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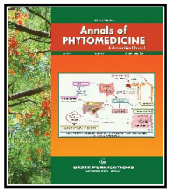

Review Article : Open Access

Metabolic profiling of plant constituents through LC-MS

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Abstract

Metabolomics is the detailed study of small molecules in biological systems and has become an important tool for understanding the complex biochemical composition of plant components. This study specifically investigates the use of liquid chromatography-mass spectrometry (LC-MS) to analyze the metabolic profiles of several plant species. Metabolites are the end results of cellular activities and play an important role in determining the properties and physiological state of plants. Metabolic profiling provides a comprehensive view of the plant metabolome and facilitates the detection and measurement of many substances, ranging from basic metabolites such as sugars and amino acids to secondary metabolites such as flavonoids and alkaloids. First and foremost, you will gain a deeper understanding of the biochemical processes and control systems that control plant metabolism. LC-MS enables the concurrent identification of several substances, giving researchers a complete overview of the plant metabolome. Overall, this study highlights the significance of metabolomics as a revolutionary method in botanical research, particularly in the context of LC-MS for the thorough analysis of plant components.

1. Introduction

In the huge and complicated field of plant biochemistry, studying metabolic components, is one of the most important ways to find out what nature is hiding. This review starts its trip through the world of plants by exploring the interesting field of metabolic profiling of plant constituents using liquid chromatography-mass spectrometry (LC-MS). We will learn about the different types of compounds, how well LC-MS works for analysis, and the useful information we can get from this state-of-the-art technology as we go through the complicated paths of plant metabolism.

1.1 Overview of LC-MS in plant research

The cutting edge of analysis methods is lquid chromatography-mass spectrometry (LC-MS), which has completely changed the way we can figure out the complex web of plant metabolomics (da Silva Oliveira *et al*., 2022). LC-MS pairs the ability to separate things like liquid chromatography does with the ability to precisely find and identify things like mass spectrometry does (Raaijmakers and Kiers, 2022). With this strong combination, scientists can look into the chemicals that plants contain in more detail than ever before (Buhner, 2002). Many important technical advances have led to LC-MS becoming an important tool in plant metabolomics. From its beginnings as a tool for separating and finding things, LC-MS has grown into an important and flexible tool for studying how plants use energy. Initial uses focused on analyzing certain groups of chemicals. However, as mass spectrometry technology has improved, like with the arrival of high-resolution mass spectrometry, the range has grown to include a wide range of metabolites (Junot *et al.,* 2014). Because it can handle complex mixtures, is very sensitive, and can find a lot of different types of metabolites, LC-MS has become an important tool in plant metabolomics study. By learning the basics of LC-MS, we open the door to a better understanding of plant biochemistry and set the stage for further research into the changing metabolic landscapes that shape the biological world.

1.2 Versatility and sensitivity of LC-MS for analyzing a wide range of metabolites

The flexibility and sensitivity of liquid chromatography-mass spectrometry (LC-MS) make it the best tool for studying a wide range of molecules in plant systems. LC-MS is great at handling the complexity of plant metabolomes because it provides a flexible tool for identifying and measuring a wide range of compounds at the same time (Patel *et al*., 2021). Its flexibility comes from the fact that liquid chromatography can sort a wide range of chemical species, and mass spectrometry can find and describe these molecules with amazing accuracy. LC-MS goes beyond the limits of standard analytical methods because it can handle many types of metabolites, such as lipids, phenolics, alkaloids, flavonoids, and terpenoids (Ganzera and Sturm, 2018). As a result of its flexibility, LC-MS has become an important tool for researchers who want to understand the complex web of plant metabolism in a way that was previously unthinkable. When looking at primary or secondary metabolites, LC-MS is a flexible and accurate tool that shows the complex biochemical patterns that make up plant life.

1.3 Diverse applications of LC-MS in plant metabolomics

The liquid chromatography-mass spectrometry (LC-MS) technique is a powerful tool in the field of plant metabolomics. It can be used in a wide range of ways to help us learn more about the complex

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molecular web that plants are made of. First, LC-MS is a dynamic tool for figuring out how plants react to signals in their surroundings. By looking at changes in metabolite composition, it lets scientists look into how plants find their way and react to their surroundings. The LC-MS method shows the molecular techniques plants use to deal with or survive environmental problems, like drought stress and pathogen attacks (Razzaq *et al.,* 2019). This application not only helps us learn more about how plants react to stress, but it also helps us make crop types that can survive in climates that are changing. Secondly, LC-MS is a very important tool for tracking how plants grow and change over time. Researchers can learn more about the biological changes that happen at important developmental stages by looking at metabolite profiles at different stages of growth (Castro-Moretti *et al.,* 2020). This helps us understand things like how seeds sprout, flowers bloom, and fruits age. With LC-MS, you can find and measure specific molecules that mark developmental stages. This helps you understand how plant growth and maturation are controlled by regulatory networks. In addition, LC-MS is very useful for figuring out how metabolism works in particular tissues. Plant parts, like leaves, roots, and seeds, have a huge range of metabolic types. LC-MS lets scientists look at specific metabolites in certain plant tissues, revealing the unique biochemical fingerprints of each (Maag *et al*., 2015). This app is very important for learning about the specific jobs that different plant parts do and how they affect the metabolism of the whole body. Finding tissue-specific secondary metabolites; for example, can help us understand how plants protect themselves and connect with other living things (Bulut *et al*., 2023). In conclusion, the many ways that LC-MS is used in plant metabolomics help us learn a lot about plant biology. From figuring out how plants react to their environment to solving mysteries about growth and studying how tissues change over time, LC-MS is an essential tool for researchers who want to get a full picture of plant metabolism. Not only do these applications add to basic information, but they also have real-world effects on farming, biotechnology, and the long-term management of plant resources in a world that is always changing.

