IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Advance and analytical tool assisted metabolomics study in plant: An Overview

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Abstract

Metabolomics is a new branch of "omics" that involves identifying and quantification of metabolites and chemical markers of various cellular administration methods biological species. Metabolome is the widespread collection of metabolites in a possible organism showing, measurement indicates genetic or environmental changes. Metabolomics playing important role for exploring genetic and environmental interactions, mutant identification, characterization, and drug discovery. The metabolites associated with various biological scientific pathways such as glycolysis, amino acid metabolism, and the Krebs cycle, etc. Advanced analytical tools, such as gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS), capillary electrophoresis mass spectrometry (CE-MS), Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), matrix -Assisted laser desorption ionization (MALDI), ion mobility spectrometry (IMS) and nuclear magnetic resonance (NMR) accelerate direct plant cell analysis. Additionally, Bioinformatics tools that have also played role in the development of plant metabolome database for the tracking of all metabolites that are used in physiology, development, and responses to biotic and abiotic stresses in model and crop plants

Keywords:- Metabolomics, Gas Chromatography, Liquid Chromatography, Analytical Technique

1 Introduction

Metabolomics is a branch of science dealing with the study which requires rapid and accurate measurement and identification of endogenous metabolites. Recent advances in metabolomics have been achieved. Mass spectrometry for complete analysis of Mass Spectroscopy (MS) data and structure identification data collected in separate experiments, or mass spectrometry data can be carried out in a single experiment. Many researchers use it as an incentive finding new drugs which can create different chemical structures. Newly developed technology allows methods such as isolation, identification, and measurement of metabolites and related metabolic pathways. Metabolite changes often referred as species` response to diseases, genetic changes, and other factors. (Dettmer, 2004). Different Analytical tools such as GC-MS/MS- GC-MS: Gas Chromatography-Mass Spectrometry, UPLC-MS: - Ultra Performance Liquid Chromatography-tandem Mass Spectrometer,

CE-MS: Capillary Electrophoresis-Mass Spectrometry along with the NMR Spectroscopy. For 2-D Gel Electrophoresis tools such as -Mass Spectrometry; MSI- MS-Imaging; MRI- Magnetic Resonance Imaging; MRS- Magnetic Resonance Spectroscopy; EI-MS: Electron Ionization Mass Spectrometry; EI- Electron Ionization; eV- electron Volt; CI- Chemical Ionization; APPI- Atmospheric Pressure Photo Ionization; APCI- Atmospheric Pressure Chemical Ionization; MALDI- Matrix Assisted Laser Desorption Ionization; GC- Gas Chromatography; LC- Liquid Chromatography; MS- Mass Spectrometry; & RPLC- Reverse Phase Liquid Chromatography tools were used (Dettmer, 2004).

Metabolomics analysis is mainly divided into two categories: (1) the first category is targeted metabolomics, (2) the second category is untargeted metabolomics. (Theodoridis et al., 2012 and Gika et al., 2014) Targeted metabolomics is characterized by focus on identification of diverse metabolites, which requires extensive sample purification as compared to non-autonomous approaches. Currently, an organized approach available to study the entire set of known metabolites of different groups in order to understand specific groups of similar metabolites, biological pathways, changes in different pathways is used. Untargeted metabolomics identifies and measures as many metabolites as possible simultaneously in a sample. Metabolites ranging from small positive molecules to large hydrophobic molecules. In general, the plants produce large amounts of metabolites due to plant development and environmental response. There are about 200,000 metabolites in the plant world, including primary as well as secondary metabolites. (Wink, 2010). Extraction methods are basically used for extraction of metabolites based on physicochemical properties; solvent properties; biochemical system composition. Plant metabolites produce a wide variety of selectable metabolites, including ionic organic compounds, hydrophilic carbohydrates, amino acids, organic compounds, and compounds related to hydrophobic lipids. The metabolite identification process is performed manually and semi-automated to obtain biologically interesting features. (Dunn, 2008). To fill this gap, several workflow documents were written to perform preprocessing, annotation, statistical analysis, and other biological interpretations of cellular data. (Chen et al., 2006) demonstrated that the use of HPLC/MS-based on metabolomics for the characterization of the nephrotoxicity induced by aristolochic acid (AA), kind of suspected kidney toxicant (Chen et al., 2006) whilst van der Greef et al. used RP HPLC/MS to examine the metabolic response to fasting in the mouse (Van Greef et al., 2007). However, the bulk of these applications still relied on the use of 10-15 min analysis times. Metabolomics is one of the most complex approaches among other omics and has received attention in agriculture science, especially for all plant selection in molecular breeding program. As we know that metabolomics is used to acquire great amount of useful knowledge by means of accurate & high throughput peak annotation of the plant metabolome for the novel genes as well as pathways elucidation (Tohge and De Souza, 2014). The combination of metabolomic integrated plus transcriptomic analysis was successfully used to find out these several possible approaches such as breeding and genome editing involved in activation of metabolic pathways and gene expression (Xu and Chen, 2020). The emerging scope as well as vast plant metabolite profiling has been resulted in the development and advancement of modern plant metabolomics approach for the study of plant biology and physiology. Recently, the conventional analytical methods have been taken over by advance analytical techniques like GC-MS/MS, UPLC-MS, CE-MS along with advanced NMR spectroscopy. The advance

technique effectively covers various processes like separation, identification, and quantification of the present metabolites and their related pathway.

