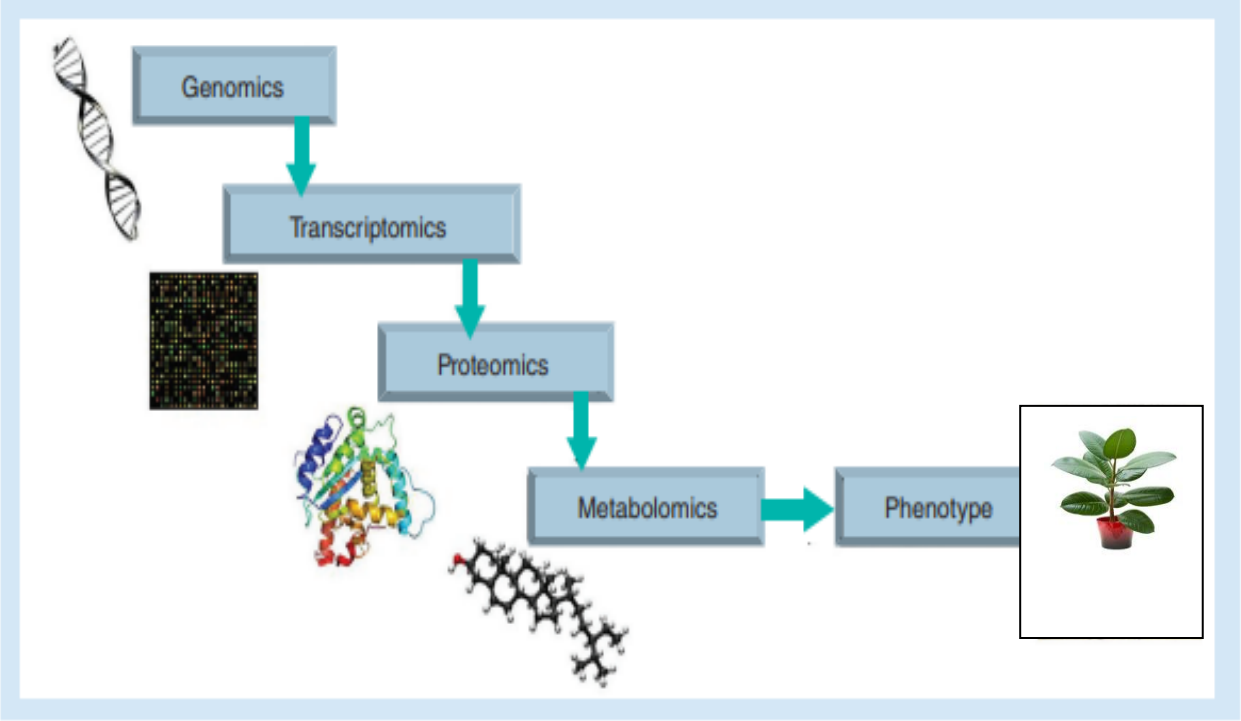


Figure 1- The Cascade of Omics

Plants are thought to be nature's most prolific biochemists (Hall, 2006), and hence constitute vast natural chemical libraries that have been inadequately mined and used to yet (Saito and Matsuda, 2010). There are many plant species which has never been studied for

biochemical insight and even if biochemical studies has been done then those biochemicals are not structurally defined. The diversity of these naturally available resources have valuable utilization in the coming era in both scientific study (allowing for a more comprehensive knowledge of plant based metabolites) and practical research (e.g., drug designing, flavor, biocidal, compound discovery).

Fields utilizing metabolomics-

Plant scientists have been able to dive deeply into cell biochemistry thanks to metabolomics, and the technique has swiftly developed a solid foothold in research as suggested by Hall (2007). These techniques have now been used to a wide range of taxa, both cultivated and wild, to address a wide range of biological issues of scientific and industrial importance. Many earlier studies have dealt with main metabolites, for which the synthesis pathways are well defined along with their genetic background. (Giavalisco et al., 2009). Authentic standards are also widely accessible, allowing for both conclusive identification and complete quantification. However, substantial research has been undertaken into the very complex and poorly understood secondary metabolite pathways. Such pathways are frequently biologically important since they are linked to abiotic or biotic stress tolerance, as well as other environmental and organism interactions, food quality attributes like taste, organoleptic qualities and Use in medicine field and other things. Polyphenolics (Tikunov et al., 2010), alkaloids (Kim et al., 2010), brassinosteroid, and glucosinolate (Hall et al., 2010) studies have all made significant progress in the field of metabolomics..

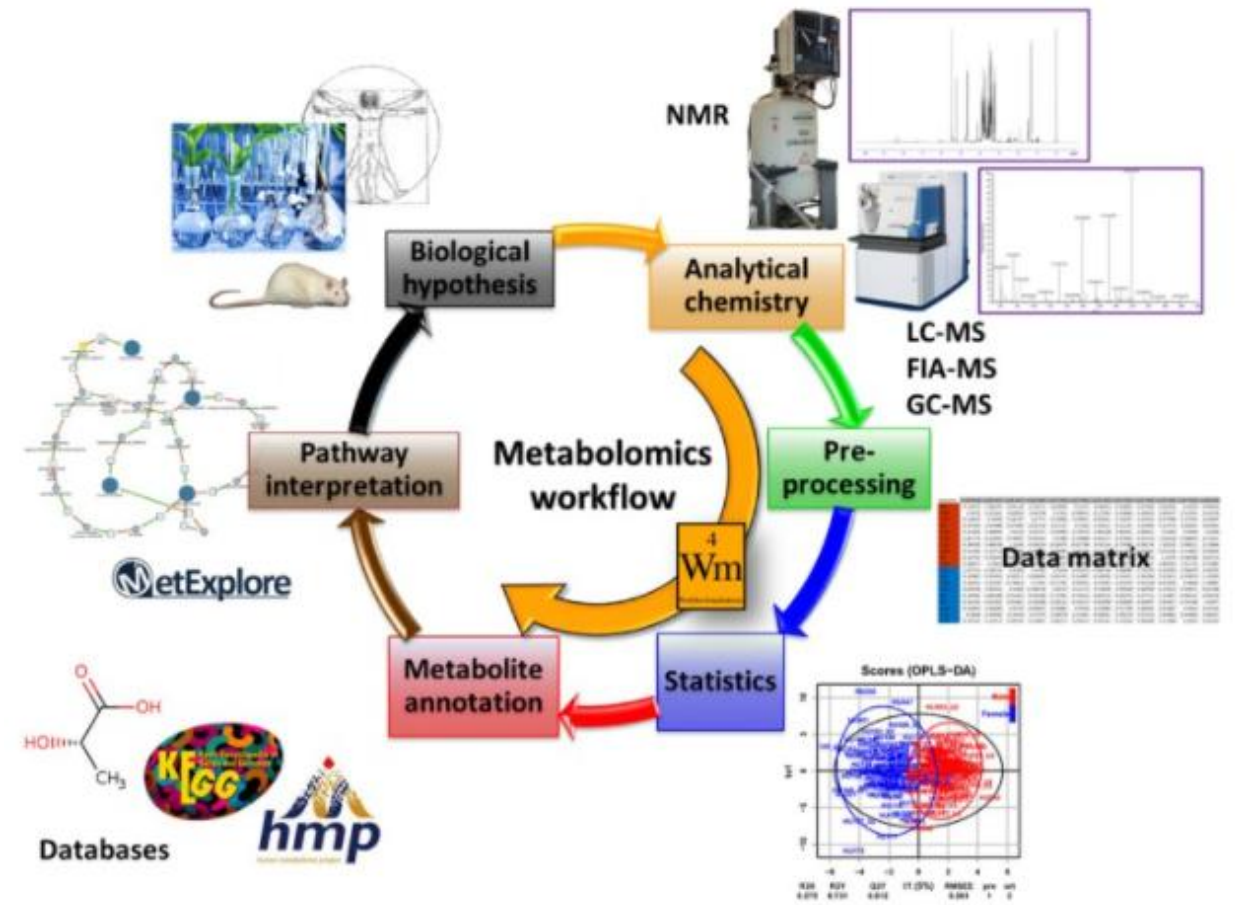


Figure 2 - Workflow in Metabolomics (Guitton et al. 2017)

General Procedure-

Researches in plant science field require variability in at least one genetic or enviromnmental variation in order to get information about response due to change. For a successful research ,before going on to chemical studies , all the prior steps should be well planned like from which tissue the sampling should be done , how sample will be taken etc.

