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Recent Progress and Prospects of Metabolomics in Crop Plants: A Review

J.P. Sahoo[1*], P. Mishra[2], P. Pradhan[3] and K.C. Samal[4]

[1]Ph.D. Scholar, Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, (Odisha) India.
[2]M.Sc. Scholar, Department of Plant Breeding and Genetics, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, (Odisha) India.
[3]Ph.D. Scholar, Department of Nematology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, (Odisha) India.
[4]Professor, Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, (Odisha) India.

(Corresponding author: J.P. Sahoo)*

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ABSTRACT: The chemical composition of the food crops is the main source to determine their nutritional value and safety for consumption. The latest development in metabolomics characterizes the metabolic profile of crop plants in a high-throughput experimental approach. It is an important branch of "omics" to identify, quantify, and characterize metabolites and cellular regulatory pathway processes in various biological species. The complete metabolite of an organism is called the metabolome. It can be assessed to know the genetic or environmental differences in plant species. The metabolomics play a significant part in finding out gene-environment interactions, mutant identification, phenotyping, and biomarkers' identification and characterization. The concept of metabolomics is an emerging method to unravel the complications of different metabolic pathway networks linked to various stress tolerance in crops. Advanced metabolomics is a term that refers to the study of the metabolic profiling of crop plants that has been investigated using analytical methods. The current challenges in the metabolomics study is being integrated with post-genomics tools, which helps in the efficient dissection of molecular markers and related trait associations in crop plant species. This review gives an overview of the metabolomics tools for crop improvement.

Keywords: Untargeted metabolomics, metabolic profiling, mass spectrometry, omics.

INTRODUCTION

In the past few years, big tendencies have been observed in different 'Omics' fields, specifically genomics, proteomics, transcriptomics, metabolomics, and epigenomics. The records developed with the help of 'Omics' techniques have superior accuracy and pace to the continued breeding applications in growing smart climate and nutritionally enriched germplasm, which is the prime step for enhancing food security (Parry and Hawkesford, 2012). In recent years, the role of phenomics-based breeding in improving agricultural performance has emerged, and genomics has also played a significant role in achieving greater genetic gains. However, the various omics systems have extremely good capacity in enhancing the knowledge of crucial traits, allowing plant breeders and biotechnologists to broaden new techniques for crop improvement. In the omics techniques, metabolomics is one of the complicated genomics studies and has acquired a low interest in crop science, especially for trait mapping and crop plant selections. Because of their impact on plant biomass and architecture, metabolites are an important part of plant metabolism (Turner *et al.*, 2016). Metabolomics has been one of the most significant scientific advances in recent years, paving the path for accurate metabolite profiling in microorganisms and plants (Wuolikainen *et al*., 2016). Because of the quick and fast advancement in metabolomics, the metabolite research of transgenic and mutant breeding holds a tremendous capacity to recognize the metabolic pathways and to point out the essential candidate genes (Hong *et al*., 2016). Metabolomics also enables researchers to understand gene function better, how a specific gene influences a metabolic route, and the interconnections between similar pathways, that are difficult by using traditional techniques like microarray (Kusano and Saito, 2012).

The last decade has been characterized through the adoption of genome-enhancing structures such as the modern discovery of TALE (transcriptional activator-like effector) proteins and the extensive adoption of the clustered regularly interspaced short palindromic repeats (CRISPR) and its associated (Cas) protein

system (Wen *et al.,* 2015). Technology inferences from genomics, proteomics, transcriptomics, and metabolomics will allow researchers to prioritize genes for enhancing critical innovations in crop species. The above-referred omics research is prolonged to discover the related regulatory steps together with epigenetic regulation, post-transcriptional and post-translation modification. To that purpose, community-based research tries to demonstrate molecular interactions between biomolecules and disprove the genotype-phenotype association (Anguraj, 2015). Thus, metabolomics can facilitate the choice of advanced developments of breeding materials. The availability of complete genome sequences, genome-extensive genetic variants, and cost-effective genotyping techniques, combined with improvements in metabolomics, present intriguing potential for effectively combining metabolomics in crop breeding programs (Fernie and Schauer, 2009; Sahoo *et al.*, 2020). The use of metabolomics research methodologies, mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy has resulted in significant crop improvement. The present framework in metabolomics research has the potential to permit comprehensive metabolite surveys.