2. Advancements in LC-MS technology

2.1 High-resolution mass spectrometry

High-resolution mass spectrometry (HR-MS) is a big step forward in the field of plant metabolomics (Mihailova *et al.,* 2021). It makes metabolite recognition much more precise and accurate. This part talks about how HR-MS has changed things and how its instruments have improved, showing how important it is for understanding how complicated plant metabolomes are. The accuracy and precision of metabolite recognition are very important for figuring out how plants' biochemical makeup works (Liu *et al.,*2019). According to its ability to measure mass-to-charge ratios very accurately, HR-MS makes metabolite detection more reliable than other mass spectrometry methods (Pengwei, 2019). Because HR-MS has better resolution, it reduces the chance of false positives and negatives. This makes it easier to build accurate metabolic profiles. Improvements in HR-MS equipment help a lot with solving the problems that come up because plant metabolomes are so complicated (Misra, 2021). New developments in mass analyzers and ionization sources have made it possible to separate and find molecules that are very similar. This better resolving power is especially important for separating isomeric species and overlapped chromatographic peaks, which happen a lot in complex plant extracts (Giesbertz *et al*., 2015). The higher precision of HR-MS makes it easier to tell the difference between small changes in mass. This makes it possible to find metabolites that look similar but may play different biological roles. This skill is very important for understanding the subtleties of plant metabolism, where chemicals with similar masses but different shapes can have different physiological roles. The more detailed information we get from HR-MS helps us understand the wide range of metabolic processes that exist in plant systems better. The experimental improvements in HR-MS go beyond resolution and include higher sensitivity and a wider mass range (Laskin *et al*., 2012). These changes make it easier for researchers to find and name a wider range of metabolites, even those that are only present in very small amounts (Xie *et al*., 2012). Especially helpful when looking into low-abundance chemicals that might be biologically important. In conclusion, HR-MS is a big step forward in the field of plant metabolomics. It has improved the precision and accuracy of metabolite identification, and improvements in instruments have made it possible to study complex plant metabolomes in more depth and with more complexity. As scientists keep trying to find new ways to analyze things, HR-MS is one of the most important tools they use. It helps them understand the complicated biology of plants in a way that has never been possible before.

2.2 Integration with chromatographic techniques

When liquid chromatography (LC) and mass spectrometry (MS) work together, they make a strong team. This teamwork is essential for getting the best separation and detection results in the field of plant metabolomics (Maciel *et al.,* 2020). This part goes into more detail about how this synergy works and how chromatographic methods play a key role in making MS better at doing complete and accurate analysis. Together with MS, liquid chromatography is the most important method because it can separate complex mixtures of compounds (Holcapek *et al.,* 2012). When these two analytical powerhouses work together, they give metabolite research a more complete look. LC is the first step and separates substances effectively based on their physicochemical qualities, like molecular size and polarity (Theodordis *et al.,* 2012). By separating the molecules first, the sample becomes simpler and easier to analyze using a mass spectrometer. Chromatographic methods, especially high-performance liquid chromatography (HPLC), help separate chemicals that elute closely together, which is often hard to do with complex plant extracts (Ahmad *et al.,* 2020). A key part of getting the best separation is fine-tuning the chromatographic conditions, which includes choosing the right stationary phase and mobile phase makeup (D'Atri *et al.,* 2018). The careful planning of chromatographic methods makes sure that each compound moves through the column at a different speed. This keeps the compounds from overlapping and allows for accurate detection by MS (Simon *et al*., 2014). Picking the right chromatographic method also affects how selective the analysis is. Assorted chromatographic modes, like reversed-phase, normal-phase, and ion-exchange chromatography, each have their own selectivity profiles (Foster *et al.,* 2022). This means that researchers can make the sorting method fit the properties of the metabolites they are studying. This ability to choose is very important when working with different types of compounds, each of which has its own chemical qualities. There is a close connection between how well chromatography separates things and how well MS can then find them. Using the mass-to-charge ratios of separated molecules, mass spectrometry is like having "eyes" that look at them. When you combine LC and MS, they work better together because LC separates chemicals based on their time differences and