Experimental design and plant growth

Plant metabolomics studies are based on thousands of metabolites that vastly out number the sample quantity, raising the possibility of false discovery. For minimizing the errors ,it is very important to know the possible cause of error at various levels like the environmental, biological, and technological levels for each stage from plant growth to sample storage and dissemination in order to reduce experimental error. Knowing the cause of error will help in

establishing "experimental design," as well as for making strategy for each step of experiment in order to find the relationships between the factors and variables influencing the process of interest and its effects (Anderson and Whitcomb, 2007). This can be achieved by doing small-scale preparatory experiments before moving on to larger-scale trials. Then, for plant growth, a design such as factorial design or optimum design, blocking, or randomization, with labelling of individual plants and maybe organs per plant, must be adopted (Hinkelmann and Kempthorne, 2008;). The environment must be managed during culture, and changes in certain main environmental variables (such as fertiliser, temperature, and/or light) must be recorded. Even in regulated conditions like greenhouses or growth chambers, slight deviations might produce biochemical status changes. Data documentation and storage are also essential for data analysis (Hannemann et al., 2009). As a result, the metadata format for plant growth should be simple to record and share. If all else fails due to high analytical costs, biological sample replication is essential and should be preferred to extraction and analytical. It's necessary to strike a balance between sample size and throughput. The experimental design must specify the number and composition of biological replicates. Replication is necessary for incorporating and measuring biological differences, as well as providing a sample.

Harvest-

For the metabolite studies, the harvesting techniques need to include several major points like at what age ,from which tissue and at what time the plant or plant part should be removed . If there is no standard technique of harvesting then there can be uncontrolled variability due to above factors . These factors become more significant when for a given experiment's harvest sessions are many, or when each session requires several operators to keep its time to a minimum. Whenever available, dedicated ontologies or reference articles (e.g., Boyes et al., 2001, for Arabidopsis, and Brukhin et al., 2003, for tomato) should be used to define the age, or preferably the developmental stage, of the plants or their organs in relation to standardised growth conditions and/or phenology de Hours for seedlings in controlled environments. If the age of the organ is uncertain, sample homogeneity can be improved by using some other defined characteristics like organ aspect, colour (e.g., a colour code with a standard scale for fruit development of a specific species), and size. Even for an organ with well defined growth stages, the sampling time and procedure must be exact (Fiehn et al., 2007). Because the metabolite composition of the leaf (Kim and Verpoorte, 2010) and also the fruit (Ma and Cheng, 2003) varies over the day and night cycle, the harvest time must be accurately set and the length limited. The organ's location (for example, fruit position) must be determined. Similar climatic conditions for each harvest on an open field are preferred. Organs should be handled with care and preserved in appropriate packaging to avoid any damage and oxidation of metabolite. For keeping the metabolite intact in the sample , the enzymatic reactions must be Enzyme activity should be prevented (AP Rees and Hill, 1994) . This can be done by keeping the sample at the freezing temperature or by keeping it in liquid nitrogen.

Sampling-

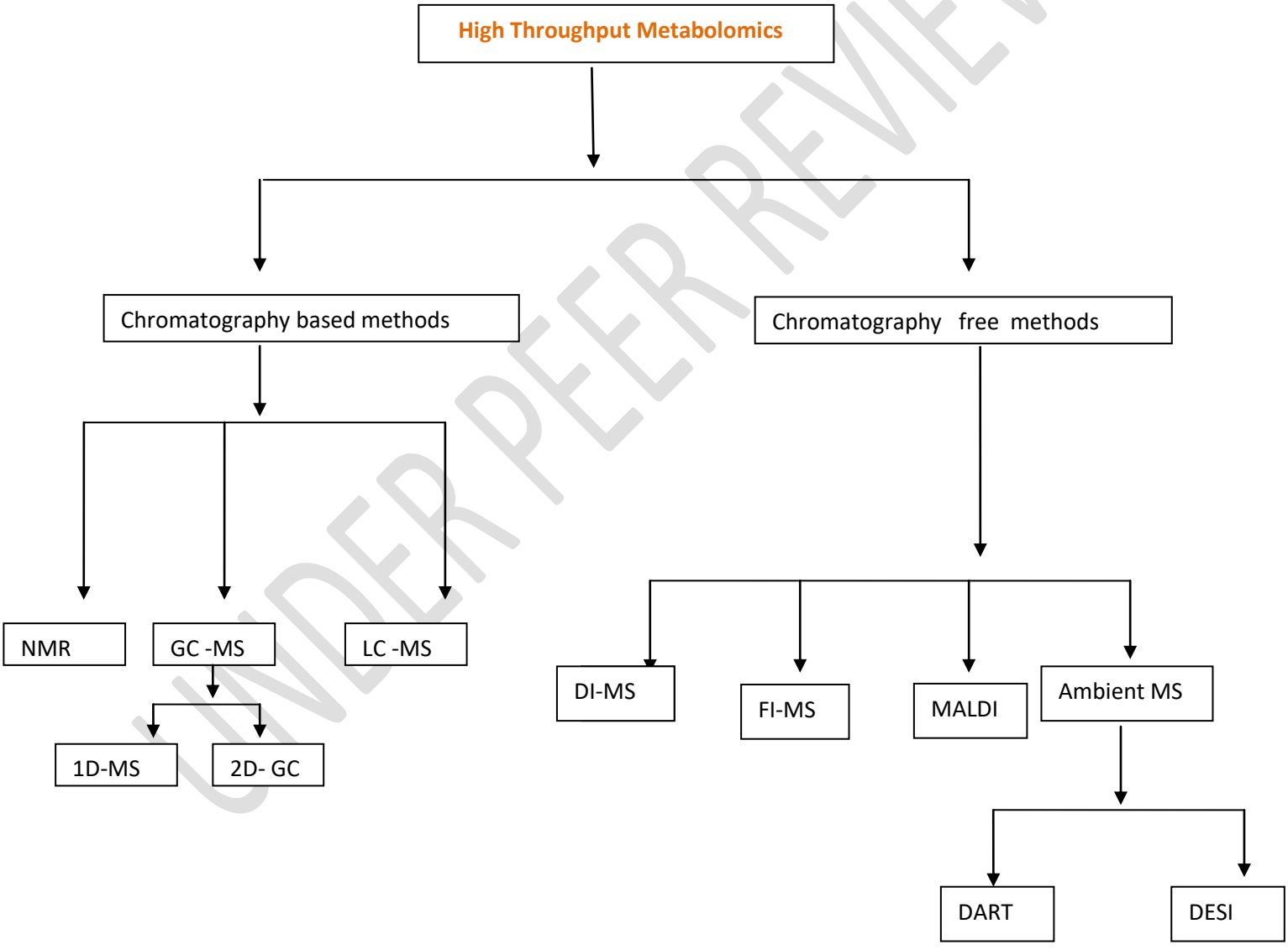
Sampling should yield samples in a form and amount adequate for metabolomic analysis, as well as samples that are representative of the overall population to be studied. If the plant material is fundamentally heterogeneous, further caution is required (e.g.,different plant parts are made of different tissues). When a specific tissue must be dissected before freezing, material must be obtained from different areas of the organ, which must be specified, because the sample must fully represent the total tissue in the organ. Working as quickly as possible is important since the use of blades and scalpels produces wound stressors that influence metabolism. Surfaces uncovered after incision should ideally be kept to a minimum, while volume should allow for quick freezing of the organ parts as described above. Microdissection necessitates the use of specialised equipment and processes (Balestrini and Bonfante, 2008; Ebert et al., 2010). The pieces can be ground right away after being completely frozen in liquid nitrogen, or put into labelled plastic bags and held at 80 C until grinding is practicable, before being distributed into tubes and stored at 80 C. Before, during, and after grinding, it is advised that all samples for a specific experiment follow the same technique. Sample grinding is normally necessary to maximise solvent extraction and to homogenise the sample material (Markert, 1995); nevertheless, contamination or volatilization of some chemicals of interest (Markert, 1995) need to be reduced. Despite the possibility of volatile component alteration during freezing, as shown for strawberry, samples are generally ground frozen for metabolomics (Douillard and Guichard, 1990). Robotized grinding automation must be considered for HTP metabolomics research. Quality assurance and quality control (QC) are critical throughout the sample preparation process, right from growing the sample in field to its storage in the laboratory and distribution to chemical analyzers. The samples should be properly identifiable. This can be achieved by barcoding or by cataloguing the information in the databases.