In this background, the development of bioinformatics coupled with the metabolomics databases, and other diverse plant species have further implications for metabolite annotation (Afendi *et al*., 2012). Metabolic research yielded a wealth of information that could improve plant growth schemes based on agricultural value, yield and stress tolerance cultivar development. Furthermore, the current era of genome-scale statistics via DNA and RNA sequencing and mass spectroscopy measurement of proteins and metabolites needs the integration of the preceding information to plot a holistic approach for crop improvement (Pandey *et al.*, 2016). The scientific community is currently facing with the enormous task of dealing with massive multi-omics data to engage in plant systems analysis (Suravajhala *et al.,* 2016). In such a scenario, an advanced statistical and bioinformatics approach might be required to research those statistical units collectively for higher consolidation that may subsequently be translated for enhancing plant performance. In this review, recent studies of plant metabolomics and the utility of metabolic engineering for plant development are outlined.

DESIGN OF EXPERIMENTS AND WORKFLOW OF METABOLOMICS ANALYSIS

A. Sample Preparation for the experiment

The preparation of samples is one of the most vital components of metabolomics because it has an excellent effect on the outcomes of metabolomics studies (Kim *et al.,* 2010). Plant tissues, including seeds, stems, and roots, may be used for sample preparation. In plant metabolomics experiments, the high-resolution spinning technique is extensively used. However, it is not always appropriate to extract secondary plant metabolites that play a vital function in plants' self-protection mechanism (Li *et al.,* 2016). The principal goal of sample preparation is to split metabolites from undesirable factors and enhance the metabolites. Therefore, the quality sample preparation approach ought to be quick, economical, simple, easy and uphold the sample integrity. Plant sample preparation for metabolic evaluation involves four steps, including collecting samples, quenching, extraction, and sample evaluation. Because the plant metabolome is vulnerable to enzymatic processes that destroy different metabolites, sampling should be done cautiously. To avoid metabolic changes, the plant sample is usually quenched in liquid nitrogen right after harvesting. Similarly, the age of the plant sample is critical since metabolic profiling of younger leaves differs significantly from the metabolic profile of mature leaves to avoid enzymatic destruction of the sample material (Li *et al*., 2016).

B. Data Mining and Processing of data in Metabolomics Assessment

New, improved metabolomics technology reveals the molecular complexity downstream of plants' genome, proteome, and transcriptome, both in normal growth and in response to various stimuli. Because of the enormous and diverse variety of metabolites present in different components of plant cells or tissues, complete metabolome analysis has generated a massive amount of data. The complexity of the nature and composition of metabolites in varied plant samples has made metabolomics data evaluation more difficult. Complete metabolome assessment aims to categorize the various metabolites of diverse plant samples brought through many factors (Aoki-Kinoshita *et al*., 2006). Effective metabolomics evaluation is based on wet and dry science (Redestig *et al*., 2018). Powerful automatic equipment is important to control large datasets and annotate to keep the unprocessed information (Doerfler *et al*., 2013). Basic steps involved in information data mining include pre-processing, pre-treatment, and statistical evaluation of information (Sun et al., 2012). As a result, advanced statistical techniques are required to target and measure all goals in a sample.

C. Statistical Tools and Characterization of Potential Biomarkers

Metabolomics measures metabolite abundance as a predictive biomarker for the diagnosis of disease. It additionally gives ratings to the genetics, in addition to environmental-caused modifications in metabolites' concentration. The identity of biomarkers is based on records, which involves the evaluation of different statistical methods. Metabolic marker probing is hooked up to the idea of linking reaction variables, including the preferred phenotype, to explanatory variables representing biomarkers. Although a couple

of metabolite evaluations are needed to layout a predictive model, canonical correlation evaluation (CCA) is frequently implemented to observe the maximum correlation among variables (Song *et al*., 2016). Many statistical tools like 'univariate evaluation' are commonly achieved for biomarker discovery at preliminary stages of structures biology, which research one variable at a particular time (Saccenti *et al.,* 2014). On the other hand, 'multivariate evaluation' can be used to screen plant cultivars and ecotypes, diagnose diseases, and uncover metabolic markers. These tools were used to quickly compare different genotypes and samples (Fiehn *et al.,* 2011).