MS separates them based on their mass differences (Kallianta *et al.,* 2023). This dynamic synergy makes the analysis process more sensitive, specific, and accurate as a whole. As the field of LC-MS-based plant metabolomics changes, new technologies in both the LC and MS parts keep making this combination better. LC-MS systems can now separate and identify things better thanks to improvements in chromatographic columns, stationary phases, and ionization techniques (Pitt, 2009). This constant improvement makes sure that scientists can get the most data from difficult plant samples, which leads to a fuller understanding of the complex metabolic patterns found in plants (Beckles and Roessner, 2012). Lastly, combining LC-MS is a great example of a working together well, as the best parts of each method make the other better. It is impossible to say enough good things about chromatographic methods for separation and detection. They are key to understanding the chemical details that make up plant metabolism.

2.3 Bioinformatics and data processing

Bioinformatics tools play a critical role in managing and organizing the vast amounts of data generated by high-throughput technologies such as liquid chromatography-mass spectrometry (LC-MS) (Sugimoto *et al*., 2012). This section discusses the complexity of bioinformatics for LC-MS-based plant metabolomics. It emphasizes how crucial it is to extract meaningful information from the massive volumes of data contained in intricate metabolic profiles. The massive volume and complexity of data produced by LC-MS investigations necessitate the use of sophisticated computational techniques for effective management. Bioinformatics tools play a critical role when working with raw LC-MS data. They adjust baselines, cut down on noise, and align peaks, among other things. Ensuring the accuracy and consistency of the data is crucial, as it establishes the foundation for further research endeavors (Campbell *et al*., 2008). One of the most significant contributions of bioinformatics to LC-MS metabolomics has been the annotation of metabolites (Blazenovic *et al*., 2018). Because researchers might detect thousands of characteristics in a single experiment, it is exceedingly difficult to identify metabolites accurately. Bioinformatics technologies facilitate the alignment of experimental data with pre-existing databases, hence simplifying the process of labeling observed peaks and providing a potential means of metabolite identification (Sugimoto *et al*., 2012). This is a critical step in determining the biological implications of the metabolome alterations. Additionally, interpreting LC-MS data requires a strong foundation in statistical analysis. To identify patterns, relationships, and significant differences in huge datasets, bioinformatics tools can be combined with multivariate statistical techniques (Cichonska *et al*., 2016). Researchers employ methods like principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and clustering algorithms to uncover important patterns and groups (Lee *et al*., 2018). These assist them in identifying the metabolites that are accountable for the majority of the observed variations. Research into metabolic pathways is another field in which bioinformatics is highly beneficial. These tools combine databases of metabolic pathways with LC-MS data to provide you with a deeper knowledge of metabolic networks (Johnson *et al*., 2015). Through pathway enrichment analysis, overrepresented pathways among the identified metabolites are identified. This aids in our comprehension of how alterations relate to function (Wieder *et al*., 2021). This broad perspective aids in our comprehension of the operation and connectivity of metabolic pathways in plants. Additionally, the integration of LC-MS data with other omics data, such as transcriptomics and genomes, is facilitated by bioinformatics tools. The integration of many omics deepens the analysis and provides a comprehensive understanding of how genetic information is translated into metabolic phenotypes. Through the combination of gene expression patterns and metabolomic data, scientists may determine the molecular mechanisms underlying metabolic alterations in response to various stimuli. Despite advancements, bioinformatics for LC-MS data processing remains plagued by issues. Constant work is being done to develop robust algorithms, standardize analysis procedures, and construct comprehensive metabolite databases (Misra, 2021). Moreover, the dynamic nature of plant metabolomes necessitates the continuous updating of bioinformatics tools to accommodate the diverse array of molecules and their behaviors (Chaleckis *et al.,* 2019; Domingo *et al*., 2018). In conclusion, a critical component of the success of contemporary plant metabolomics understands how bioinformatics tools may be utilized to handle and interpret LC-MS data. These instruments enable researchers to link raw data to biologically meaningful information, allowing them to better understand the metabolism of plants. Making the most of LC-MS technology will be crucial as bioinformatics advances since it will provide us with greater insight into the molecular complexity of plants.Figure 1 illustrates bioinformatics analysis using mass spectrometry.

3. Obstacles in plant metabolomics utilizing LC-MS

Standardization is very important in LC-MS-based plant metabolomics because different studies need to be able to repeat and compare their results. It can be hard to draw useful conclusions from different studies because sample preparation, data collection, and analysis are all very different. To fix this problem, people are still working on making sure that all parts of the LC-MS process follow the same set of rules. Sample preparation is the most important part of standards. Improving the consistency of sample handling, picking the right chemicals, and coordinating the extraction methods are all very important steps. Different plant parts need different protocols. The goal is to get a wide range of metabolites while reducing the amount of bias that comes from the extraction process. Standardization in data acquisition means using standardized methods and adjusting instrument settings to get the best results. This makes sure that the data generated is the same in all labs and instruments. To make results more reliable, people are working on making standards that include quality control measures, instrument calibration, and performance validation. To properly analyze the huge amounts of data that LC-MS produces, we need to use common methods. There are ongoing efforts to improve protocols for peak detection, alignment, and metabolite identification to make sure that data processing is consistent. As open-access databases and standardized spectral libraries grow, they make it easier to label metabolites and give the metabolomics community a shared point of reference. By pushing for standardization in LC-MS-based plant metabolomics, researchers can safely compare results from different studies, which makes findings more reliable and strong. Setting rules not only improves the quality of each experiment, but it also makes it easier to combine data from different sources, which leads to a deeper understanding of how plants work.