Sample Storage-

An experiment's samples should all be stored in the same way. Sample storage conditions and duration must be monitored and recorded. Plant samples are rarely studied for the influence of long-term preservation on their metabolites (Ryan and Robards, 2006). As a result, while dealing with several families of compounds utilising a mix of extractions and analytical procedures, it is worthful to suggest a few compounds as "markers" of excellent storage conditions and duration. Albeit, solid phase micro extraction trapping of volatile chemicals (Tholl et al., 2006) may be conducted on full organs when tiny or tissue bits, samples can be kept as organs, tissue pieces, or powdered tissue at 80 C. Tissue samples can be preserved as raw or lyophilized samples, depending on the required analysis. Freeze-drying will result in the loss of volatiles, hence fresh-frozen samples are required for analytical evaluation of highly volatile chemicals (Keinanen and Julkunen-Titto, 1996). It should be kept in mind that freeze-drying might result in the permanent loss of certain metabolites due to irreversible attachment to cell walls or membranes, despite the fact that freezing disrupts enzymatic reactions and microbial degradation during storage (Dunn et al., 2005a). Stability during sample storage is an important component that is seldom assessed, hence storage conditions must be regulated. Fruit that has

been frozen for a long period has been found to change several aromatic components (Ma et al.2007). Typically, metabolomics samples are kept at 80 degrees Celsius. It is advised that lyophilized samples be maintained at 20°C and in dry circumstances (Salminem, 2003). Tissue samples and extracts may need to be preserved in tubes filled with nitrogen or argon gas to prevent oxidation for certain reasons (Erban et al., 2007).

Chart 1 : High Throughput Metabolomics



Some important high throughput metabolomics techniques are explained in the following section

A) Chromatography based Metabolomics-

1 .GC- MS Metabolite Profiling

Metabolic phenotyping through the use of GC–MS profiling has become an integral feature of plant functional genomics (Fernie et al., 2004) and is about to become a standard technology with the potential to generate comparable data across laboratories (Allwood et al., 2009). This finding adds significantly to the growth of metabolomics as a fourth Rosetta stone for plant functional genomics and molecular physiology (Fiehn et al., 2000). Six general steps are involved in metabolite profiling using GC–MS:

1) Extraction of metabolite from the plant or organ
2) Derivation of metabolite in the form which can be used for gas chromatography
3) GC separation- Because it uses automated sample insertion robots, highly standardised gas flow settings, temperature programming, and uniform capillary column material, GC may be extremely reliable.
4) Ionization of compound- Electron impact (EI) method is widely used as it is highly reproducible.
5) Detection of molecule and fragment ions with time resolution. TOF detectors that can be set to rapid scanning speeds are preferred for mass separation and detection.
6) GC–MS data file acquisition and assessment

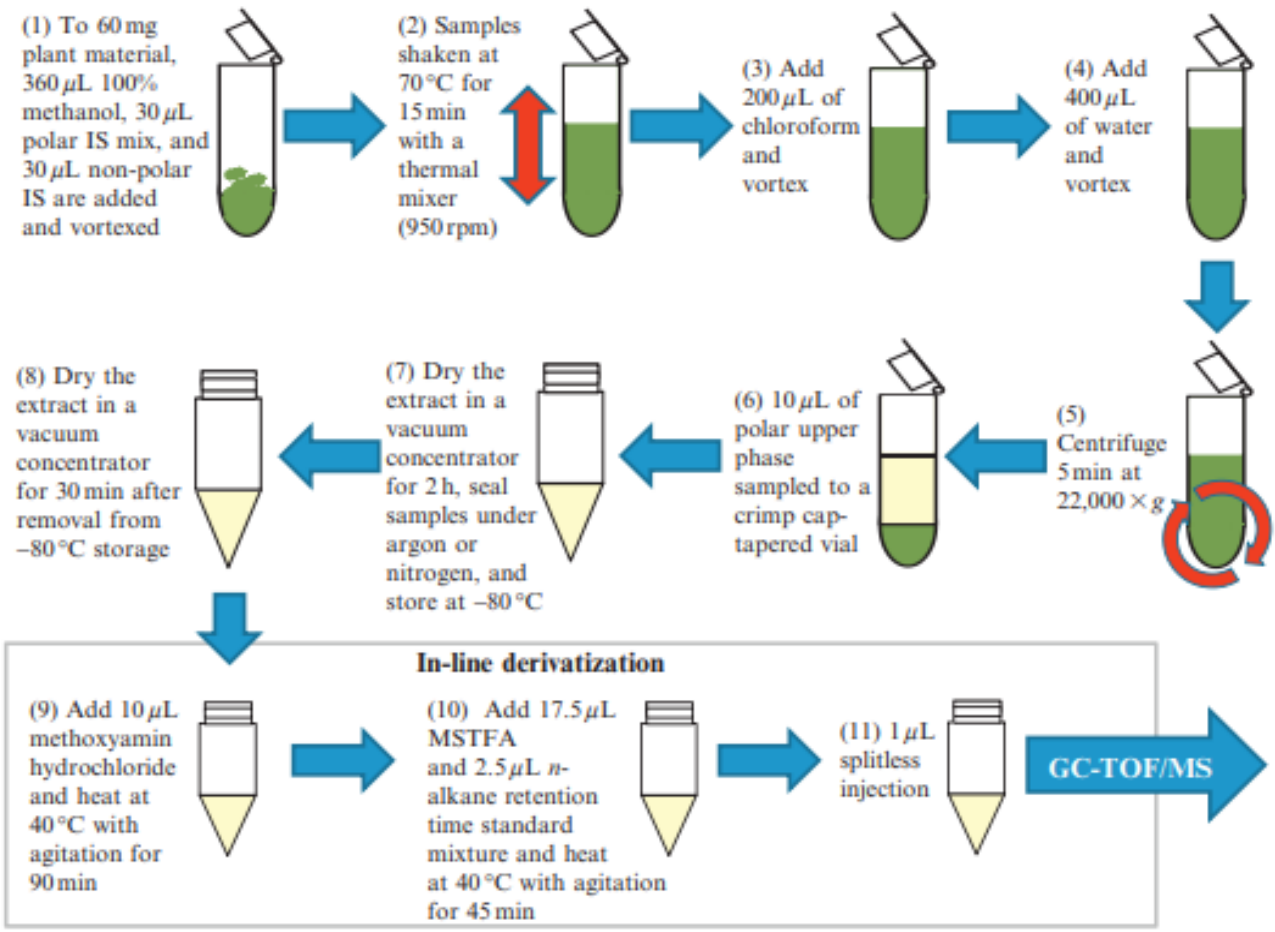


Figure 3 -Preparation of polar plant extracts and chemical derivatization for GCTOF/MS analysis. An illustration of the plant polar metabolite extraction scheme and in-line derivatization (gray box) as presented in the reference protocol of Erban et al. (2007).