Several multivariate statistical tools are available, such as ANOVA, evaluation of variance-simultaneous element evaluation (A-SCA), principal component analysis (PCA), partial least squares-discriminant evaluation (PLS-DA), and heat map evaluation. PCA is identified as a crucial unsupervised multivariate statistical tool used for the multidimensional reduction technique (Xu *et al*., 2012). Orthogonal PLS techniques also supply massive statistics useful for metabolic marker selection (Chun *et al.,* 2010). R programming software has been developed, and the R package language statistical tools are developed and designed to offer statistical computing. A wide range of statistical evaluation strategies is hired in R package programs (Spicer *et al*., 2017). A few R software programs were recently designed for reproducible records evaluation, pathway-based modelling, and linear modelling for quantitative records evaluation. MetabR (Ernest *et al.,* 2012), MetaboAnalystR (Chong *et al.,* 2018), Lilikoi (AlAkwaa *et al.,* 2018), and MetaboDi (Mock *et al.,* 2018) are a few crucial R software programs to be used for metabolomics evaluation.

D. Bioinformatics Tools and Databases Searching

Computational informatics is a pre-requirement of metabolomics studies (Wishart *et al.,* 2007). The disposal of correct and monetary assessable systems has pretty eased the layout and renovation of internet tools that may be utilized by many researchers with little bioinformatics capabilities and restrained computational facilities (Gardinassi *et al*., 2017). XCMS is an internet bioinformatics tool, which lets unprocessed information be uploaded immediately and helps in statistical evaluation and information processing (Tautenhahn *et al.,* 2012). However, XCMS servers cannot manage large information due to finite space. Recently, the XCMS has been installed for programmed information switch in LC-MS experiments, which reduced information processing time and improved the efficacy of an internet system (Montenegro-Burke *et al.,* 2017).

MetaGeneAlyse is an internet-based bioinformatics tool that applies standard clustering techniques, like unbiased aspect evaluation and k-means. This internet device additionally offers many approaches for statistical evaluation, consisting of pathway enrichment evaluation, PLS-DA, and t-test (Daub *et al.,* 2003).

A comprehensive internet-based platform that has been hired in plant metabolomics for information assessment, processing, and statistical evaluation is MeltDB (Kessler *et al*., 2013). Other databases, consisting of iMet-Q, MS-Dial, and MetAlign, are operated through home windows graphical user interfaces (Chang *et al.,* 2016). MZedDB and KEGG were specifically implemented to examine the metabolome with a species-nonspecific or species-specific origin (Draper *et al.,* 2009). Galaxy-M, a fresh new instrument, was recently developed to look at untargeted metabolites using LC-MS techniques (Davidson *et al*., 2016). Babelomics (Alonso *et al*., 2015) and GenePattern (Reich *et al*., 2006) are omics-internet-based programs that have been used to make univariate and multivariate statistical analysis data interpretation and data visualization.

PLATFORMS FOR METABOLOMICS ANALYSIS

The description of plant metabolites in metabolic profiling is drastically tough because of an inadequate connection between the proteome and metabolome. In metabolomics, no single method or technique may be used to research all of the metabolites found in a metabolome. Different metabolomics strategies consist of mass spectrometry (Yadav et al., 2019), non-destructive nuclear magnetic resonance spectroscopy (Cuperlovic-Culf *et al.,* 2019), high-performance thin-layer chromatography (HPTLC), capillary electrophoresis-mass spectrometry (Komatsu et al., 2014), gas chromatography-mass spectrometry (Chang *et al.,* 2019), liquid chromatography-mass spectrometry (Zhou *et al*., 2019), direct infusion mass spectrometry, ultra-performance liquid chromatography, high-resolution mass spectrometry (Thomason et al., 2018) and fourier transform ion cyclotron resonance mass spectrometry (Seybold *et al.,* 2019). Table 1 lists out the benefits and drawbacks of certain common metabolomics testing techniques. NMR-based metabolic profiling is a quick, easy, and effective method for screening and identifying similar biological samples. It maps metabolic pathways in a non-destructive, selective, and extremely efficient manner (Boiteau *et al.,* 2018). The mass spectrometry method benefits quick sample preparation and examination in their natural state (Kang *et al.,* 2019). For metabolic profiling, GC-MS has been recognized as a high-throughput analytical method, due to an electronic impact ionization factor of supply provides exceptionally accurate detection, separation, and identity. Amino acids, natural acids, sugars, alkaloids, lipids, ketones, esters, peptides, and sugar-phosphate can all be probed by GC-MS (Jorge *et al*., 2016).