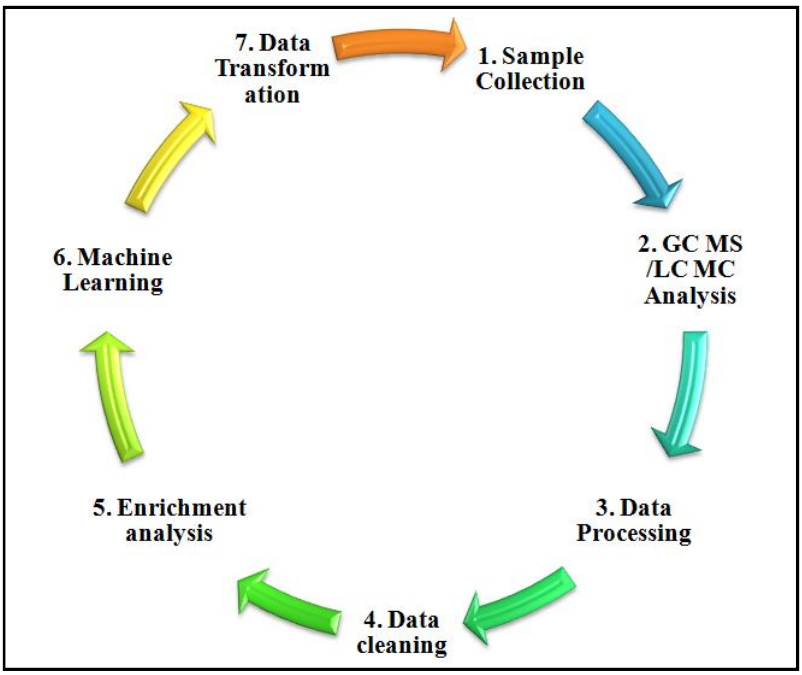

Figure 1: Bioinformatics analysis using mass spectrometry.

4. Prospects for the future of plant metabolomics using LC-MS

4.1 Integration of multiple omics

Putting together genetics, transcriptomics, and metabolomics data is the start of a new era in plant systems biology. It gives us a full picture of how genes, transcripts, and metabolites work together. This method goes beyond the limits of individual omics datasets and gives a full picture of how plants use energy. The basic blueprint of a creature is its genomic information. Researchers can look into how genetic differences affect the plant metabolome by combining genetics and metabolomics. For targeted breeding efforts that want to improve traits like stress resistance or nutritional content, it is important to understand the genetic basis of metabolic diversity. Transcriptomics shows how gene expression changes over time. When you combine transcriptomic data with metabolomics data, you can see how metabolic processes are controlled. This working together makes it possible to find important regulatory points, which helps us figure out how molecules control how plants react to environmental cues or developmental cues. Even though, multiomics integration has a lot of benefits, it also has a lot of problems. Bioinformatics frameworks must be strong in order to coordinate the collection, storage, and processing of data across multipleomics platforms. Standardizing data types and ontologies is necessary for integration to go smoothly. It can also be hard to combine the dynamic nature of transcriptomic and metabolomic information because changes in gene expression over time may not always match up with changes in the abundance of metabolites. Multiomics methods open up a huge number of possibilities. They help us understand plant biology on a systems level, which makes it easier to find biomarkers, understand metabolic processes, and find new ways to control plants. Multi-omics integration is going to be very important for figuring out how plants work as tools and analysis methods keep getting better. Figure 2 presents a step-by-step workflow for plants.

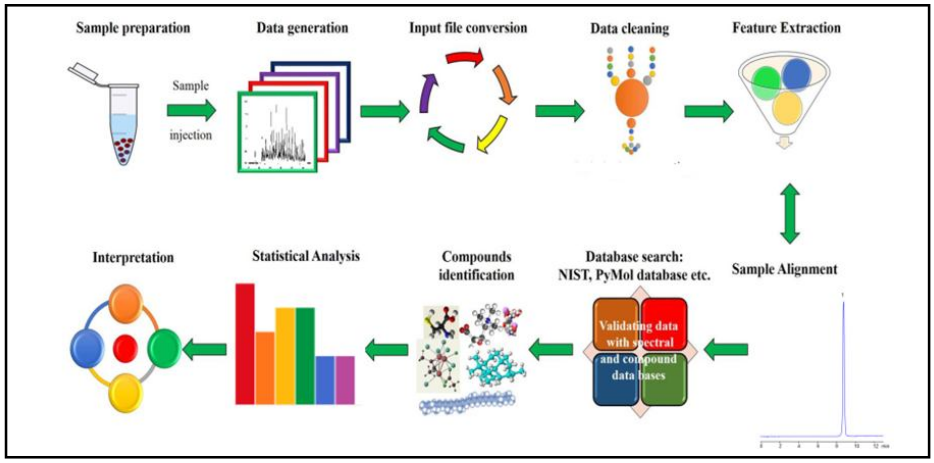

Figure 2: Schematic diagram of the process of metabolomic profiling of plants.