Data preprocessing in GC-MS

To construct numerical metabolite or chemical feature matrices for statistical computations, and to give a way of associating such chemical properties to a metabolite identification, automated data processing tools are required. Retention indices and mass spectra are employed in GC–MS profiling investigations for this reason (Wagner et al., 2003). Tools for mass spectral deconvolution and retention index computation for compound recognition, peak height or peak area retrieval for relative or absolute quantification, and mass isotopomer distribution analysis for stable isotope tracing and flux investigations have all been created. In academia, many data preparation procedures have been used. One basic technique employs mass

spectrum deconvolution first, then quantifies based on deconvoluted (mass fragment) abundances; nevertheless, caution must be exercised. If the goal is to conduct a long-term metabolomic profiling investigation, it is advisable to try to detect all metabolite characteristics, which necessitates the use of whole data set deconvolution through AMDIS (Lisec et al., 2006) or LECO ChromaTOF (Lisec et al., 2006). However, if the experiment involves metabolite fingerprinting and the investigator only wants to find statistically significant metabolite differences between experimental groups, such as in gene function analyses, a more rapid approach would be to use generic software like TagFinder (Luedemann et al., 2008, 2011) or MetAlign (Luedemann et al. 2011).The present more pressing problem is identifying metabolites in complicated combinations in an automated and precise manner. Even today, human judgements are crucial for harmonizing and categorization of mass spectra or mass spectral tags (MSTs). Many software tools are available that either support MST recognition in comparison to reference libraries like NIST or freely available libraries like the GMD (Golm Metabolome Database: Kopka et al., 2005) or aid the interpretation of unknown mass spectral features that cannot be linked to a compound structure in the absence of authenticated reference compounds (e.g., Hummel et al., 2010). A laboratory undertaking routine metabolic profiling, on the other hand, should build an in-house library of pure authenticated reference standards that are examined after being subjected to the same derivatization and analytical parameters as the profiled samples for unambiguous identification.

2. LC–MS Metabolite Profiling

For metabolic profiling of semi-polar secondary metabolites such as phenolic acids, flavonoids, alkaloids, polyamines, saponins, and glucosinolates, HPLC together with MS (in short LC–MS) is the recommended approach. These chemicals can be extracted with aqueous alcohol solutions and evaluated directly without the need for previous derivatization. In LC–MS , ionization is commonly done through soft ionisation methods, such as electrospray ionisation (ESI) or atmospheric pressure chemical ionisation, which either produce protonated (in positive mode) or deprotonated (in negative mode) molecular ions, in contrast to EI used in GC-TOF/MS. Modern high-resolution mass spectrometers, such as TOF/MS, ion cyclotron FT-MS, or Orbitrap FT-MS, can now profile hundreds to thousands of compounds in crude plant extracts while also calculating elemental formulas from the discovered masses (Allwood and Goodacre, 2010). A comprehensive information about the relative abundance of hundreds of known and undiscovered semi-polar metabolites may be acquired through an essentially unbiased technique that undertakes all metabolite mass signals from the LC–MS raw data files (de Vos et al., 2007). C18-based reversed phase columns are often used in LC–MS-based metabolomics techniques to achieve optimum separation of large quantity of semi-polar chemicals that can be there in raw plant extracts (Hanhinevaet al., 2008). This method has been used to profile secondary metabolites in a variety of plant species, including Arabidopsis, cabbage, tomato, potato, strawberry, apple, lettuce etc as well as plant tissues (Adato et al., 2009).

Data preprocessing in LC–MS profiling

MetAlign is a software which is freely available tool for untargeted processing of raw data files from LC–MS and GC–MS instruments from a variety of vendors (download at www.metalign.wur.nl). For up to hundreds of samples, it conducts various operations like correction of local baseline, selection of unbiased peak, and alignment of mass peak, which altogether results in a matrix of mass signal intensities. It is ideal for those mass spectrometry instruments that have limited dynamic range in mass accuracy, such as the QTOF Ultima, since it allows you to choose an intensity window for precise mass computation. The data from the quality control samples can be used for consistent peak selection and alignment after processing. For recognised chemicals, around 70–80% of all peaks should be present in all quality control samples, with an overall intensity variation of less than 20% and a mass deviation of less than 3 ppm. Thus the resulted peak table can be further utilized to remove signals that are inconsistent, low (noisy), or saturated. By adopting a mass spectra reconstruction technique, mass signals arising from the same sample can be classified as per their related retention duration and relative intensities across samples (Tikunov et al., 2010). LC–MS signals from the same metabolite (molecular ion and its fragments, adducts, and isotopes) are grouped and replaced by a single representative metabolite signal in this method. To identify the important secondary metabolites, data-dependent tandem-MS/MS and MSn fragmentation studies can be used (de Vos et al., 2007).

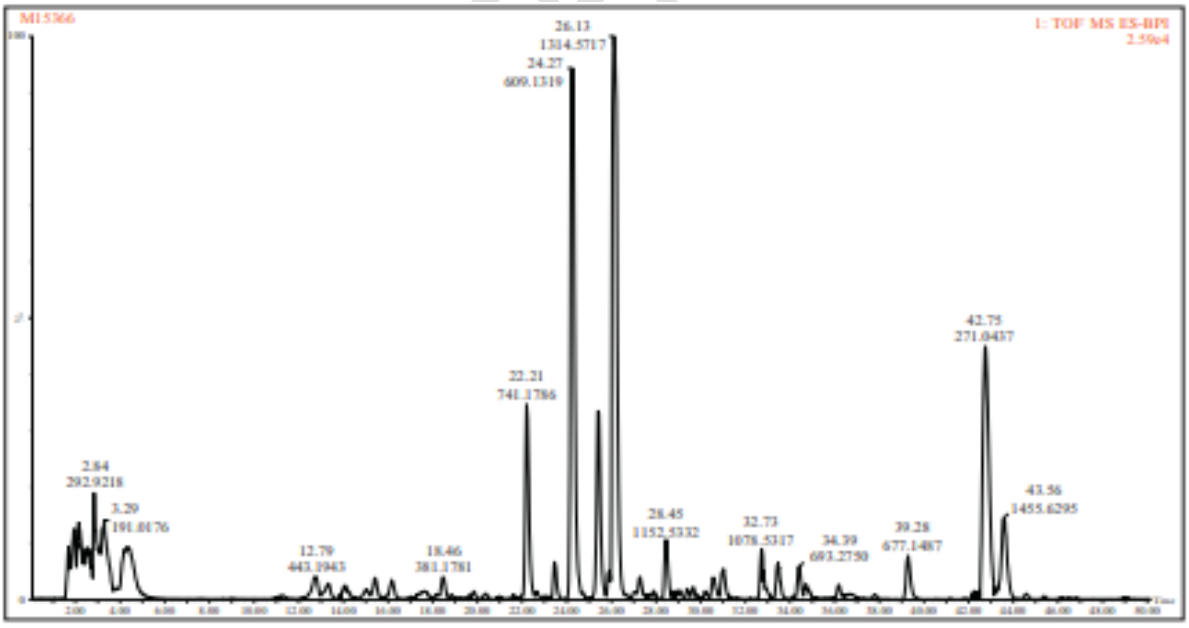


Figure 4 -LC- MS chromatogram (base-peak intensity) of an aqueous-methanol extract from peel of ripe tomato fruit numbers at peaks indicate retention time and accurate mass on top of peak. (Iijima et al., 2008)

