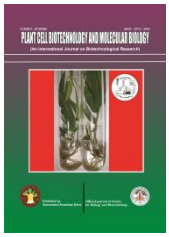

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Metabolomics and Metabolic Engineering for Crop Improvement: Current Trends and Future Prospects

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Metabolomics is a rising field within the realm of “omics,” focusing on the detection and measurement of metabolites and chemical markers associated with cellular regulatory mechanisms across various biological organisms. The exploration of metabolomic control in plant life plays a imperative role in comprehending their ability to adapt, acclimate, and defend against environmental pressures by generating a diverse array of metabolites. Furthermore, the application of metabolomics holds promise in the characterization of plant traits, offering significant prospective for amalgamation into genome editing initiatives aimed at advancing the development of enhanced, future-generation crops. The forefront technologies have introduced economical and high-capacity methods to molecularly analyze the operation of cells or organisms. Cutting-edge analytical methods in metabolomics, such as nuclear magnetic resonance spectroscopy (NMR), liquid chromatography mass-spectrometry (LC-MS), gas chromatography-mass (GC-MS) and high performance liquid chromatography (HPLC) have accelerated metabolic profiling. This review provides an insights into the latest tools in plant metabolomics for enhancing crops and process of plant metabolome research, engaging in plant mechanisms especially for tolerating biotic and abiotic stresses. This review also provide potential approaches to metabolomics through metabolic engineering such as miRNA- and RNAi-Mediated Metabolic Engineering, Genome editing mediated metabolic Engineering etc

Keywords: Metabolomics; metabolite quantification; metabolite engineering.

1. INTRODUCTION

In the present era of smart breeding which emphasis on development of plants with sophisticated traits such as climate smart,multiple biotic and abiotic resistance, nutrionally enrichment have lead to significant advancements in form of “Omics” includinggenomics,transcriptomics,epigenomics, proteomics,metabolomics and phenomics which have revolutionized the breeding activities crucial for ensuring the unavoidable evergrowing food security to sustain livelihood of exponentially increasing population. Structural genomics relies on molecular markers that have pratical applications in tagging and mapping gene of interest. These markers are instruments in crop breeding programs, where they can be strategically deployed to improve crop varieties [1].Genomics encompasses the exploration of gene and genomes, delving into area such as structure, function, evolution mapping, epigenomic, mutagenomic and genome editing [2] its crucial role lies in deciphering genetic variation ,a key feature that can significantly boost crop breeding efficiency and ultimately lead to the genetic enhancement of various crop species. Proteomics is a methodology employed for the comprehensive analysis of all expressed proteins within an organism. It encompasses four main aspects which includes sequence proteomics, structural proteomics, functional proteomics and expression proteomics for comparison of different expression in plants and use it for selection indices [3]. Transcriptomics is a field that revolves around the transcriptome, encompassing the hole collection of RNA transcripts generated by any crop plant’s genome in a specific cell or tissue [4]. By adopting transcriptome profiling, researchers gain access to dynamic and promising technique that facilitates the analysis of gene epression alterations in response to various stimuli. This approach is invaluable in observing the differential expression of genes providing important insights for further research [5 and 6]. Epigenetics are the heritable changes that occur beyond alterations in the DNA sequences, which include DNA methylation and post translational modification of chromosomal proteins [7,8]. The combination of epigenetics and genomics has given rise to novel omics technique known as epigenomics, which aims to unravel the genetic regulation and its role in plant breeding unlike genomics, epigenomics is also influenced by biotic and abiotic stress. Metabolomics is an exciting and rapidly advancing omics technique that has been extensively utilized to improve crops. It entails a thorough examination of metabolites engaged in diverse cellular processes within a biological system. The metabolome on the other hand, encompasses all the metabolites synthesized through metabolic pathways within a plant system. Contemporary metabolomics platforms are now being utilized to carry out metabolite profiling, metabolite identification and metabolite quantification [9,10].

Metabolomics, an emerging and captivating approach among omics tools, has found extensive application in enhancing crop productivity. It perform a important role in studying various aspects of crop improvement, inclusive of biotic and abiotic strain in plants [11], as this factors involves complex responses breeding for such traits are challenging. Over time, noteworthy advancements have been made in developing modern metabolomics tools dedicated to crop enhancement.

The crop plant have immense diversity of metabolites, most of which remain unidentified. These metabolites exhibit diverse biochemical properties and functions, making them highly significant in plant biology and crop breeding as a tool for tapping the variability at metabolite level through selection [12] , with the utilization of modernized metabolomics platforms, researchers are now able to decipher complicated biological pathways of crop growth and development. This enables a deeper understanding of how crops respond to different factors and helps in developing strategies for crop improvement.Crop production is adversely affected by biotic and abiotic stresses, resulting in a significant reduction in annual crop production [13]. These stresses include herbivores, insects, pathogens, salinity, trace metal contamination, drought, and extreme temperatures. They disrupt various physiological and morphological aspects of plants, hindering the function of crucial molecules such as enzymes, polynucleotides, and transport of ions and nutrients, along with overall growth and metabolic activities of plant [14].To combat these stresses, biologists, especially agriculturists, are seeking alternative approaches. Plants have developed several mechanisms to handle with these challenges, including metabolomics, genomics, proteomics and transcriptomics, either independently or in collaboration. Metabolomics focuses on analyzing the plant metabolome, which comprises primary plant metabolites and secondary plant metabolites. Plants and microorganisms require primary metabolites for optimum growth and development. Secondary metabolites, on the other hand, are formed during the stationary phase of growth and do not directly contribute to growth, reproduction, or development. By investigating the metabolic profiles of primary and secondary plant metabolites. Researchers learn a lot about the physio-chemical and biochemical processes that are concerned in plant's total metabolism. This understanding assists in the creation of measures to mitigate the detrimental effects of biotic and abiotic factors during crop production.