1.1 Gas Chromatography- Mass Spectrometry (GC-MS)

Gas chromatography is the best technique for the identification and quantification of small metabolites (~500 Daltons) also used in plant metabolomics. These molecules include amino acids, fatty acids, hydroxyl acids, alcohols, sugars, sterols, and amines, which are identified mostly using chemical derivation to make them volatile enough for gas chromatography (Fiehn, 2016). It is largely used to quantify volatile compounds at higher temperatures and derived from polar metabolites (Dunn, 2008). Sample separation in GC occurs at a higher temperature; followed by reprivatisation form volatile to non-volatile, prior to the analysis. The spectral data are compared with the NIST database to ascertain unknown metabolites. Nevertheless, molecular ions are often undetected which largely reduces the elemental composition, and henceforth, this technique is often used for the selective study of known primary metabolites. Alternative techniques such as positive chemical ionization and negative ion chemical ionization are employed to enhance the separation and sensitivity of metabolites in complex mixtures (Raina, 2008). GC-MS is the preferable chromatographic technique for identifying low molecular weight compounds which are either volatile neither converted into volatile & thermally stable metabolites using chemical derivation for analysis. Gas Chromatography- Mass Spectrometry is the robustness, outstanding ability to separate compounds, highly selective nature, responsiveness and also the re-producible results (Tsugawa et al., 2011 and Hall, 2006). GC-MS has the availability for huge number of well-established libraries of both commercial as well as in-house metabolite databases (Koek et al., 2011; Mastrangelo et al., 2015) and (Beale et al., 2018). Metabolite profiling is utilized as an important tool for screening of GM crops with regard to quality and health requirements and in categorization to an investigation of potential changes in metabolic contents. For e.g.; *T. aestivum* (Stamova et al., 2009), *O. sativa* (Zhou et al., 2009), and *Z. mays* (Sissener et al, 2011). The GC-MS technique is the most systematized techniques in the field of metabolomics, with established analytical protocols for carrying out such metabolite analysis occurs in the medicinal plants such as amino acids, sugars, sterols, hormones, catecholamines, fatty acids and aromatics.

1.2. Liquid Chromatography- Mass Spectrometry (LC-MS)

LC- MS is the coupling between Liquid Chromatography and Mass Spectrometry (Warren CR, 2013). In general, separation procedure is performed prior to MS (Mass spectrometry) analysis of most metabolomics studies in the LC-MS. LC-MS used as a flexible and efficient separation process. To analyse polar compounds in plant extracts, hydrophilic interaction chromatography columns are often used (Tolstikov et al., 2002). In liquid chromatography mass spectrometry, the ionization techniques include electrospray ionization and atmospheric chemical ionization coupled with LC–ESI–MS (Codrea et al., 2007; Dunn, 2008); and Grandori et al., 2009). LC–ESI–MS produces molecular ions namely a few like [M + H] +, [M + 2H] +, [M + NH_4] + ions in positive mode of ionization and [M–H] −, [M–2H] −, [M–NH_4]− ions in negative mode, for the identification of the proposed compounds. The method of LC-MS is suitable for metabolites with high molecular weight and thermosensitive and chemically unstable functional groups as it does not need volatilization of the compound. LC-MS is also very useful in the profiling of secondary metabolites (e.g.,

alkaloids, saponins, and phenyl propanidids) and lipids (e.g., sphingolipids and glycerol lipids) (Matsuda et al., 2010); (Okazaki et al., 2011). LC-MS can also be used with various ionization methods and depending on the choice of specific separating columns based on the chemical characteristics of both mobile and stationary phases (Okazaki et al., 2012). Metabolite profiling utilized as an essential tool for screening of GM crops with respect to quality and health requirements and in categorization to an investigation of potential changes in metabolic content by Liquid Chromatography- Mass Spectrometry (LC-MS), e.g., T. aestivum (Niu et al., 2020), O. sativa (Chang et al., 2012), and Arabidopsis (Grata et al., 2008). Several metabolites were analysed using LC-MS were depicted in figure 1.