3. ^{1}H Nuclear magnetic Resonance (NMR)

Despite the fact that NMR spectroscopy has been a prominent technology in metabolomics since 2000 (Ludwig and Viant, 2010), it is underutilised in plant metabolomics compared to mammalian metabolomics. NMR can be either monodimensional or multidimensional. HSQC (heteronuclear singlequantum coherence spectroscopy) and 2D JRES (2D 1 H 1 J-resolved) spectroscopy are powerful and frequently crucial methods for metabolite identification, although 1D 1 H NMR has mostly been used in metabolomics until recently. Rapid spectrum collection and the ability to perform relative or absolute quantification are key factors in 1D 1 H NMR's success in metabolomics. Furthermore, NMR gives comprehensive molecule structure information and does not degrade the sample during the measurement.

Plant sample preparation for NMR usually involves many procedures. The technique of extraction has a significant impact on the subsequent identification of metabolites, and samples are always tested promptly after extraction. Although the processing of sap or fruit juice samples is faster than that of tissues, enzymes in sap or juice need to be deactivated , either with alcohol like mathenol or by high temperature treatment (Biais et al., 2009).The amount of sample needed in NMR method is quite higher (200–500 mg fresh weight) than that chromatographic procedures with or without mass spectrometry(60 mg fresh weight) .As water is the most abundant component of plant tissue and is made up of two hydrogen atoms that may be detected using NMR, it is critical to remove it using lyophilization or cryodessication. This step should be conducted after cryogrinding for maximum efficiency, since fine plant powder aids in the freeze-drying and extraction processes (Kim and Verpoorte, 2010).

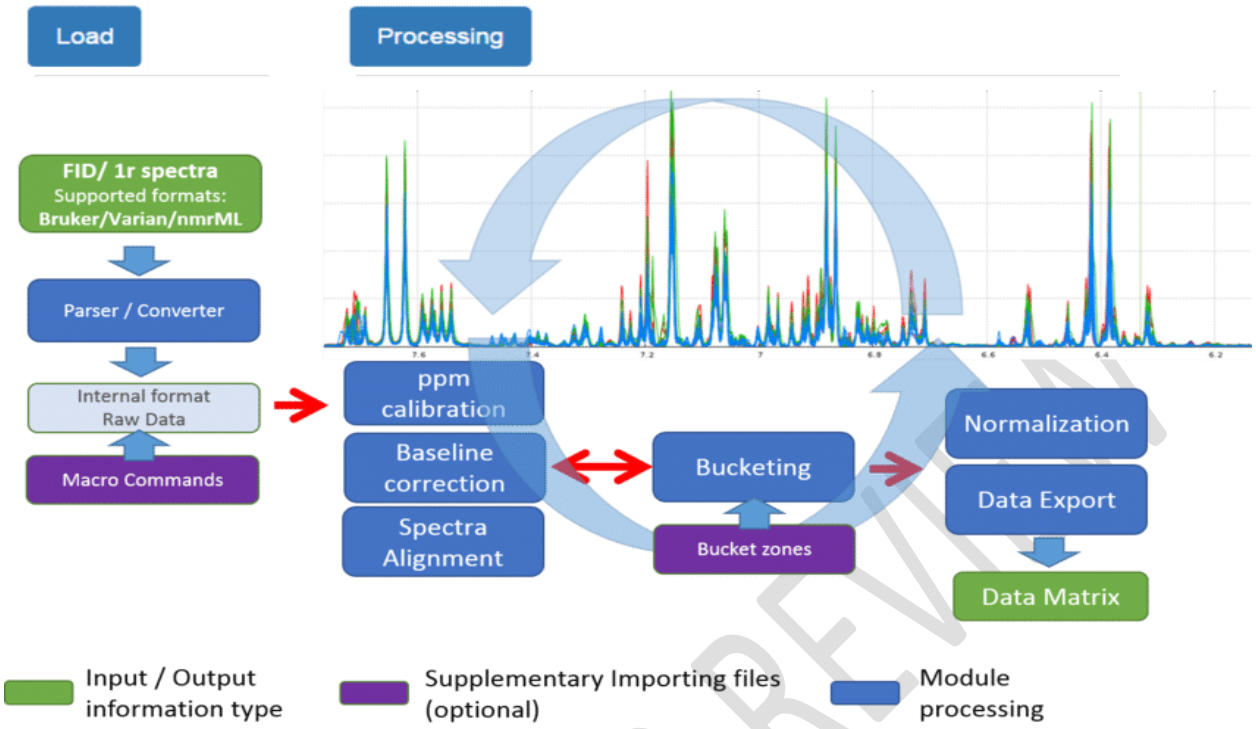


Figure 5 - NMR workflow

For NMR based metabolite profiling. high-field devices with superconducting magnet coils operating at liquid helium temperature should be used. The highest commercially accessible field is currently 23.5 T, which corresponds to a 1 H frequency of 1 GHz. The routine fields utilised for plant metabolite profiling have a 1 H frequency of 400–600 MHz. A console (high-frequency channels for excitation and acquisition, gradient amplifier unit), a probe head, and an autosampler are also part of an NMR spectrometer. Tunable broadband inverse gradient probe heads (e.g., Bruker's ATMA-BBi and Varian's ProTune Auto-X ID) flushed with nitrogen gas (from a nitrogen separator enriching N2 to roughly 98 percent of compressed air) and temperature regulated are advised automatically. Water's chemical change is temperature dependent. For 1 H detection, inverse or indirect detection probe heads give the best signal-to-noise ratio. Standard probe heads have a diameter of 5 mm and need a sample volume of 500–600 mL. (Ross et al., 2007). HTP sample handling is made possible by autosamplers or robotic sample changers, which allow for the mechanical changing of NMR tubes according to a predefined programme. In terms of tubes, it is advised to always use same quality tube and cap from the same vendor for the whole series of tests.