Table 1: Benefits and drawbacks of some analytical techniques used in metabolomics.

Tools for Analysis	Benefits	Drawbacks	Application
Liquid Chromatography-Mass Spectrometry (LC-MS)	Good selectivity, Minimal sample preparation, Covers a large portion of the metabolome Less volume of sample required, Highly sensitivity.	Suitable for targeted profiling, Destructive, Ion suppression, Laborious sample preparation	Appropriate for secondary metabolite analysis
Nuclear Magnetic Resonance Spectroscopy (NMR)	Accurate quantification, Highly reproducible, Provide rich information about metabolite structure, Ease of sample preparation, Quantitative	Low Sensitivity, High cost of the instrument, Large volume of sample is required	Comparative analysis of samples, Non-destructive
Gas Chromatography-Mass Spectrometry (GC-MS)	High resolving Power, Supported by bioinformatics and databases, More accurate, Suitable for volatile compound analysis,	Destructive, Possible loss of pseudo molecular ion, Unsuitable for non-volatile compounds,	Good for polar and hydrophobic compounds such as sugars, vitamins, organic acids
Fourier-Transform Infrared Spectroscopy (FT-IR)	Cost-effective, Provide more information about data Direct characterization and separation in mixed samples, High-throughput analysis.	Isomer-related issues, Not feasible for wet samples, Less specificity	Recognition of unfamiliar metabolites analysis

UNTARGETED DATA INTERPRETATION AND ANALYSIS

High-resolution platforms like MS and NMR give rise to spectral datasets, which are multidimensional and require respective processing stages before interpretation (Sevin *et al*., 2015). The pre-processing of MS dataset begins with the use of open-online data sources like XCMS (Forsberg et al., 2018), MetAlign (Lommen and Kools, 2012), or Open MS (Rost *et al*., 2016). Commercial software is more widely used in the NMR platform, although open-source tools are available for analysis. The significant metabolic alterations between data sample groups are usually detected using univariate methods such as Welch's t-test (pairwise analysis) and ANOVA (multi-group analysis) or various multivariate statistical methods to identify significantly disregulated metabolite features and allow visualization of metabolomics datasets by analysing multiple variables (Liland, 2011).

Clustering a group of samples can be significantly found by using principal component analysis or partial least squares analysis methods.

Such approaches were commonly used to compare genetically modified varieties based on metabolite profile fingerprints (Ren *et al.,* 2015). Untargeted metabolomics can determine the upregulated and down regulated metabolites in a sample group with the controls in combination with statistical analyses. The molecular formula of the analyte can be inferred from precise mass measurements and isotope abundance ratios in circumstances where annotation using chemical formulae is appropriate (Pluskal *et al*., 2012). Still, the accurate confirmation of the identity of the analyte relies on NMR and crystallographic methods. Many publicly accessible spectral databases can be available for finding out the mass spectral similarity (Vinaixa *et al.,* 2016). A list of widely used bioinformatics and statistical tools for plant metabolomics workflow is indexed in Table 2.

Table 2: Plant metabolomics analysis using bioinformatics tools.