Table 1: Comprehensive list of plants that were examined for metabolomics using LC-MS

Plant species	Metabolites	MS-based method
Cucurbita maxima leaves (phloem) (Vladimir Tolstikov *et al.*,2002)	sucrose, raffinose, stachyose, maltoheptose, UDP-glucose, 1,4-dideoxy-1,4-imino-d-arabinitol, *N*-methyl-1-deoxynojiri-mycin, 2-amino-2-deoxy-d-glucose, *N*-acetyl-d-glucosamine, glucosaminic acid, l-methionine, l-alanyl-l-alanine	HILIC-ESI-QIT-MS
Arabidopsis thaliana leaves (Antonio *et al.,* 2007)	fructose, glucose, maltose, sucrose, trehalose; fructose-6-phos-phate, glucose-1-phosphate, glucose-6-phosphate, sucrose-6 phosphate, trehalose-6-phosphate	PGC-ESI-QIT-MS/MS
Lupinus albus stems (Antonio *et al.,* 2008)	galactinol, inositol, maltitol, mannitol, sorbitol, fructose, glucose, maltose, raffinose, stachyose, sucrose, verbascose	PGC-ESI-QIT-MS/MS
Arabidopsis thaliana leaves (Antonio *et al.,* 2008)	glucose, raffinose, sucrose, verbascose, mannitol, maltitol, glucose-6-phosphate, trehalose-6-phosphate	HILIC-ESI-QIT-MS/MS
Arabidopsis thaliana leaves (Behmuller *et al.,* 2014)	UDP-arabinose, UDP-galactose, UDP-galacturonic acid, UDP-glucose, UDP-glucuronic acid	PGC-LTQ-Orbitrap-MS/MS
Tritium aestivum grains (Warth *et al.,* 2015)	deoxynivalenol, DON-3-glucoside, UDP-glucose, UDP-glucuronic acid	HILIC-ESI-QqQ-IT-MS/MS
Arabidopsis thaliana leaves (Ito *et al.,* 2014)	GDP-fucose, GDP-glucose, GDP-mannose, UDP-arabinofur-anose, UDP-arabinopyranose, UDP-galactosamine, UDP-galactose, UDP-galacturonate, UDP-glucosamine, UDP-glucose, UDP-glucuronate, UDP-xylose	HILIC-ESI-QqQ-MS/MS
Arabidopsis thaliana seedlings (Lunn *et al.,* 2006)	trehalose-6-phosphate	AEC-MS-QqQ-MS/MS
Arabidopsis thaliana seedlings (Umesh Prasad Yadav *et al.,* 2006)	trehalose-6-phosphate	AEC-ESI-QIT-MS/MS
Arabidopsis thaliana seedlings (Chiwocha *et al.,* 2003)	trehalose-6-phosphate	HILIC-ESI-QIT-MS/MS
Glochidion velutinum leaves (Syed Luqman Shah *et al.*, 2022)	benzoic acid derivatives, flavans, flavones, O-methylated flavonoids, flavonoid O- and C-glycosides, pyranocoumarins, hydrolysable tannins, carbohydrate conjugates, fatty acids, coumarin glycosides, monoterpenoids, diterpenoids, and terpene glycosides	LC-MS/MS
Morus alba L. roots, leaves, twigs, and fruits (Zhou *et al*., 2022)	Chlorogenic acid, rutin, isochlorogenic acid A, kuwanon G, kuwanon H, and morusin	HPLC-Q-TOF-MS
Andrographis paniculata aerials parts (Dwivedi *et al*., 2021)	Neoandrographolide, andrographolactone, 14-dehydroxy-11,12- didehydroandrographolide, skullcapflavone I, and 5-Hydroxy-2¡ä,7,8-tri methoxy flavone	HPLC-LC-MS/MS