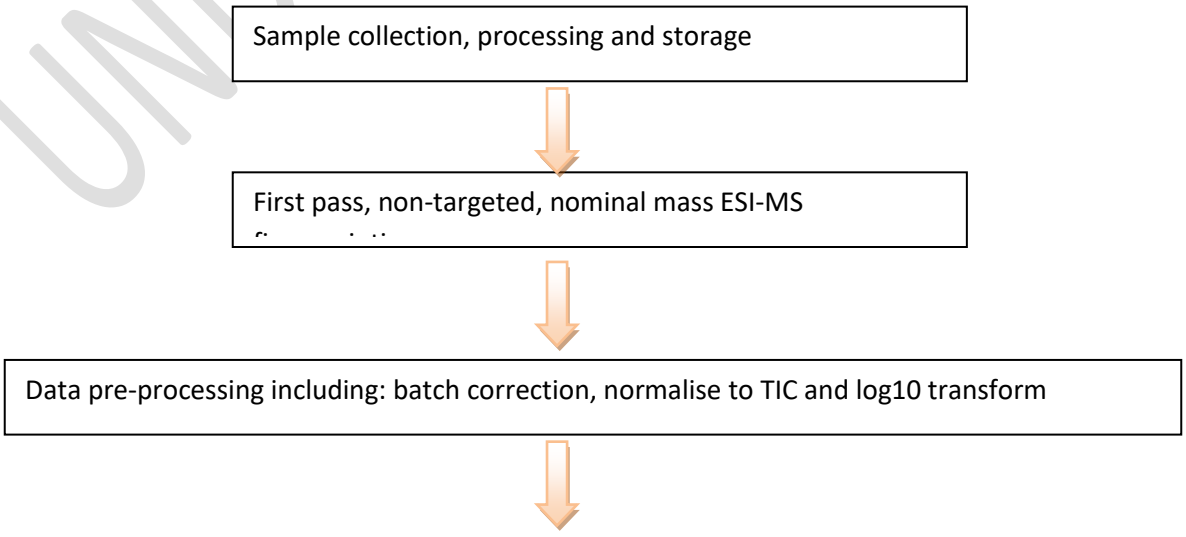
B) Chromatography-free metabolomics

Given that chromatography tends to be the most time-consuming aspect of quick analysis and smooth peak identification, omitting chromatographic separation entirely in metabolomics seems like a natural way to boost sample throughput. Direct infusion and flow injection are two methods for introducing liquid samples into mass spectrometers.

1.Flow injection

It involves injecting a sample into a continuous stream of organic phase running at a rate of 100–1000 L/min to the electrospray interface. Peak width varies depending on flow rate, sample concentration, and electrospray source, but it usually falls between 5 and 15 seconds. With a conventional LC–MS equipment, this enables for the injection of 50–100 samples each hour, resulting in 1000–2000 injections per day (Furher et al ,2011). Despite the great sensitivity of current mass spectrometers, such high throughput may be maintained for long periods of time by performing routine electrospray maintenance (Furher et al ,2011).

Flow injection techniques have coverage and sensitivity that are equivalent to rapid chromatography-based approaches. Thousands of m/z signals were detected in yeast extracts by Madalinski et al., with 400 being detected after intensive filtering (Madalinski et al.2008). Sévin et al. 2014discovered more than 1000 chemicals in E. coli by matching a substantially higher number of characteristics to theoretical m/z within 0.001 Da tolerance. It shows that state-of-the-art devices may provide deep coverage of important pathways in primary metabolism even at very high throughput. The detection of analytes with low abundance or wide chromatographic elution, which are difficult to distinguish in, for example, LC–MS, partially compensates for the loss of detectable characteristics induced by the removal of chromatography. Because there is less noise in peak integration, flow-injection analysis has a competitive repeatability (Nanita et al, 2009). Furthermore, due to the short cycle duration and high frequency of analysis, most signal, background, and noise drifts that are prevalent in electrospray mass spectrometry of complex materials may be detected and corrected efficiently (Kirwan et al,2013)



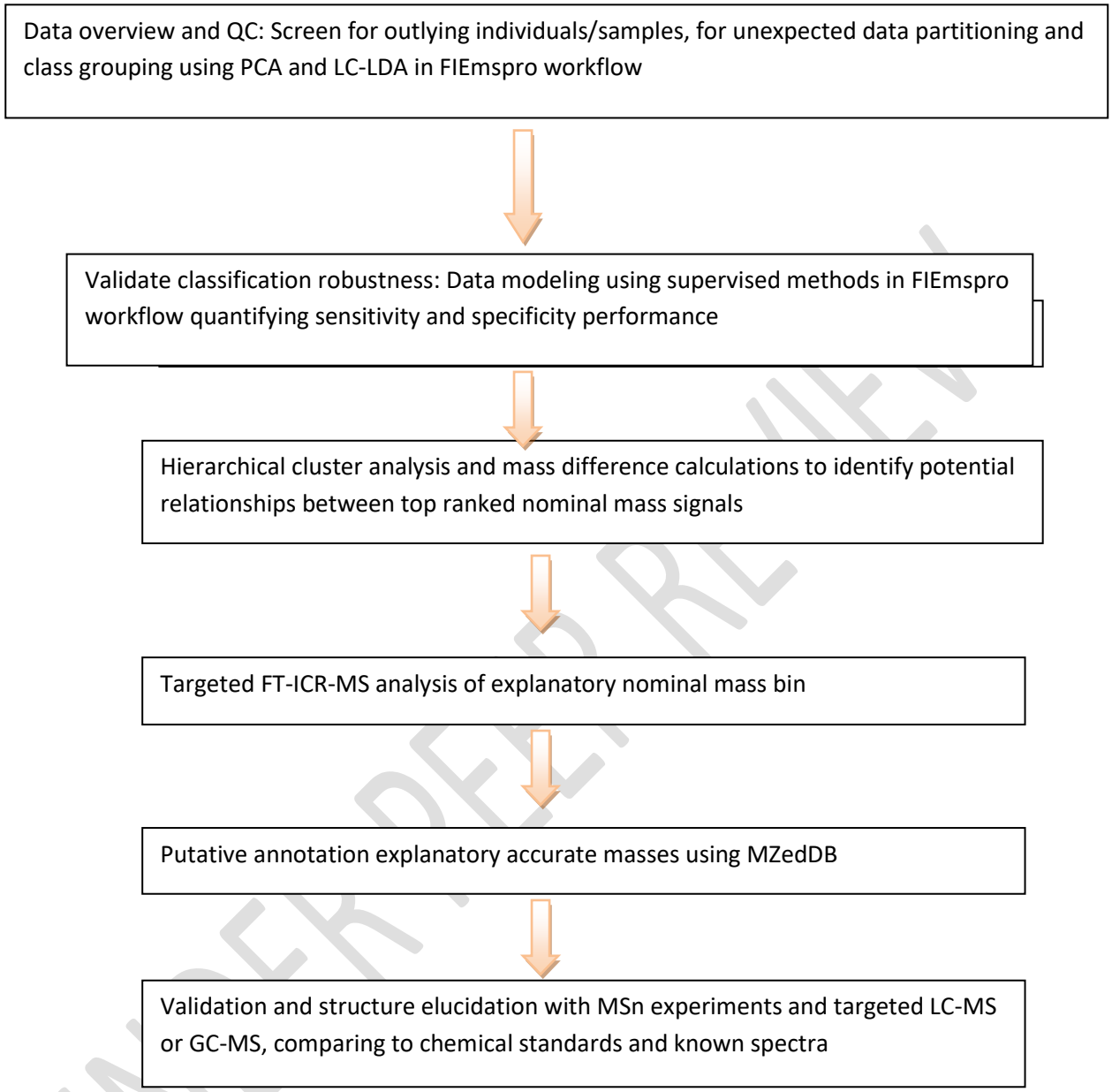


Figure 6- pipeline for non targeted metabolomics study using Flow infusion(Draper et al. 2013)

2 Direct infusion (DI)

The injection or infusion of a sample into the mass spectrometer's ionisation source without previous chromatographic separation is known as direct injection analysis. This method is frequently used in conjunction with atmospheric pressure ionisation methods, namely ESI. Direct injection is a high-throughput method that may handle a sample in a matter of minutes. The quick analysis time enhances inter-sample reproducibility and cluster analysis accuracy. ESI and nominal mass resolution mass analyzers were used for direct injection analyses. For metabolic fingerprinting in crude fungal extracts, Smedsgaard et al. 1997 employed ESI in positive mode and a single-stage quadrupole apparatus. Castrillo et al. (2003) used direct injection ESI in positive mode and triple quadrupole MS to examine yeast intracellular metabolites.