1.3. Nuclear Magnetic Resonance (NMR-Spectroscopy)

NMR is another popular analytical tool for investigating the varied metabolome in plants, involving the structure, content, and purity of molecules in the sample. As a result, metabolic profiling can provide qualitative and quantitative data from biological extracts (Kim, 2010). Furthermore, the easy sample preparation procedure with excellent repeatability, non-destructive nature enables high throughput and quick analysis in NMR metabolomics but has less sensitivity than Mass Spectroscopy (Emwas, 2015) and (Deborde et al., 2017). NMR is pH sensitive, buffered solutions usually required to maintain the pH stable. A combination of methanol and aqueous phosphate buffer (i.e., pH 6.0, 1:1 v/v) or ionic liquids such as 1-butyl-3-methylimidazolium chloride has been showed to be the most effective in providing a comprehensive overview of both primary and secondary metabolites (Kim and Choi, 2011). There are several merits of this technique which includes considerable reproducibility, high throughput, easy identification of metabolite, and non-destructive sample preparation (Markley et al., 2017). This technique over a period of time has become the preferred approach for evaluating plant metabolites due to its simple and uncomplicated sample preparation protocols and the quick assessment of the NMR spectrum. Advantage of NMR is not confined that the analysis of tissue or bio fluid extract only but analysis of whole tissues, solid and semisolid specimens can easily be made through solid-state NMR as well as magic-angle sample spinning (Blondel et al., 2016); (Diserens et al., 2015); (Hong et al., 2009); (Jang et al., 2016) which can help in de-cluttering the spectra under special conditions. Transgenic maize plants showed lower levels of pyruvic, iso-butyric, succinic, lactic, and fumaric acids than non-transgenics (Piccioni et al., 2020). During seed germination in chickpea, the exogenous uptake of glucose in presence of nitric oxide donor was quantified by using 1H-NMR (Pandey et al., 2019).

1.4. High Performance Liquid Chromatography (HPLC)

HPLC analytical technique lower particle dimension induces a concomitant increase in efficiency and optimal velocity, due to improved mass transfer (Nguyen et al., 2006). Analytical columns with diameters of 1 to 4.6 mm are commonly employed. Short columns are selected for metabolite fingerprinting and longer column with higher plate number for profiling plant metabolomic studies. Since this approach provides very narrow LC peaks as MS detectors with very fast response which is generally mandatory in the recent years. UHPLC-TOF-MS has been recognized to be very efficient for studies of both plant and mammalian metabolomes (Grata et al., 2009 and Wilson et al., 2008) Metabolomic profiling also demonstrated that introduction of the cold and drought Regulatory-protein encoding CORA- gene (*Sb CDR*) encoded from *S. brachiata* into tobacco which enhances salt and drought tolerance by increasing the stress related metabolites such as proline,

threonine, valine, glyceryl acid, fructose, 4-aminobutanoic acid, asparagine (Jha et al., 2021). The overexpression of native *UGPase2* gene induced several metabolites related to Amino Acids, phenolic glycosides such as asparagine, -amino-butyric acid, aspartic acid, glutamine, 5-oxo-proline, 2-methoxyhydroquinone-1-*O*-glucoside, 2-methoxyhydroquinone-4-*O*-glucoside, salicylic acid-2-*O*-glucoside, 2, 5-dihydroxybenzoic acid-5-*O*-glucoside, salicin in transgenic *Populus* plants (Payyavula et al., 2014)

1.5. Capillary Electrophoresis- Mass Spectrometry (CE-MS)

CE-MS is a strong analytical technique for evaluating a large variety of ionic metabolites based on the proportion of charge and size ratio (Obata et al., 2012). This analytical technique provides fast and high resolution of charged compounds from small injection volumes and enables the metabolites characterization based on mass fragmentation (Salem et al., 2020). The use of in-capillary pre-concentration techniques can give further gain of sensitivity and the use of MS detection provides a powerful combination for performing rapid, efficient and sensitive analysis. Ionic compounds are separated into capillaries are based on their charge and size of ions. The samples in CE–MS are separated into the cationic and anionic analysis. In Soga's method for cationic analysis, formic acid used as the electrolyte provides good reproducibility of metabolites (Soga et al., 2006). The metabolites identified using the CE–MS technique physiologically important and similar in organisms. Hence, this technique is employed to profile metabolites of all organisms (Urano et al., 2009); (Ishikawa et al., 2010). CE-MS analyses the metabolites which are important from the physiological point of view and that are similar in all organisms. Furthermore, CE has low sensitivity and reproducibility, poor migration time and lack of Reference libraries so it should be at least an appropriate platform for studying different metabolite compounds from such complex plant samples (Soga et al., 2001), (Williams et al., 2007). Metabolite profiling utilized as an essential tool for screening of GM crops with respect to quality and health requirements and in categorization to an investigation of potential changes in metabolic content by Capillary Chromatography- Mass Spectrometry (CE-MS), e.g., *Oryza sativa* (Sato et al., 2004), *Aeluropus lagopoides*, (Sobhanian et al., 2010), and Maize (Levandi et al., 2008).