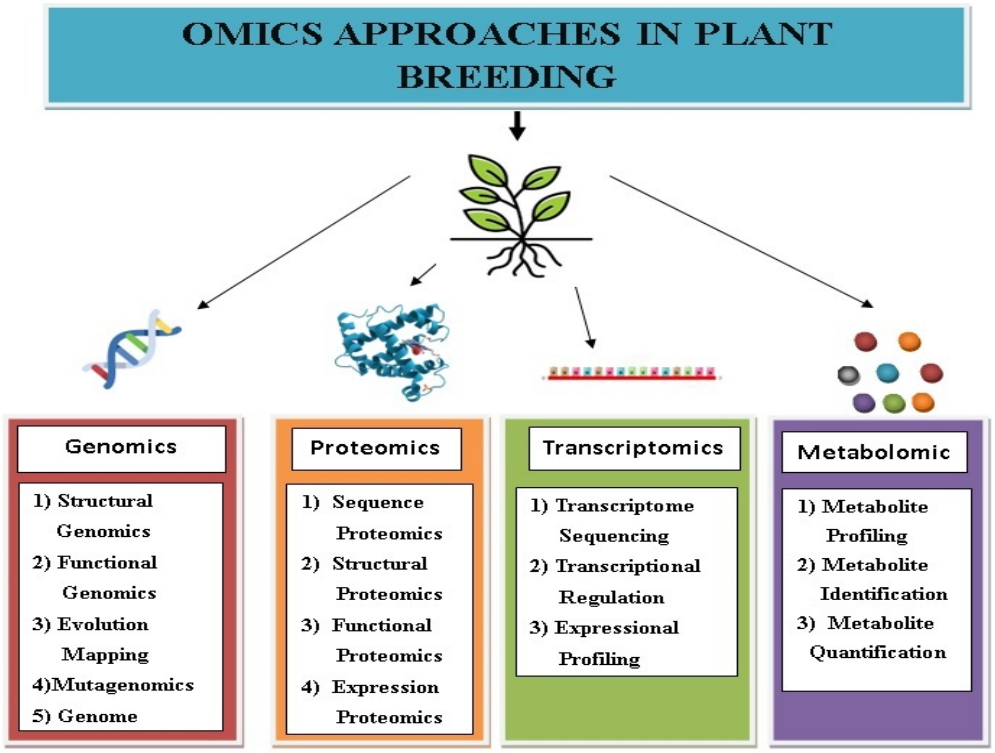

Fig. 1. Chart showing different omic approaches and there scope in plant breeding

Table 1. Difference between primary and secondary metabolite found in plants.
Secondary metabolites (SM's)

Features	Primary metabolites	Secondary Metabolite
Defination	Organic compounds required for the maintenance and growth of cellular functions, generally initiated when nutrients required are present in the medium for plant.	Organic substances that are not directly involved in plant growth or development and reproduction.
Quantity produced	Produced in large quantities.	Produced in small quantities.
Extraction process	Easy to extract.	Difficult to extract.
Differential production	These produce same products in every plant species.	These produce different products in every plant species.
Examples	Carbohydrates, Proteins, Lipids, Vitamins etc..	Alkaloids, Phenolic compounds, Terpenoids etc.

2. NITROGEN-CONTAINING SM's

2.1 Alkaloiods

These are the SM's that are synthesized from amino acids and contain one or several nitrogen atoms as a constituents of heterocycles.

Examples- Coniine, Nicotine, Cocaine, Atropine, Morphine, Caffeine etc.

2.2 Nitrogen and Sulphur containing SM's

These are SM's are naturally occuring sulphur linked glucosides found in *Brassicdeae sps*.

Examples- Glucosinolates.

2.3 Phenolic Compounds

Plants are known to synthesize a wide range of SMs with a phenol group, which is a hydroxyl functional group linked to an aromatic ring and is sometimes referred to as phenol. This class of chemicals is highly varied and plays an important function in plant defense mechanism.

Examples- Phenolic acids,Coumarins, Lignans, Flavonoids, Tannins, Xanthones, Quinonoids etc…

2.4 Terpenoids

These are also known as isoprenoids derived from isoprene.

Examples- Monoterpenes, Sesquiterpenes, Diterpenes, Sterols, Triterpenes etc…

3. EVOLUTIONARY DIVERSIFICATION OF METABOLITES

Plants produce diverse lineage specific metabolites. Plant lineage have modified the fundamental metabolic pathway of photosynthesis carbon fixation in various ways, giving rise to C_4 photosynthesis and crassulacean acid metabolism. Despite of its limitations in terms of high metabolic costs involved, such as carbon fixation occuring twice and the need to regenerate phosphoenol pyruvate. These modifications provide adaptive advantages, by concentrating CO_2 and reducing the oxygenation side reaction of RubisCo(Ribulose bisphosphate carboxylase oxygenase), photorespiration is attenuated making it advantageous under specific environment [15,16] comparative analysis shows that presence of " pre-conditions" or "enabling traits" in specific plant lineages that facilitated this process [17,18]. These enabling traits are "genetic enablers" for expression of C_4 enzymes [19] and "anatomical enablers" such as kranz anatomy in C_3 ancestoral plants [20]. Further "metabolic enablers" involving transportation of glycine from mesophyll to bundle sheath cells resulting in C_2 photosynthesis and found in many closely related C_4 lineage [21].Similar diversification is observed in isopentenly pyrophosphate(IPP) and phenylalanine pathways through methylerythritol phosphate (MEP) and mevalonate (MVA) pathway for synthesis of terpenoids and phenylpropanoids [22]. Plant lipid metabolism is also characterized by vast diversity. Most lipids contain major acyl chains such as linolenic acid (18:3), linoleic acid (18:2) and oleic acid (18:1). However, some plants, such as castor and vernonia, generate atypical fatty acids composed primarily of epoxy fatty acids. This modification helps us to understand the variability among plant lineage.Since, SM's are derived from primary metabolites these also show variable diversification . Plant kingdom harbors an extensive array of metabolites with majority remaining unidentified to the date . Plant metabolites posses distinct structural, functional, biochemical properties,which can be utilized as a possible tool for elucidating biochemical pathways and uncovering unnown regulatory networks and pathways that affect crop life cycle