Function	Bioinformatics Tool	Weblink
R package	MetabR	http://metabr.r-forge.r-project.org/
	MetaboAnalystR	https://github.com/xialab/MetaboAnalystR/
	Lilikoi	https://github.com/lanagarmire/lilikoi/
	MetaboDi	http://github.com/andreasmock/MetaboDi/
Statistical analysis	MetaboAnalyst	www.metaboanalyst.ca/
	MetAlign	www.metalign.nl
	Babelomics 5.0	http://www.babelomics.org/
Data annotation	MetaboSearch	http://omics.georgetown.edu/metabosearch.html
	MetiTree	http://www.metitree.nl/
	Metacrop 2.0	http://metacrop.ipk-gatersleben.de
	MetAssign	http://mzmatch.sourceforge.net/
	MZedDB	http://maltese.dbs.aber.ac.uk:8888/hrmet/index.html
	MaxQuant	https://www.maxquant.org/
Workflow analysis	Metab	www.metabolomics.auckland.ac.nz/index.php/ downloads
	Galaxy-M	https://github.com/Viant-Metabolomics/Galaxy-M
	Metabox	https://github.com/kwanjeeraw/metabox
Metabolite annotation and Metabolite data analysis	METLIN	https://metlin.scripps.edu/
	MetFrag	http://c-ruttkies.github.io/MetFrag
	MetaGeneAlyse	http://metagenealyse.mpimp-golm.mpg.de/
	MassBank	http://www.massbank.jp/
	MarVis	http://marvis.gobics.de/
	MMCD	http://mmcd.nmrfam.wisc.edu/
	CFM-ID	http://cfmid.wishartlab.com
Structural annotation and	CDK	https://cdk.github.io

Metabolic models	KEGG	http://www.genome.jp/kegg/
Pathway analysis	MetExplore MetPA MSEA Mummichog	http://metexplore.toulouse.inra.fr http://metpa.metabolomics.ca http://www.metaboanalyst.ca/ http://mummichog.org
Integrated compound detection	MetFusion	http://mgerlich.github.io/MetFusion/
Data processing and Data analysis	MeltDB 2.0 metaP-server MET-COFEA iMet-Q XCMS MAVEN MZmine2	https://meltdb.cebitec.uni-bielefeld.de http://metabolomics.helmholtz-muenchen.de/metap2/ http://bioinfo.noble.org/manuscript-support/met-cofea/ http://ms.iis.sinica.edu.tw/comics/Software_iMet-Q.html https://xcmsonline.scripps.edu https://maven.apache.org/ http://mzmine.github.io/

MoNA and METLIN (Guijas *et al.,* 2018) are two extremely used important databases containing huge verified experimental mass spectra datasets. In addition, the GNPS (Global Natural Products Social Molecular Networking) database allows uploading and sharing of unidentified spectra datasets (Wang *et al.,* 2016). Although many plant metabolites are known to date, only a small number of these can be annotated and characterized using spectral databases. In silico prediction statistical algorithms for spectral MS Data Interpretation, such as MetFrag (Rtttkies *et al.,* 2016), CFM-ID (Allen *et al.,* 2015), MS2LDA (Vander Hoofl *et al.,* 2016), and CSI: FingerID (Duhrkop *et al*., 2015) are designed to find out the most identical chemical structure that corresponds to a given experimental mass spectrum using the chemical databases (Kim *et al*., 2016).

DATA STORAGE ARCHIVING, SHARING, AND CLOUD STORAGE

Data sharing is seen as an important part of scientific research since it encourages the dissemination of lengthy study findings and conclusions and the reuse and repurposing of data. Most archives allow for data sharing while yet allowing the owner to maintain control over their information. Information sharing is carried out by Email request, site, and archiving. FAIR is a set of guiding principles for making data Findable, Accessible, Interoperable, and Reusable for scientific data management and stewardship, launched at Lorentz workshop in 2014 (Wilkinson *et al.,* 2016). Dataverse, FigShare (http://figshare.com), Dryad, Mendeley Data (https://data.mendeley.com/), Zenodo (http://zenodo.org/), DataHub (http://datahub.io), DANS (http://www.dans.knaw.nl/), GitHub (https://github.com/), and EUDat are just a few of the many general-purpose data repositories Zenodo ("Zenodo" n.d.) provides for the sharing of raw data and codes, whereas OSF (Open Science Framework) (Foster, MSLS and Deardorff, MLIS) can assist in the hosting of projects using a variety of data types and file formats, and both provide digital object identities (DOIs).