4.2 The functions of machine learning

In the complicated world of LC-MS metabolomics, machine learning proves to be a useful tool that helps us figure out how complex metabolic networks work. Artificial intelligence plays a bigger and bigger role in finding important patterns and insights in datasets as they get bigger and more complicated. Machine learning algorithms, which include both supervised and unsupervised methods, are very good at dealing with the complicated links in metabolomic data. These algorithms are very good at finding patterns, grouping metabolites, and finding important details that might be hard for standard statistical methods to find in metabolic networks that are very complicated. In particular, supervised machine learning makes it easier to build prediction models for identifying and measuring metabolites. Using machine learning along with LC-MS metabolomics makes data processing faster and more accurate. In order to help find biomarkers linked to certain conditions or phenotypes, algorithms can learn from trends in the data. This speeds up the analysis process and also gives us new information about how certain metabolites work in the setting of plant physiology. Machine learning can be used for more than just analyzing data. It can also be used to create new methods and make existing ones better. Based on feedback, algorithms can change and improve LC-MS methods, making the best conditions for experiments with higher sensitivity and specificity. It is this ability to change that helps make analytical methods in plant metabolomics better all the time. Adding machine learning to LC-MS metabolomics does come with some problems, though. Researchers have to think about things like the need for annotated datasets for training models, how easy it is to understand complicated algorithms, and the possibility of overfitting. Also, because plant metabolism is always changing, it is hard to make models that work well for a wide range of species and environmental situations. To sum up, the most important things for progress in LC-MS-based plant metabolomics are standardization, multiomics integration, and machine learning. Standardized protocols make sure that results are reliable and can be compared. Multiomics integration gives us a full picture of how

plants work, and machine learning improves our ability to analyze data, giving us new insights into the complicated world of plant biochemistry. Together, these methods from different fields move the field forward and promise a better understanding of how dynamic and complicated plant metabolism is.

5. Conclusion

Significant developments in metabolomic platforms have been achieved through analytical progress and the improvement of data processing. Metabolomics analysis has quickly become an essential tool for systems biology studies in medicinal research, including medicinal plants. The metabolomes of medicinal plants are valuable for the development of evidence-based new phytotherapeutics. As we reach the end of our journey through the botanical world with LC-MS as our guide, it becomes abundantly clear that the coming together of technology and biology has ushered in a new era of understanding plant metabolism. In the exciting subject of plant metabolomics, the dynamic interaction of recent accomplishments, ongoing problems, and potential opportunities for the future sets the stage for an ongoing cycle of innovation and discovery.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Ahmad Dar, A.; Sangwan, P. L. and Kumar, A. (2020). Chromatography: An important tool for drug discovery. J. Sep. Sci., **43**(1):105-119.

Antonio, C.; Pinheiro, C.; Chaves, M.M.; Ricardo, C.P.; Ortuño, M.F. and Thomas-Oates J. (2008). Analysis of carbohydrates in *Lupinus albus* stems on imposition of water deficit, using porous graphitic carbon liquid chromatography: Electro spray ionization mass spectrometry. J. Chromatogr. A., **11**(87):111-118.

Antonio, C.; Larson, T.; Gilday, A.; Graham, I.; Bergström, E. and Thomas-Oates J. (2007). Quantification of sugars and sugar phosphates from *Arabidopsis thaliana* tissues using porous graphitic carbon liquid chromatography: Electrospray ionization mass spectrometry. J. Chromatogr. A., **11**(72):170-178.

Antonio, C.; Larson, T.; Gilday, A.; Graham, I.; Bergström, E. and Thomas-Oates J. (2008). Hydrophilic interaction chromatography/electrospray mass spectrometry analysis of carbohydrate related metabolites from *Arabidopsis thaliana* leaf tissue. Rapid Commun. Mass. Spectrom., **22**:1399-1407.

Beckles, D. M. and Roessner, U. (2012). Plant metabolomics: Applications and opportunities for agricultural biotechnology. In Plant Biotechnology and Agriculture, (Academic Press), pp:67-81.

Behmüller, R.; Forstenlehner, I.C.; Tenhaken, R and Huber, C.G. (2014). Quantitative HPLC-MS analysis of nucleotide sugars in plant cells following off-line SPE sample preparation. Anal. Bioanal. Chem., **406**:229-3237.

Blazenovic, I.; Kind, T.; Ji, J. and Fiehn, O. (2018). Software tools and approaches for compound identification of LC-MS/MS data in metabolomics. Metabolites, **8**(2):31.

Buhner, S. H. (2002). The lost language of plants: The ecological importance of plant medicines to life on Earth (Chelsea Green Publishing), pp:56-58.

Bulut, M.; Wendenburg, R.; Bitocchi, E.; Bellucci, E.; Kroc, M.; Gioia, T.; and Alseekh, S. (2023). A comprehensive metabolomics and lipidomics Atlas for the legumes common bean, chickpea, lentil and lupin. Plant J., **116**(4):1152-1171.

Campbell, N. D.; Vogiatzis, G.; Hernandez, C. and Cipolla, R. (2008). Using multiple hypotheses to improve depth-maps for multiview stereo. In Computer Vision-ECCV 2008: 10th European Conference on Computer Vision, Marseille, France, October 12-18, 2008, Proceedings, Part I (Springer Berlin Heidelberg), **10**:766-779.

Castro-Moretti, F. R.; Gentzel, I. N.; Mackey, D. and Alonso, A. P. (2020). Metabolomics as an emerging tool for the study of plant–pathogen interactions. Metabolites, **10**(2):52.