3.1 The Workflow of Metabolomics Analysis

Plant metabolomics studies involve several critical steps, including prompt sample harvesting, storage, metabolite extraction, quantification, and subsequent data interpretation. Sample preparation is a crucial aspect, as it can significantly impact the metabolite levels. Therefore, it is essential to minimize the duration between harvesting and storage to prevent biochemical reactions in plant [43]. Inadequate sample management during collection can lead to erroneous plant meta bolomic research. The steps are described as follows:

3.2 Sample Preparation

Sample groundwork plays a essential role in metabolomics and significantly influences the outcome [44]. plant tissues, including seeds,

stems, and roots, are suitable for sample preparation [45]. The goal of any sample preparation involves separation metabolites from undesired components and increase the concentration of the desired or targeted metabolites. The ideal preparation method must be quick accessing, cost-effective and non-disruptive to maintain sample integrity [46].

3.2.1 Sample harvesting and storage

In plant metabolomics, the manner usually entails four principal steps: harvesting, storage, extraction, and analysis. During plant harvesting, caution is necessary, as enzymatic reactions can degrade various metabolites due to the sensitivity of the plant's metabolome. Moreover, metabolite composition can vary depending on factors such as growth stage, age of sample plant, and the time of harvest. Metabolomics studies usually require ten to hundred mg of biological sample. After harvesting, it is common practice to rapidly freeze the plant samples in liquid nitrogen to avoid metabolic changes.

3.2.2 Sample extraction

The selection of solvents is a key aspect since a single solvent may not be able to extract various metabolites, including polar, nonpolar, and hydrophilic compounds. A widely used solvent system in plant metabolomics is composed of chloroform: methanol: water, capable of extracting a wide variety of metabolites [47]. Other solvent systems reported in plant metabolomics include pure methanol, methanol: water mixture, and methanol: methyl-tert-butyl-ether (MTBE): water [48-51]. Some specialized methods, like specific solvent gradient extraction, hot methanol extraction, and various extraction techniques, such as microwave assisted sample extraction technique, ultrasound assisted extraction technique, Swiss rolling extraction technique, and finally enzyme assisted sample extraction technique, are used [52-55].

Table 2. Response of metabolites in plants during variable environmental conditions

Metabolite	Function	Reference
Primary metabolites		
1) Amino acids (Proline)	Osmoprotectant during osmotic stress, redox balancing, ROS scavenging, pH buffering,protein structural stabilization and molecular chaperon.	[23-25]
2) Polyamines (Putrescine,Triamine spermidine,Tetramine spermine)	Act against drought stress, chilling, heat, salinity stress by suppressing ROs production.	[26- 28]
3) Carbohydrates Soluble sugars Sugar alcohols	Act as osmoprotectant during osmotic stress, scavenging of ROs, mentain turgor Act as osmoprotectant during low temperature stress and avoid adhesion of ice.	[30, 31]
4) Lipids	Act as signalling molecules during temperature, drought, heavy metal, salinity stresses by scavenging ROs molecules.	[32-34]
Secondary Metabolite		
1)Phenolic compounds (Caffeicacid, Flavonoids,Suberin, Lignin, Coumaric acid)	Act against heavy metals, ROs, water stress, drought, UVr, low temperature stresses by scavenging ROs molecules, regulation of antioxidants mechanisms, lignification of cell wall.	[35- 37]
2) Terpenoids (Tocopherol, Saponins,Gossypol, Momilactones)	Act against biotic and abiotic stresses by stabilizing cell membranes, enhancing antimicrobial properties, antioxidant.	[38- 40]
3) Nitrogen containing SM's (Alkaloids, Glucosinolates)	Act against drought, herbivores through osmoprotection and defense properties	[41,42]