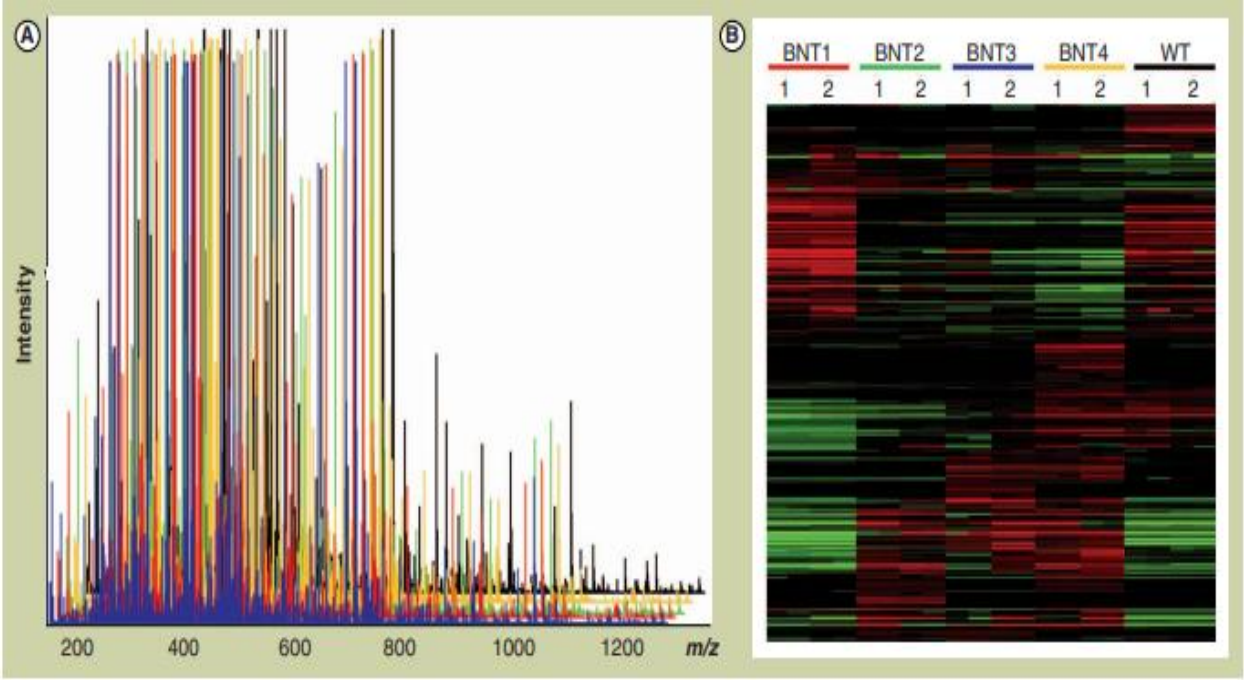


Figure7 A) Direct infusion mass spectra of aqueous phase metabolite extracted from 4 different lines of Brassica napus seeds. B) The clustering heap map

3.MALDI-MS

MALDI, or matrix-assisted laser desorption/ionization, is a mass spectrometry soft ionisation method. This approach may be used to examine biomolecules (proteins, peptides, and sugars) as well as bigger chemical compounds (polymers and dendrimers). These compounds are ionised using MALDI because they are delicate and will fragment if ionised using other, more traditional ionisation techniques. The biomolecule is protected by a matrix to prevent it from being damaged and to allow for optimum vaporisation and ionisation using a laser beam. The most frequent three crystallised molecules employed in MALDI-MS are 3,5-dimethoxy-4-hydroxycinnamic acid, alpha-cyano-4-hydroxycinnamic acid, and 2,5-dihydroxybenzoic acid

(DHB). Purified water and an organic solvent such as ethanol or acetonitrile are combined together to make a solution.

Youanqquan et al. 2020 tested Citrus Huanglongbing (HLB) infected citrus leaves caused by the phloem bacteria *Candidatus Liberibacter*. Since this disease is asymptomatic ,its phenotypic detection is not easy. Thus ,they developed the fast detection method by testing metabolite differences between healthy and unhealthy leaves using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The result showed the infected and noninfected leaves were distinguishable on the basis of MALDI-TOF-MS along with multivariate analysis. It was found that 32 MS peaks of metabolite showed the clear cut differences among healthy anf unhealthy plants . Among these peaks, in 9 substances the upward trend was observed and for 19 metabolites, downward trend was observed which included a significant decrease in malate content of infected leave regardless the symptom appeared or not . Thus ,regulation pattern of metabolites can be used as biomarkers for early detection of infected plant.

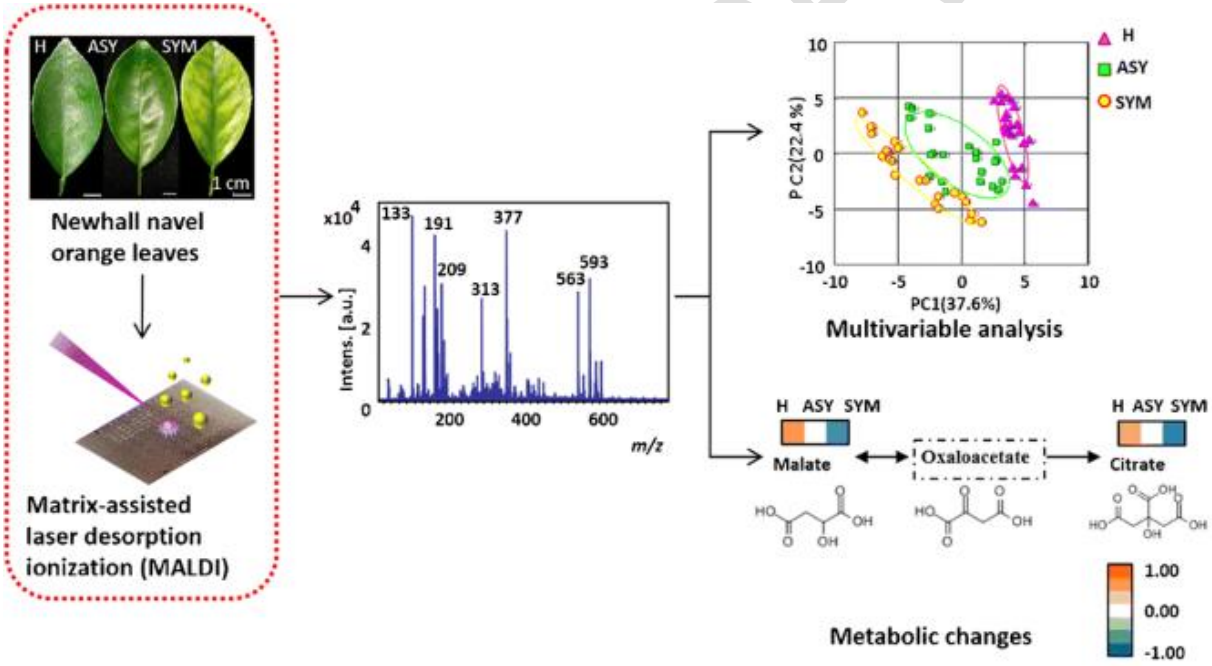


Figure 8– MALDI analysis result by Youangquan et al.2020

3. Ambient mass spectrometry-

Ambient ionization/sampling mass spectrometry (also known as "ambient mass spectrometry") is a kind of mass spectrometry that allows for high-throughput surface

examination of native materials. Direct analysis in real time (DART) and desorption electrospray ionisation (DESI), two flagship ambient mass spectrometry techniques, have not only enabled previously impossible experiments, but have also been surrounded by a plethora of other techniques, each with their own advantages and applications. This chapter discusses the kind of studies that are at the heart of ambient mass spectrometry and includes a number of specific examples to help readers unfamiliar with the topic have a better understanding of it.