Chaleckis, R.; Meister, I.; Zhang, P. and Wheelock, C. E. (2019). Challenges, progress and promises of metabolite annotation for LC–MS-based metabolomics. Curr. Opin. Biotechnol., **55**:44-50.

Chiwocha, S.D.; Abrams, S.R.; Ambrose, S.J.; Cutler, A.J.; Loewen, M.; Ross, A.R. and Kermode, A.R. (2003). A method for profiling classes of plant hormones and their Metabolites using liquid chromatography–electrospray ionization tandem mass spectrometry. an analysis of hormone regulation of thermo dormancy of lettuce (*Lactuca sativa* L.) seeds. Plant J., **35**:405-417.

Cichonska, A.; Rousu, J.; Marttinen, P.; Kangas, A. J.; Soininen, P.; Lehtimäki, T. and Pirinen, M. (2016). metaCCA: summary statistics-based multivariate meta-analysis of genome-wide association studies using canonical correlation analysis. Bioinformatics, **32**(13):1981-1989.

D'Atri, V.; Fekete, S.; Clarke, A.; Veuthey, J. L. and Guillarme, D. (2018). Recent advances in chromatography for pharmaceutical analysis. Analyt. Chem., **91**(1):210-239.

Da'Silva Oliveira J. P.; de Oliveira, R. T.; Guedes, A. L.; da Costa Oliveira, M. and Macedo, A. F. (2022). Metabolomic studies of anthocyanins in fruits by means of a liquid chromatography coupled to mass spectrometry workflow. Curr. Plant Biol., **32**(2):100260.

Domingo-Almenara, X.; Montenegro-Burke, J. R.; Benton, H. P. and Siuzdak, G. (2018). Annotation: a computational solution for streamlining metabolomics analysis. Analyt. Chem., **90**(1):480.

Dwivedi, M.K.; Sonter, S.; Mishra, S.; Priyanka Singh. and Prashanth Kumar Singh. (2021). Secondary metabolite profiling and characterization of diterpenes and flavones from the methanolic extract of *Andrographis paniculata* using HPLC-LC-MS/MS. Futur. J. Pharm. Sci., **7**(184):1-28.

Foster, S. W.; Parker, D.; Kurre, S.; Boughton, J.; Stoll, D. R. and Grinias, J. P. (2022). A review of two-dimensional liquid chromatography approaches using parallel column arrays in the second dimension. Anal. Chim. Acta., **1228**:340300.

Ganzera, M. and Sturm, S. (2018). Recent advances on HPLC/MS in medicinal plant analysis: An update covering 2011-2016. J. Pharm. Biomed. Anal., **147**:211-233.

Giesbertz, P.; Ecker, J.; Haag, A.; Spanier, B. and Daniel, H. (2015). An LC-MS/MS method to quantify acylcarnitine species including isomeric and odd-numbered forms in plasma and tissues. J. Lipid Res., **56**(10):2029-2039.

Holcapek, M.; Jirasko, R. and Lisa, M. (2012). Recent developments in liquid chromatography: Mass spectrometry and related techniques. J. Chromatogr A., **1259**:3-15.

Ito, J.; Herter, T.; Baidoo, E.E.; Lao, J.; Vega-Sánchez, M.E.; Michelle Smith-Moritz, A.; Adams, P.D.; Keasling, J.D.; Usadel, B.; Petzold, C.J. and Heazlewood, J.L. (2014) Analysis of plant nucleotide sugars by hydrophilic interaction liquid chromatography and tandem mass spectrometry. Anal. Biochem. **448**:14-22.

Johnson, C. H.; Ivanisevic, J.; Benton, H. P. and Siuzdak, G. (2015). Bioinformatics: the next frontier of metabolomics. Analyt. Chem., **87**(1):147-156.

Junot, C.; Fenaille, F.; Colsch, B. and Bécher, F. (2014). High resolution mass spectrometry based techniques at the crossroads of metabolic pathways. Mass Spectrom. Rev., **33**(6):471-500.

Kallianta, M.; Pappa, E.; Vastardis, H. and Rahiotis, C. (2023). Applications of mass spectrometry in dentistry. Biomedicines, **11**(2):286.

Laskin, A.; Laskin, J. and Nizkorodov, S. A. (2012). Mass spectrometric approaches for chemical characterisation of atmospheric aerosols: critical review of the most recent advances. J. Environ. Chem., **9**(3):163-189.

Lee, L. C.; Liong, C. Y. and Jemain, A. A. (2018). Partial least squares-discriminant analysis (PLS-DA) for classification of high-dimensional (HD) data: A review of contemporary practice strategies and knowledge gaps. Analyst, **143**(15):3526-3539.

Liu, X.; Zhou, L.; Shi, X. and Xu, G. (2019). New advances in analytical methods for mass spectrometry-based large-scale metabolomics study. Trends Anal. Chem., **121**:115665.