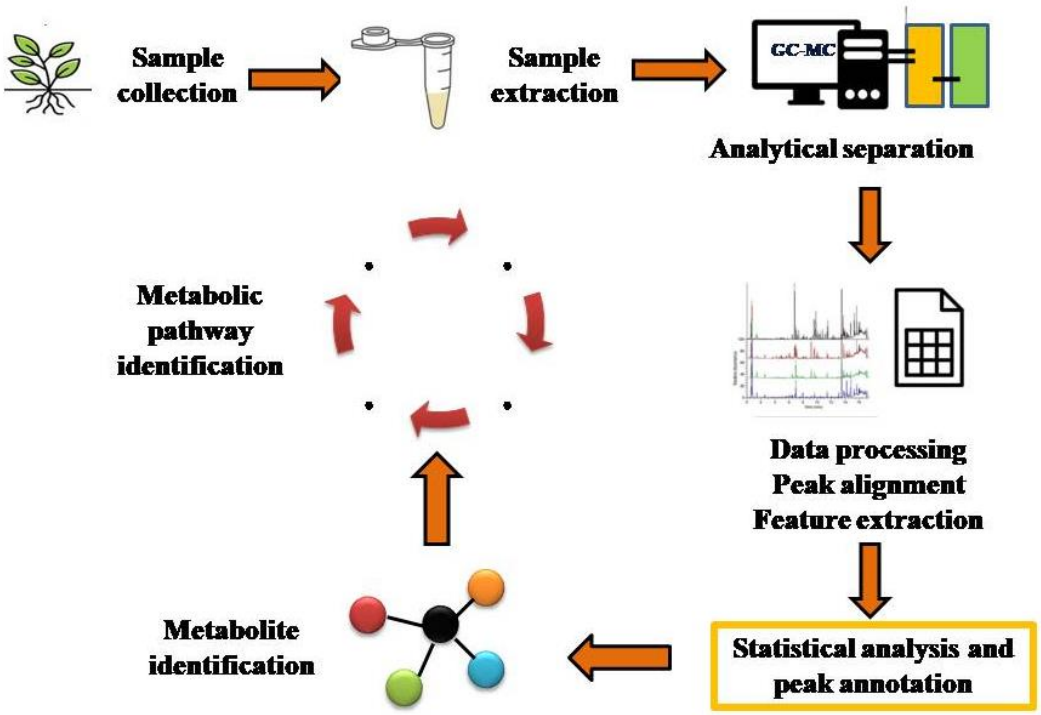

Fig. 2. Flowchart of procedures involved in plant metabolomics for crop improvement

For metabolomics investigations, targeted and untargeted metabolite identification and quantification of such metabolites are the primary objectives [56]. Sample preparation for target metabolites involves enriching the desired metabolites and removing contaminants, like proteins and salts, which may hinder with the analysis. The choice of extraction protocol depends on dissolution rate and solubility of metabolites, considering interactions with biological components such as cellulose or lignin wchich are major constituents of plant cell. Traditional methods like Soxhlet extraction, solid-phase microextraction, laser microdissection (LMD), and microwave-assisted extraction (MAE) are utilized for sample extraction. To analyze volatile metabolites, the supercritical fluid extraction method is efficient[57] . LMD is particularly useful in isolating desired cells from microscopic samples without affecting the chemistry and morphology of the metabolites.

3.3 Analysis of Plant Metabolites

Analyzing plant metabolites in metabolomics presents significant challenges due to the limited concentration of many SM's , detecting certain metabolites , instrument incompatibility, absence of accurate and standardized protocols, and the volatility nature of desired metabolites. In metabolomics, it is important to use a combination of different technologies instead of relying on a single technique to ensure the most comprehensive coverage of metabolites.Metabolomics techniques encompass various methods, including mass spectrometry (MS) , nuclear magnetic resonance spectroscopy (NMR) non distructive,liquid chromatographymass spectrometry (LC-MS) ,gas chromatography-mass spectrometry (GC-MS) ,Fourier transform ion cyclotron resonance mass spectrometry (FI-ICR-MS) ,capillary electrophoresis-mass spectrometry (CE-MS) .

3.4 Nuclear Magnetic Resonance (NMR)

NMR is a widely used coherenttool for studying the diverse metabolome of crop plants, enabling investigation of molecular structure, purity, and molecular content. Metabolic profiling through NMR provides both quantitative and qualitative data from plant biological extracts (<~50 kDa)[58].NMR-based metabolite identification relies on detecting radio frequency electromagnetic emissions from atomic nuclei having an odd atomic number (e.g., ^{1}H) or with odd mass number (e.g., ^{13}C,^{15}N and ^{31}P) in presence of strong magnetic field. The appeal of

NMR lies in its ability to avoid the need for chromatographic sample derivatization, leading to its significant growth in recent years [59,60]. Further NMR-based metabolic profiling proves to be a rapid, efficient, and non-destructive technology, needs less sample preparation making it highly suitable for screening and identifying similar biological samples.NMR has a narrow range, lower resolution, and sensitivity compared to MS,resulting in limited coverage of primary and secondary plant metabolites during any metabolic experiment [61].Developments such as miniaturized radiofrequency coil based,superconducting magnets [62], cryogenic probes based and multi-dimensional based NMR techniques [63] have significantly improved NMR technology.

3.5 Gas Chromatography and Mass Spectrometry (GC-MS)

GC-MS is an optimal method for identifying and quantifying small metabolites, (usually about 500 Da in molecular weight).Thes e molecules encompass sugars,amino acids, fatty acids, hydroxyl acids, alcohols,aminesand sterols,often chemically derivatized**,**to perkup thei r volatility for gas chromatographic analysis [64]. GCMS extraction and metabolite identification inv olve two derivatization steps. The first step uses methoxamine hydrochloride to convert all carbon yls to their corresponding oximes.The second step involves the use of derivatization reagents such as N-methyl-N (trimethylsilyl) trifluoroacetamide (MSTFA) and N,O- bis-(trimethylsilyl)- trifluoroacetamide (BSTPA) for Trimethylsilylation to increase the volatility of derivatized metabolites [65-67].GC-MS is the technique used mainly for the identification of lower molecular weight compounds which can be converted to inactive and thermally stable with help of derivatization process prior to sample analysis [68]. unique methods are required for the identification of primary plant metabolites.

The identification of different classes of plant metabolites often relies on a blending of mass spectrometry and chromatography. It is vital to choose the correct ionization technique and analyte for metabolite identification in mass spectrometry technique. The method involves counting ionized molecules with mass spectrometry (MS), followed by measuring the mass-to-charge ratio of the ions formed in a smaller or higher resolution mass spectra in mass spectrometer Analyzer. High resolution mass analyzers can determine the elemental composition of identified ions present in the mass spectrum. Today, MS systems loaded with electron spray ionization system and matrix assisted laser desorption sources are used. Basically two types of ionization are used in GC-MS technique chemical ionization electron ionization. Currently, most methods in metabolomics analysis use electron ionization [69].