S.L. No: -	Plant Species	Analytical Techniques (GC-MS)	Type of Metabolite	References
1	*Plantago ovata*	GC-MS	Primary Metabolite	(Patel et al.,)
2	*Triticum aestivum*	GC-MS	Secondary Metabolite	(Stamova et al.,)
3	*Paeonia rockii, P. potaninii, and P. lutea*	GC-MS	Primary Metabolite	Zhang et al.,
4	*Cicer arietinum*	GC-MS	Primary Metabolite	(Pandey et al, 2019)

5	*Fritillaria thunbergii*	GC-MS	Primary Metabolite	(Cui)
6	*Ocimum gratissimum*	GC-MS	Secondary Metabolite	(Hazzoumi)
7	*Lycopersicon esculentum*	GC-MS	Secondary Metabolite	(Roessner-Tunali)
8	*N. tabacum*	GC-MS	Secondary Metabolite	(Jha et al, 2021)
9	*Oryza sativa*	GC-MS	Secondary Metabolite	(Zhou)
10	*Populus*	GC-MS	Secondary Metabolite	(Payyavula et al, 2014)
11	*P. ovata*	LC-MS	Primary Metabolite	(Jha et al.,)
12	*O. sativa*	LC-MS	Secondary Metabolite	(Chang et al.,)
13	*Solanum tuberosum*	LC-MS	Secondary Metabolite	(Iwaki et al.,)
14	*T. aestivum*	LC-MS	Primary Metabolite	(Niu et al.,)
15	*Strawberry*	LC-MS	Primary Metabolite	(Hanhineva et al.,)
16	*Arabidopsis*	LC-MS	Secondary Metabolite	(Grata et al., 2008)
17	*Green Tea*	LC-MS	Primary Metabolite	(Pongsuwan et al.,)
18	*Panax ginseng*	LC-MS	Secondary Metabolite	(Chan et al.,)
19	*Lactuca Sativa*	NMR	Secondary Metabolite	(Sobolev et al.,)
20	*Nicotiana tabacum*	NMR	Secondary Metabolite	(Choi et al.,)
21	*Dendrobium* Snowflake 'Red Star'	NMR	Secondary Metabolite	(Morita et al.,)
22	*Zea mays*	H^1NMR	Primary Metabolite	(Piccioni et al, 2019)

23	*Mulberry*	NMR	Primary Metabolite	(Fukuda et al.,)
24	*P. ovata*	HPLC	Primary Metabolite	(Patel et al)
25	*Amaranthus hypochondriacus*	HPLC	Secondary Metabolite	(Xie et al.,)
26	*Achyranthes bidentata*	HPLC	Secondary Metabolite	(Li et al.,)
28	*Populus*	HPLC	Secondary Metabolite	(Payyavula et al., 2014)
29	Brassica napus	HPLC	Secondary Metabolite	(Feng et al.,)
30	*Aeluropus lagopoides*	CE-MS	Secondary Metabolite	(Sobhanian et al., 2010)
30	*Oryza sativa* leaves	CE-MS	Primary Metabolite	(Sato et al.,)
31	Catharanthus roseus cell cultures	CE-MS	Primary Metabolite	(Harada et al.,)
32	*Arabidopsis thaliana* seedlings	CE-MS	Primary Metabolite	(Delatte et al.,)
33	*Maize*	CE-MS	Secondary Metabolite	(Levandi et al.,)
34	*Polygala tenuifolia Willd*	UHPLC	Secondary Metabolite	(Xue et al)
35	*Rehmannia glutinosa*	UHPLC	Primary Metabolite	(Li, 2010)
36	*Salvia miltiorrhiza*	UHPLC	Primary Metabolite	(Zhan et al., 2019)
37	*Panax Noto ginseng*	UHPLC	Primary Metabolite	(Toh et al., 2010)
38	*Noto pterygium Franchetti*	UHPLC	Secondary Metabolite	(Su et al.,2019)

Conclusion

In conclusion, metabolomics is an extremely important field that deals with the identification and quantification of metabolites in diverse biological species, making a huge impact on our understanding of gene-environment interactions, mutant characterization, and biomarker discovery as well as bioactive drug compound development. For these reasons, understanding plant metabolism has been a difficult task and the ability to study it through metabolomics represents an essential tool for research on plant adaptation, resistance responses to environmental stress, and for improving plant phenotypes in genome editing programs. High-throughput analytical tools and technique have increased the capability to directly analyze plant samples, which has driven the creation of plant metabolome databases and aided research in various plant physiologies as well as contributing to plant biology more broadly within systems biology.

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