Lunn, J.E.; Feil R.; Hendriks, J.H.; Gibon, Y.; Morcuende, R.; Osuna. D.; Scheible, WR.; Carillo P. and Hajirezaei, M.R. (2006). Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADP glucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. Biochem. J., **397**:139-148.

Maag, D.; Erb, M. and Glauser, G. (2015). Metabolomics in plant-herbivore interactions: challenges and applications. Entomol. Exp. Appl., **157**(1):18-29.

Maciel, E. V. S.; de Toffoli, A. L.; Sobieski, E.; Nazario, C. E. D. and Lancas, F. M. (2020). Miniaturized liquid chromatography focusing on analytical columns and mass spectrometry: A review. Anal Chim Acta, **1103**: 11-31.

Mihailova, A.; Kelly, S. D.; Chevallier, O. P.; Elliott, C. T.; Maestroni, B. M.; and Cannavan, A. (2021). High-resolution mass spectrometry-based metabolomics for the discrimination between organic and conventional crops: A review. Trends Food Sci. Technol., **110**:142-154.

Misra, B. B. (2021). New software tools, databases, and resources in metabolomics: Updates from 2020. Metabolomics, **17**(5):49.

Patel, M. K.: Pandey, S.; Kumar, M.; Haque, M. I., Pal, S. and Yadav, N. S. (2021). Plants metabolome study: Emerging tools and techniques. Plants, **10**(11):2409.

Pengwei, Z. (2019). Reliability of high resolution mass spectrometry in metabolite identification and untargeted metabolomics studies (Doctoral Dissertation, University of Macau). 30530508.

Pitt J. J. (2009). Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. Clin. Biochem. Rev., **30**(1):19-34.

Raaijmakers, J. M. and Kiers, E. T. (2022). Rewilding plant microbiomes. Science, **378**(6620):599-600.

Razzaq, A.; Sadia, B.; Raza, A.; Khalid Hameed, M.; and Saleem, F. (2019). Metabolomics: A way forward for crop improvement. Metabolites, **9**(12):303.

Simon J.; Hird, S. J.; Lau, B. P. Y.; Schuhmacher, R. and Krska, R. (2014). Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food. TrAC Trends Anal. Chem., **59**:59-72.

Sugimoto, M.; Kawakami, M.; Robert, M.; Soga, T. and Tomita, M. (2012). Bioinformatics tools for mass spectroscopy-based metabolomic data processing and analysis. Curr. Bioinform., **7**(1):96-108.

Syed Luqman Shah.; Kashif Bashir.; Hafiz Majid Rasheed.; Jamil Ur Rahman.; Muhammad Ikram.; Abdul Jabbar Shah.; Kamlah Ali Majrashi.; Sulaiman Mohammed Alnasser.; Farid Menaa. and Taous Khan. (2022). LC-MS/MS-based metabolomic profiling of constituents from *Glochidion velutinum* and its activity against cancer cell lines. Molecules, **27**(24):9012.

Theodoridis, G. A.; Gika, H. G.; Want, E. J. and Wilson, I. D. (2012). Liquid chromatography-mass spectrometry based global metabolite profiling: A review. Analytica. Chim. Acta., **711**:7-16.

Umesh Prasad Yadav.; Alexander Ivakov.; Regina Feil.; Guang You Duan.; Dirk Walther.; Patrick Giavalisco.; Maria Piques.; Petronia Carillo.; Hans-Michael Hubberten.; Mark Stitt. and John Edward Lunn (2014). The sucrose-trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signaling by Tre6P. J. Exp. Bot., **65**(4):1051-1068.

Vladimir, V.; Tolstikov, V.V. and Fiehn O.(2002). Analysis of highly polar compounds of plant origin: Combination of hydrophilic interaction chromatography and electrospray ion trap mass spectrometry. Anal. Biochem., **301**:298-307.

Warth, B.; Siegwart, G.; Lemmens, M.; Krska, R.; Adam, G. and Schuhmacher, R.(2015). Hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry for the quantification of uridine diphosphate-glucose, uridine diphosphate-glucuronic acid, deoxynivalenol and its glucoside: in-house validation and application to wheat. J. Chromatogr. A.,1423:183-189.

Wieder, C.; Frainay, C.; Poupin, N.; Rodríguez-Mier, P.; Vinson, F.; Cooke, J. and Ebbels, T. (2021). Pathway analysis in metabolomics: Recommendations for the use of over-representation analysis. PLOS Comput. Biol., **17**(9):e1009105.

Xie, C.; Zhong, D.; Yu, K.; and Chen, X. (2012). Recent advances in metabolite identification and quantitative bioanalysis by LC-Q-TOF MS. Bioanalysis., **4**(8):937-959.

Zhou, Q.Y.; Liao, X.; Kuang, H.M.; Li, J.Y. and Zhang, S.H. (2022). LC-MS metabolite profiling and the hypoglycemic activity of *Morus alba* L. extracts. Molecules, **27**:5360.

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