3.6 Liquid Chromatography and Mass Spectrometry (LC-MS)

LC-MS is one of the most extensively used methods plant metabolome analysis, capable of measuring a wide range of metabolites. This method is particularly suitable for metabolites with higher molecular weight (> 500 kDa), compounds which are thermally liable and chemically unstable and higher vapour content in them .Unlike other methods, LC-MS does not require volatilization of metabolites. LC-MS is effective in identifying many metabolites, including secondary metabolites (SM's) like phenols, alkaloids, flavonoids and terpens along with lipids such as glycerolipids, phospholipids, sphingolipids, steroids and sterols[70,71].LC-MS uses an electron spray ionization source to analyze polar and thermally unstable higher molecular weight metabolites.A distinct feature of LC-MS is its ability to directly probe metabolites.Further it’s has Easy sample preparation no need of derivatization, presence of multiple MS detectorsand can detect large number of metabolites important for plant metabolism[72].

3.7 Capillary Electrophoresis and Mass Spectrometry (CE-MS)

CE-MS stands as a powerful analytical method often employed to assess a wide variety of ionic plant metabolites taking into account the charge-to-mass ratio [73]. This procedure allows for rapid and higher-resolution separation of charged molecules from small sample volumes [74]. Further it is fast due to absence of derivatization process making it more efficient.

3.8 Fourier Transform ion Cyclotron Resonance and Mass Spectrometry (FTICR-MS)

FTICR-MS stands out as a mass spectrometry technique that offers unparalleled resolving power and mass accuracy compared to other methods [75]. Its distinctive analytical capabilities

have established FTICR as a crucial tool in proteomics and metabolomics, especially for molecules with a range below 1500 Dalton. The inherent capacity and ability of FTICR-MS to provide qualitative and quantitative data has become widespread practice in metabolomics research [76]. Additionally, it can be used to identify unknown metabolites based on mass-to-charge ion chemistry using higher resolution. However, difficulties such as higher magnetic field requirement, ion to ion interactions and higher costs prevent it from being widely used in plant metabolomics research [77].

3.9 Statistical Analysis

Identifying metabolites relies on analyzing data using various statistical methods. Connecting metabolic markers to phenotypic variables involves utilizing a multidimensional statistical platform for efficient analysis and understanding the associations between metabolites and phenotypic traits. One way to discover specific biomarkers is by employing pairwise Pearson's correlation, which identifies metabolites linked to the desired phenotype. However, developing a predictive model often requires analyzing multiple metabolites, and this is where canonical correlation analysis (CCA) comes into play, aiming to maximize correlations between variables [78].To handle high-throughput metabolomics data, statistical tools originally designed for other omics technologies, such as transcriptomic analysis, can be adapted. Generally, researchers first use different univariate techniques such as analysis of variance (ANOVA),t-test and Mann-Whitney U-test to look for groupwise differences in metabolomic data. Univariate analysis focuses on individual variables at specific time points and aids in biomarker discovery and validating potential metabolic markers [79]. In contrast, multivariate analysis is valuable for tasks such as screening plant cultivars, disease diagnosis, and discovering metabolic markers. There are many diverse multivariate statistical tools such as Principal Component Analysis (PCA), ANOVA, ANOVA-Concurrent Component Analysis (A-SCA), Heat Map Analysis and Partial Least Squares Statistical Analysis (PLS-DA). The selection of appropriate analysis tools should align with the experimental design of the study [80]. A number of exceptional R programming packages have emerged to meet a broader range of applications in metabolomics . This R package provides rich graphics including functions and various statistical tools [81].

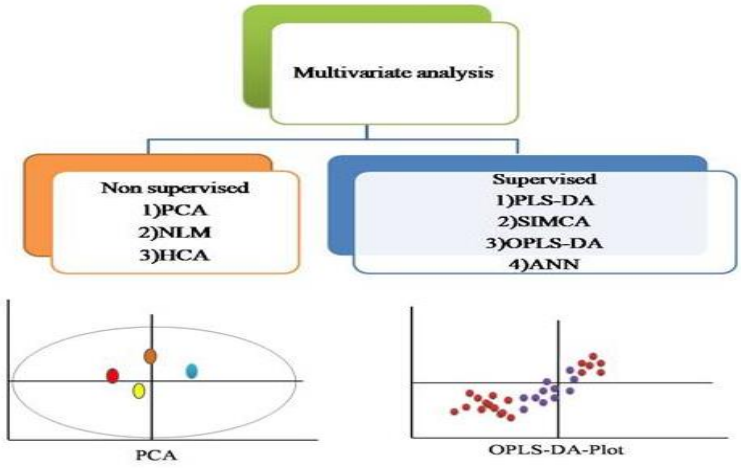

Fig. 3. Different statistical tools with graph

Table 3. List of bioinformatics tools for statistical analysis

Tool	Link	Reference
MetabR	http://metabr.r-forge.r-project.org/	[82]
MetaboDiff	http://github.com/andreasmock/MetaboDiff/a	[83]
MetaboAnalystR	https://github.com/xialab/MetaboAnalystR	[84]
Lilikoi	https://github.com/lanagarmire/lilikoi	[85]
MetaboAnalyst	www.metaboanalyst.ca/	[86]
Babelomics 5.0	http://www.babelomics.org/	[87]

4. BIOINFORMATICS ANALYSIS

Various software's are available especially for in silico data analysis of large amounts of metabolite profiling data. Web based tools are predominantly useful for tasks such as mining raw data processing, and metabolite integration. Data processing includes baseline correction, background noise reduction, peak detection of metabolite, alignment, and mass spectral deconvolution. Due to rapid advances in analysis and technology, time constraints are no major concern for metabolomics data mining. Computational computing plays an chief role in metabolomics experiments.In current years, many online services have been developed to facilitate metabolomic data mining, data analysis, data processing and data interpretation. These user friendly platforms make the creation and maintenance of web based tools accessible to researchers with narrow bioinformatics skills and computing equipment.