However, other public databases have been established to store and share specific types of omics data as public repositories throughout time. (e.g., genomics data in NCBI-SRA ("SRA" n.d.) and EBI-ENA (European Bioinformatics institute), proteomics data at PRIDE ("PRIDE - Proteomics Identification Database" n.d.), or metabolomics data at MetaboLights ("MetaboLights" n.d.) (https://www.ebi.ac.uk/metabolights/), Metabolomics Workbench ("Metabolomics Workbench (Webpage)" n.d.) (https://www.metabolomicsworkbench.org/), and GNPS-MASSIVE ("GNPS" n.d.) (https://gnps.ucsd.edu/), and other efforts on bringing this together multi-omics data in a linked and discoverable manner, in the form of OmicsDI ("OmicsDI" n.d.) (Perez-Riverol *et al.* 2017).

XCMSOnline (https://xcmsonline.scripps.edu) also offers data storage and a variety of analyses, including targeted and untargeted data analysis. Biological Magnetic Resonance Data Bank (BMRB: http://www.bmrb.wisc.edu/deposit/) is a repository for data from NMR Spectroscopy that accepts NMR spectral parameters such as chemical shifts, coupling constants, time-domain data, spectral peak lists, relaxation data, other kinetic and thermodynamic data. Unfortunately, like other omics domains such as genomics, metabolomics suffers from a lack of data reproducibility problems coming from a variety of challenges, including the accessibility and archiving status of computational tools and resources (Mangul *et al*., 2018). Some of the available data repositories which are dedicated to metabolomics data interpretation are indexed in Fig. 1.

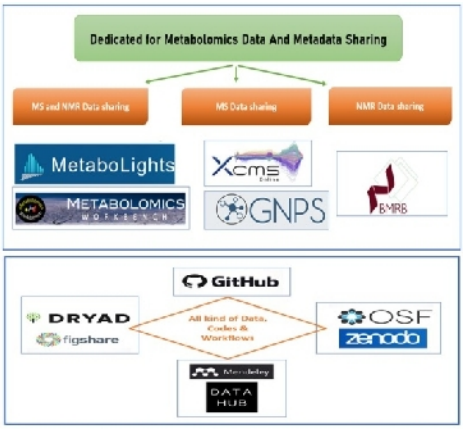

Fig 1. The available data repositories which are dedicated to metabolomics data interpretation.

APPLICATION OF METABOLOMICS APPROACH IN CROP IMPROVEMENT

The most important biotechnological tool for deciphering diverse stress tolerance in crop plant species is metabolomics. During the life cycle of various crop plants, metabolomics was frequently employed to look for unique metabolites. Plants respond similarly to biotic and abiotic stresses, but these stresses cause changes in the plants' biochemical and physiological processes. The activation of particular metabolic networks in crop plants' cellular mechanisms results in forming a novel bioactive metabolic agent. Table 3 lists out the role of metabolomics and the application of recent metabolomics approaches in crop improvement.

Table 3: Metabolomics for stress management in crop plants.