DART-

DART-MS (direct analysis in real time mass spectrometry) is a well-established technology for performing quick mass spectrum analysis on a wide range of substances. DARTMS can analyse a sample under atmospheric pressure, allowing it to be analysed in an open laboratory setting. DART MS may be used to analyse chemicals that have been deposited or adsorbed on surfaces, as well as compounds that are being desorbed into the atmosphere. This makes DART-MS ideal for analysing plant elements, pesticide monitoring on crops, forensic and safety applications, such as screening for residues of explosives, warfare chemicals, or illegal substances on baggage, clothing, or bank notes, and so on. DART can also be used to analyse solid or liquid bulk materials for quality control purposes, or to swiftly determine the identify of a product derived via chemical synthesis. DART-MS may be used on live creatures as well. The combination of the DART ionisation source and interface may be customised in several geometries and with various accessories to adjust the arrangement as needed, driven by varied demands in analytical practise. DART-MS depends on a gas-phase ionisation process to do its analysis. The ionising species are first generated in DART using a corona discharge in a pure helium environment, which yields excited helium atoms that, when released into the atmosphere, start a cascade of gas-phase events. Finally, reagent ions formed from atmospheric water or (solvent) vapour at the surface are subjected to analysis, where they cause a chemical ionisation process.

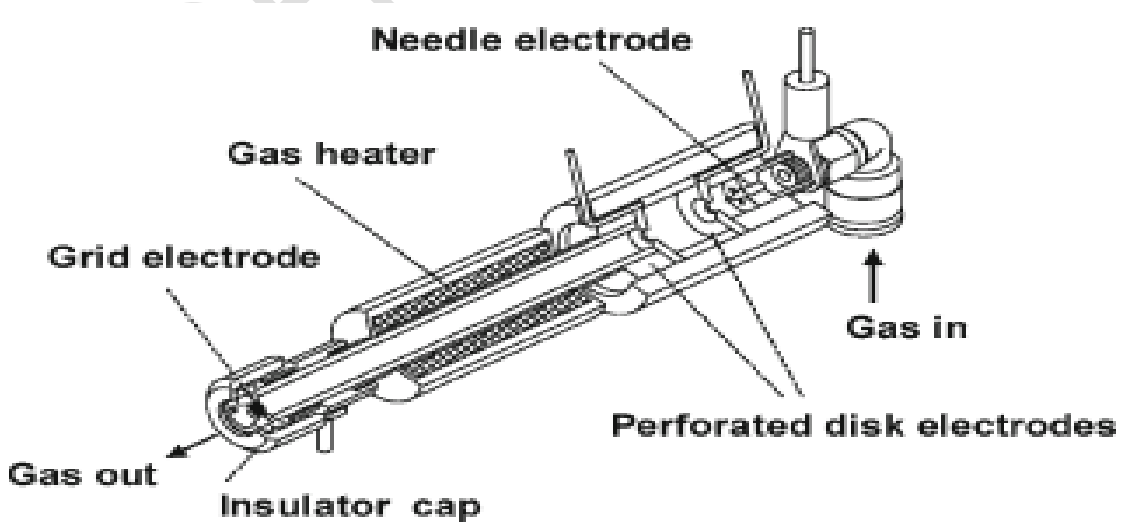


Figure 9 - Cutaway view of the DART ionization source. The exiting gas stream effects ionization of sample in the gap between exit and entrance into the mass spectrometer vacuum interface (Cody et al.2005)

DESI-

Traditional electrospray ionisation (ESI) is the source of DESI (Dole,1997; Pramanik et al. 2002). DESI employs a pneumatically assisted electrospray of a solvent containing a low concentration of an electrolyte to produce an electrically charged aerosol (Takats et al . 2004). In contrast to ESI, the spray capillary in DESI is pushed back from the API interface's orifice, and the aerosol (devoid of analyte species) is directed at a 45° angle toward the item to be examined. The distance between the spray capillary and the item has been reduced to a few millimetres, and the API interface's entry has been positioned at the same angle and distance. Sprayer, object, and orifice create a compact V-alignment as a result of this. The highly charged aerosol droplets are then propelled by the intense pneumatic spray onto the surface of the item to be studied, resulting in a thin liquid coating covering the surface. DESI makes a surface "moist." The solvent will be able to remove analytes from the item in this manner. The API interface samples analyte-containing charged droplets released from the surface by the combined effect of the persistent aerosol stream and the electrostatic charging of the surface. The same mechanisms as in ESI are used to liberate isolated gas phase ions. DESI, like ESI, can analyse substances in the ultra-low to ultra-high mass range while keeping the molecules intact (Watson et al.2002)

Table 1 : Some commonly used Metabolome Databases

Name	Content
Compound and species specific Databases	
PubChem	A collection of chemical structure and biological properties of small molecules
HMDB	A comprehensive data of more than 6800 human metabolites
LIPID MAPS	Structure and annotation of more than 9000 lipids
KNApSAcK	A cross species metabolite data base of more than 28500 compounds with approximately 1000 masses , mostly from plants and microorganism
Reference spectral Library	
NIST08	Commercial library of 2,20000 EI mass spectra from more than 190,000 pure chemical compounds contaiong 14,802 spectra of positive ions and 1410 negative ions
GMD	Mass spectra and retention indices of known plant metabolites analyzed by GC –TOF-MS
METLIN	A database of LC-MS mass spectra of metabolites from human

	and microbial species
MassBank	A high resolution MS/MS database standard chemical substances
MS2T	A MS/MS spectral tag library of phytochemical compounds
MMCD	A NMR and LC-MS spectral library of metabolite standard
	Pathway specific Databases
KEGG	A composite database consisting of collections of pathway maps, genes, organisms, enzymes and ligands
BioCyc	A collection of 506 pathway/genome databases of organisms with completely or partially sequenced genome,database of metabolite compounds
EcoCyc	A scientific database from E.coliK-12 MG1655
MetaCyc	A database of nonredundant, experimentally elucidated metabolite pathways

Source- Han et al. 2009

Conclusion-

Model species have been used to achieve a substantial percentage of plant metabolomics' success. However, improving the applicability of metabolomics in agricultural species has a lot of demands, possibilities, and obstacles. The complicated genetics commonly linked with crops poses a significant difficulty. Metabolomics, on the other hand, has been effectively employed to improve the breeding of vital crops. The identification of genomic areas and genes linked with metabolic quantitative loci (mQTL) or the production of specialised metabolites has been done with key crops using current genetic resources as a foundation for future breeding initiatives. With multiple examples , metabolomics has considerably increased our understanding of plant specialised metabolism and natural product production at the molecular and biochemical levels. Metabolomics is also allowing researchers to gain a better knowledge of medicinal plants and to identify key metabolic QTLs for improved breeding. Despite the fact that metabolomics has demonstrated its worth, it still confronts significant hurdles, such as large-scale metabolite identifications. The discipline of metabolomics can only improve as improved technology continue to advance. This shows that metabolomics has a bright future ahead of it.

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