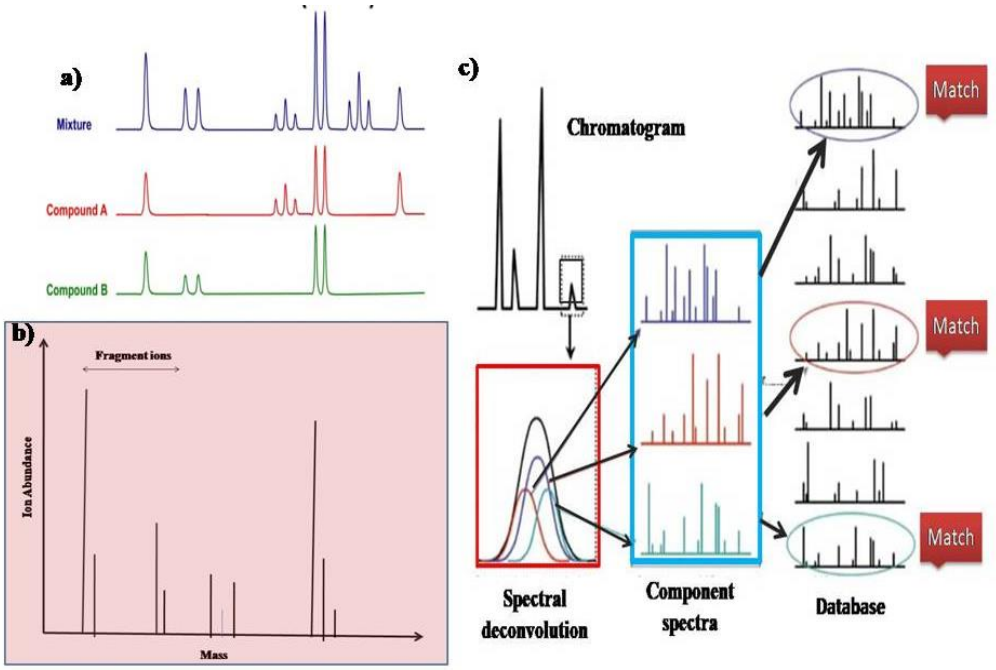

Fig. 4. a) Graph depicting spectral deconvolution of NMR ,b) Spectrum graph (m/z) of GC-MS and c)Metabolite identification by GC-MC output

Table 4. List of bioinformatics tools for plant metabolomics

Tools	link	Use	Reference
ADAP	http://www.du-lab.org/software.htm/	GC/TOF-MS - Data processing	[88]
AllCSS	http://allccs.zhulab.cn/	Metabolite prediction andannotation for DTIM-MSTWIM-MS	[89]
AMDIS	http://www.amdis.net/	Data Processing for GC-MS-	[90]
BinBase	https://fiehnlab.ucdavis.edu/projects/binbase-setup	Metabolite Annotation for GC-MS-	[91]
FiehnLib	http://fiehnlab.ucdavis.edu/db	Metabolic	[92]

Tools	link	Use	Reference
		profiling for GC-qTOF-MS	
GMDB	https://jcggdb.jp/rcmg/glycodb/Ms_ResultSearch	Metabolite annotation for MALDI-TOF	[93]
GNPS	https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp	Data processing, visualization and metabolite annotation GC-MS-EI-LC-MS	[94]
KNApSAcK	http://kanaya.naist.jp/KNApSAcK/	Metabolite Database for FT/ICR-MS	[95]
MarVis	http://marvis.gobics.de/	Metabolite annotation for LC-MS	[96]
MassBase	http://webs2.kazusa.or.jp/massbase/	Metabolite annotation	[97]
MAVEN	https://maven.apache.org/	Data processing for LC-MS	[98]
MeltDB 2.0	https://meltdb.cebitec.uni-bielefeld.de	Data processing for GC-MS and LC-MS	[99]
Metabolome Express	https://www.metabolome-express.org	Data processing, Visualization and statistical analysis for GC-MS	[100]
MetaboSearch	http://omics.georgetown.edu/metabosearch.html	MS Data annotation	[101]
MetAlign	www.metalign.nl	Data processing and Statistical analysis for GC-MS and LC-MS	[102]
MetAssign	http://mzmatch.sourceforge.net/	Data annotation for LC-MS	[103]
METLIN	https://metlin.scripps.edu/	Metabolite annotation for LC-MS and MS	[104]
Molfind	http://metabolomics.pharm.uconn.edu/Software.html	Metabolite annotation for HPLC/MS	[105]
NIST	http://www.nist.gov/srd/nist1a.cfm	Metabolite annotation for GC-MS and LC-MS	[106]

Tools	link	Use	Reference
PRIMe	http://prime.psc.riken.jp/	Metabolite annotation for GC-MS,LC-MS and CE-MS	[107]
XCMS	https://xcmsonline.scripps.edu	Data processing of GC-MS and LC-MS	[108]

4.1 Network Analysis

Pathway analysis is integration of biochemical knowledge with gathered metabolomic data for identifying metabolite patterns that align with specific metabolic pathways. Metabolic pathways can be understood as clusters of metabolites linked by one or more enzymatic reactions, sharing a common biological process. Comprehensive metabolic pathway databases encompass a ample range of these metabolic pathways.