Crop Plants	Type of Stress	Target Tissue	Platform of Analysis	The platform for Data Analysis	Metabolite Products	Reference
Rice	Drought	Leaf	GC/EI-TOF-MS, GC/MS	PCA, PLS-DA, Tag Finder and NIST	Proline, GABA, Glutamate and spermidine, Serine, threonine arginine, and asparagine	Ma *et al.*, (2016)
Rice	Salinity	Leaf, Seedling, Leaf, and root	GC/MS, NMR	ANOVA, MS, PLS-DA, PCA	Mannitol and sucrose, Leucine, GABA, proline, isoleucine, valine	Chang *et al.*, (2019)
Rice	Waterlogging	Leaf	GC/MS, NMR	PCA	GABA, glycine, alanine, 6-phosphogluconate, phenylalanine, and lactate	Locke *et al.*, (2018)
Soybean	Drought	Leaf	H-NMR, GC/MS	PCA PC-DFA	GABA, Sugars, and sugar alcohols	Ogbaga *et al.*, (2016)
Soybean	Waterlogging	Leaf and Roots	NMR	ANOVA, PCA, and MATLAB	Isoflavones and kaempfero	Coutinho *et al.*, (2018)
Maize	Drought	Immature kernels, Leaf-blades	MS/MS, GC/MS	PLS-DA, KEGG, ANOVA, PCA	Carbohydrates, Myoinositol, and glycine	Yang *et al.*, (2018)
Maize	Salinity	Leaf and Root	GC-MS	PCA, PLS-DA, and SIMCA	Auxin, ABA, Proline, sucrose, xylose and maltose	Zorb *et al.*, (2013)
Maize	Heat	Leaf	NMR	PCA	GABA, inositol, fructose, aspartate, sucrose, asparagine, analine, valine, and proline	Sun *et al.*, (2016)
Wheat	Waterlogging	Shoot	GC/MS, LC/MS	ANOVA, PCA	Tryptophan and methionine	Zorb *et al*., (2013)
Wheat	Drought	Roots and leaves	GC-MS	PLS-DA, KEEG, PCA	Tryptophan citric acid, fumaric acid, malic acid, and valine	Kang *et al.*, 2019
Wheat	Salinity	Root, Shoot, and Leaves	HPLC, GC-MS	ANOVA, PCA, METABOLOME EXPRESS	Fructose, Malic acid, glycine, proline, Glutamic acid, Auxin, ABA, lyxose, lysine, mannose, proline, sorbitol, and sucrose	Che-Othmen *et al.*, (2019)
Wheat	Heat	Flag Leaf, Filling grains	LC-MS/MS, HPLC	PLS-DA, KEGG	Pipecolate and L-tryptophan, G1p, and sucrose	Thomason *et al.*, (2018)
Barley	Drought	Fifth leaf	MS-EI	PROC UNIVARIATE	Aromatic amino acids	Hein *et al.*, (2016)
Wheat	*Fusarium graminearum*	Leaf	NMR	PCA	Trehalose, 3-hydroxybutarate, asparagine, phenylalanine, myoinositol, and L-alanine	
Wheat	Wheat streak mosaic virus	Leaf	UPLC-QTOF/MS	PCA	Reduction in tryptophan, isoleucine, and phenylalanine	Farahbakhsh *et al.*, (2019)
Rice	*Orseolia oyzae*	Leaf	GC/MS	ANOVA	Threonic acid and heneicosanoic acid	Agarrwal *et al*., (2014)
Rice	*Xanthomonas oryzae pv. oryzae*	Leaf	GC/TOF and LC/TOF	KEGG, MassHunter	Tyrosine and phenylalanine	Sana *et al*., (2010)
Rice	*Magnaporthe grisea*	Leaf	NMR, GC/MS, and LC/MS	PCA, MATLAB	Cinnamate, proline, glutamine, and malate	Jones *et al.*, (2011)
Maize	*Fusarium graminearum* and *Bipolaris maydis*	Root and Leaf	LC/MS, FT-IR, and NMR	ANOVA, PCA	flavonoids and polyphenols, metabolites smiglaside and smilaside Alignin	Figueroa *et al.*, (2018)
Wheat	*Lolium rigidum, Urochloa panicoides*	Root and Shoot	LC-MS/MS, Q Trap	Analyst Software	Hydroxamic acids and Benzoxazinoids	Mwendwa *et al*., (2016)
Legumes	Weeds	Root and shoot extracts	UHPLC, QTOF-MS	METLIN	Flavonoids	Berrabah *et al.*, (2019)

CONCLUSION

Domestication and plant breeding have resulted in large-scale genome duplication mutagenesis and rearrangement events in ancestral crop genomes, resulting in present-day plants. From *Agrobacterium*-mediated T-DNA insertions to more recently improved genome-modifying technologies, new genetic engineering technique allows scientists to improve plants by carefully introducing relevant improvements. The loss of social recognition of genetic engineering technology is essentially due to public worries approximately whether or not present-day breeders can completely apprehend the complexity of the brand-new phenotypes from diverse genetic engineering strategies and the ability dangers related to them. The metabolite-focused framework provided here commonly targets enhancing the present risk assessment method to deal with the ever-developing complexity of biotech plants, each with the strategies used (like multiplexed gene modifying, epigenetic modifications) and with the developments advanced. It is important to show that the integration of metabolomics with other approaches such as quantitative genetics, transcriptomics, and genetic modification is very important for plant improvement. With an effective combination of these modern approaches, researchers can identify functional genes, characterize large numbers of metabolites, prioritize candidate genes for downstream analysis, and ultimately commercialize them. It provides trait-specific markers for enhancing metabolically important traits.

Conflict of Interest. The authors declare no conflict of interest.

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