4.2 Crop Improvement through Metabolomics

Metabolomics has seen significant advancements in software tools and instrumentation, allowing high-throughput scanning of complete metabolome in various plant species. It has become a commanding tool for deciphering abiotic stress tolerance in plants and studying distinctive metabolites during their growth and development. Both biotic and abiotic stresses can impact crop yield by affecting biochemical and physiological processes, and metabolomics aids in detecting these changes at the molecular level. By combining metabolomics with other profiling technologies like transcriptomics and proteomics, scientists gain a comprehensive understanding of an organism's biological responses to environmental changes. This versatile approach finds applications in gene annotation, metabolic pathway unraveling, biomarker evaluation from transgene expression, clinical diagnostics, environmental research, drug action research, plant taxonomy, biotechnological engineering, food nutritional science, and more.Metabolomics provides a comprehensive perspective on cellular metabolites, such as small organic compounds, involved in various cellular processes, reflecting the cell's physiological state. The rapid advancements in metabolomics offer the potential to study mutants and transgenic lines, aiding in understanding metabolic networks and identifying candidate genes [115,116]. Additionally, it helps reveal how specific genes impact metabolic pathways and uncover regulation and interactions between linked pathways, which is challenging with conventional assays like microarray [117]. Integrating genomics, transcriptomics, proteomics, and metabolomics enables researchers to prioritize genes for enhancing essential traits in crop species.

The combination of post-genomic approaches and metabolomics has speed up the screening process, leading to the development of improved crop plants with better tolerance against various stresses. The time needed for this development can be reduced by integrating metabolomics with other high throughput tools. Metabolomics offers a comprehensive analysis of metabolites, aiding in diagnosis and phenotyping. Understanding metabolic networks can play a crucial role in breeding programmes using metabolomics assisted breeding to create superior cultivars with improved quality and yield. Multi-omics tools, combined with forward and reverse genetic approaches, can identify candidate genes responsible for secondary metabolite biosynthesis [118]. Metabolomics shows promise in aiding the selection of superior traits and enhancing breeding materials [119]. Alongside the progress in metabolomics, the accessibility of whole genome sequences, genome-wide genetic variants, and affordable genotyping assays create an exciting chance to seamlessly incorporate metabolomics into crop breeding initiatives [120].

4.3 Metabolic Engineering

Metabolic engineering plays an vital role in improving plant diversity against climate change [142,143]. This technology improves plant biotic and abiotic stress tolerance, contributes to sustainable agriculture. Advances in genomic technology allow us to identify genes and metabolic processes responsible for the increase

of biotic and abiotic tolerance. However, this process is complex and requires a deep understanding of the core pathway, transcriptional factors and genetics. Several genes associated with abiotic stress have been identified and their expression patterns during stress are being investigated. Plants naturally produce osmoprotectants in response to abiotic stress., but their concentration is often insufficient to provide complete protection. Variations in osmoprotectant levels exist even among cultivars of the same species. By utilizing cultivars with high osmoprotectant production, there is an opportunity to develop tolerance to adverse environmental conditions [144]. Progress erstwhile made identifying and characterizing genes responsible for osmoprotectant biosynthesis [145]. This knowledge can facilitate the advance in transgenic plants with increased osmoprotectant concentrations, making them better in harsh environmental conditions and potentially leading to increase in yield and biomass.

Agricultural crops face numerous threats from diseases and pests, causing significant damage to yields worldwide. More than half of the world's crop production is destroyed each year due to these issues. Farmers encounter various pests and diseases, including pesticide-resistant microorganisms, posing additional challenges.To address these challenges, genetic modifications are exploited in commercially cultivated and important crops, such as insectresistant traits through the Bt gene producing endotoxin and herbicide tolerance technique using glyphosate. The successful incorporation of these traits in crops like cotton, soybeans, and maize has been significant.One widely studied metabolic engineering approach to enhance plant defense against pests and diseases is the incorporation and stilbene synthase gene product. Stilbenes, characterized by presence of central ethylene group covalently bonded to a phenyl group in each division[146,147], play a crucial role in plant defense. Stilbenoids, like resveratrol, are derived from phenylalanine metabolism via the phenylpropanoid pathway. Stilbene synthase uses the metabolites malonyl-CoA and p-coumaroyl-CoA as substrates for the manufacture of resveratrol. Conversion of stilbene biosynthetic genes to other types stabilizes stilbene-bound phytoalexins. For example, stilbene synthase from peanut induced resveratrol production in unhoused tobacco plant cells using fungal elicitors [148]. Advances in biotechnology and bioinformatics tools have made it easier to predict and develop plant based gene expression system.For example, artificial gene cluster and specific transcription factors can be used to control the regulation of natural controled processes in plants, bring aboutg in a better results.

However, notable differences arise when metabolic processes are developed to protect against disease or increase the amount of the compound. In these cases, the production of metabolites must clearly occurs during infection or pest infestation. Continued production of metabolites is detrimental because high levels can have adverse effects on plants. Therefore, providing the right balance and time in metabolite production is important for good preservation without problems. In anabolic engineering, several techniques are employed to improve the synthesis of products:

1. First approach involves increasing the expression of upstream genes involved in encoding key enzymes in the target pathway. Ensuring adequate supply of key precursors and boosts the metabolic rate in the mark pathway.
2. Second approach involves suppressing the enzyme gene expression in competitive way or branch points to prevent the abstraction of intermediates and preserve the target metabolite.
3. Third approach involves over expression of transcription factors or impor tant enzyme genes to open many pathway s related to endogenously important genes leading to potent metabolite synthesis.
4. Fourth approach involves CRISPR/dCas9 based activation/repression system for metabolic engineering [149].

Scientists employ these methods to establish efficient metabolic processes, enhance desired outcomes, and achieve specific goals within synthetic biology. Metabolic engineering holds promise in enhancing crop nutrition via genetic modification. This approach involves modifying inherent metabolic pathways by introducing foreign elements, aiming to boost target product output, reduce undesired substances, or redirect flux to amass stable compounds. To conduct successful metabolic engineering, a profound comprehension of involved metabolic pathways proves crucial. This understanding facilitates the formulation of effective engineering tactics, focusing on the upregulation or downregulation of key enzymes.

This precise approach enhances the synthesis of micronutrients in crops without compromising growth and yield, ensuring improved nutritional benefits.

Table 5. List of bioinformatics tools for pathway analysis

Tool	Link	Reference
iPath	http://pathways.embl.de/	[109]
KEGG	http://www.genome.jp/kegg/	[110]
MetaCrop	http://metacrop.ipk-gatersleben.de	[111]
MetPA	http://metpa.metabolomics.ca/MetPA/	[112]
PathwayExplorer	http://genome.tugraz.at/pathwayexplorer/pathwayexplorer_description.shtml	[113]
WikiPathways	http://wikipathways.org	[114]

Table 6. Some applications of metabolic engineering in crop improvement

Crop	Type of stress	Metabolites Produced	Reference
Abiotic Stress			
Maize	Drought	Lipids,carbohydrates metabolismandglutathione cycle.	[121]
Maize	Drought	Glycine and myoinositol	[122]
Maize	Heat	Proline ,Sucrose, fructose, aspartate, valine, inositol andanaline.	[123]
Barley	Drought	Aromatic amino acids andproline.	[124]
Barley	Salt	Proline, sucrose, xylose and maltose	[125]
Wheat	Drought	Glutamine, serine, methionine,	[126]
Wheat	Salt	Proline,glycine, fructose, mannose, Glutamic acid and Malic acid	[127]
Rice	Drought	Stearic acid,ferulicacid,xylitol and 4-hydroxycinnamic acid	[128]
Rice	Salt	Mannitol and sucrose	[129]
Rice	Salt	stearic acid ,4-hydroxybenzoic acid, palmitic acid, Vanillicacid,ramnose, L-tryptophan and pyruvic acid	[130]
Soybean	Heat	Naringenin-7-O-glucoside,ferulate, glycitein, apigenin and genistein	[131]
Soybean	Waterlogging	Glycine, phosphoenol pyruvate, NADH2,.	[132]
Sunflower	Metal	Fatty acids	[133]
Biotic stress			
Wheat	*Fusarium graminearum*	3-hydroxybutarate,L-alanine, asparagine,rehalose,phenylalanine and Myoinositol	[134]
Wheat	Wheat streak mosaic virus	Reduction of phenylalanine, L-tyrosine, tryptophan and isoleucine	[135]
Rice	*Xanthomonas oryzaepv. oryzae*	Tyrosine and phenylalanine	[136]
Rice	*Magnaporthe grisea*	Glutamine, cinnamate, proline, and malate	[137]
Rice	*Rhizoctoniasolani*	Jasmonic acid, mucic acid.	[138]
Legumes	*Weeds*	Flavonoids	[139]
Maize	*Bipolarismaydis*	Lignin, flavonoids and polyphenols	[140]
Maize	*Fusarium graminearum*	Metabolites smiglaside	[141]

miRNA-mediated metabolic engineering involves use small RNA as inactivating tools like miRNA target mimicry, miRNA sponge and short tandem target mimic for functional categorization of miRNAs and improving miRNA related traits [150-153]. Downregulation of miRNAs can be achieved by artificial micro RNAs and synthetic transacting siRNAs [154-156] Metabolic engineering involves manipulating complex biosynthetic pathways to increase production rate of novel beneficial metabolites in plants and microbes. such as golden rice, artemisinin, and flavonoids, and to improve tolerance to stress in plants while reducing harmful metabolites in specific crop plants [157,158]. By using plant miRNA gene regulator tools, researchers can gain better understanding of biosynthetic pathways and modify them to achieve desired characteristics [159]. This approach holds promise for advancing crop improvement and bioengineering efforts.

5. CONCLUSION

Metabolomics holds a crucial role in plant science, encompassing diverse applications from stress-specific metabolite exploration under varying climates to unraveling genetic regulations and metabolic networks. Taking into consideration of above aspects of metabolomics this review provides information about sample collection, storage, preparation methods, and widely used tools like NMR ,GC-MS, LC-MS, CE-MS and FTICR-MS. Integration with other omics principles which includes genomics, proteomics and transcriptomics enhances our comprehension of genetic processes, growth, and stress responses in crops. The amalgamation of GWAS with metabolomics and other omics offers potential for uncovering biochemical processes and stress tolerance mechanisms. As high-throughput technologies advance, combining multi-omics techniques in a panomics platform holds promise for predicting essential crop traits and enhancing precision breeding. Although metabolomics has made strides, further research is needed for effective data mining, annotation, and processing. Its integration with genetic and post-genomics tools approaches paves an exciting path for studying genetic regulations and metabolism. The future of metabolomics includes identifying metabolic markers for stress prediction, assisting breeding programs also called metabolomics assisted breeding, and assessing genetically engineered crops. The emerging field of speed breeding also offers distinct scope for metabolomics in crop improvement.

Genetic modifications that engineer metabolites offer a promising avenue to enhance the nutritional value of plants. This method involves modifying existing metabolic pathways within the plant, introducing external components to amplify target product production, decrease undesirable molecules, or redirect flux to accumulate more potent and stable compounds. Successful metabolic engineering hinges on a comprehensive understanding of the underlying metabolic pathways. This knowledge guides the strategic manipulation of key enzymes, either by enhancing their expression or suppressing it, facilitating increased biosynthesis of essential micronutrients, phytochemicals etc... Importantly, these modifications can be made without adverse effects on crop growth and yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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