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REVIEW

Metabolomics: a systems biology approach for enhancing heat stress tolerance in plants

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Abstract

Key message **Comprehensive metabolomic investigations provide a large set of stress-related metabolites and metabolic pathways, advancing crops under heat stress conditions. Metabolomics-assisted breeding, including mQTL and mGWAS boosted our understanding of improving numerous quantitative traits under heat stress.**

Abstract During the past decade, metabolomics has emerged as a fascinating scientific field that includes documentation, evaluation of metabolites, and chemical methods for cell monitoring programs in numerous plant species. A comprehensive metabolome profiling allowed the investigator to handle the comprehensive data groups of metabolites and the equivalent metabolic pathways in an extraordinary manner. Metabolomics, together with transcriptomics, plays an influential role in discovering connections between stress and genes/metabolite, phenotyping, and biomarkers documentation. Further, it helps to decode several metabolic systems connected with heat stress (HS) tolerance in plants. Heat stress is a critical environmental factor that is globally affecting the growth and productivity of plants. Thus, there is an urgent need to exploit modern breeding and biotechnological tools like metabolomics to develop cultivars with improved HS tolerance. Several studies have reported that amino acids, carbohydrates, nitrogen metabolisms, etc. and metabolites involved in the biosynthesis and catalyzing actions play a game-changing role in HS response and help plants to cope with the HS. The use of metabolomics-assisted breeding (MAB) allows a well-organized transmission of higher yield and HS tolerance at the metabolome level with specific properties. Progressive metabolomics systematic techniques have accelerated metabolic profiling. Nonetheless, continuous developments in bioinformatics, statistical tools, and databases are allowing us to produce ever-progressing, comprehensive insights into the biochemical configuration of plants and by what means this is inclined by genetic and environmental cues. Currently, assimilating metabolomics with post-genomic platforms has allowed a significant division of genetic-phenotypic connotation in several plant species. This review highlights the potential of a state-of-the-art plant metabolomics approach for the improvement of crops under HS. The development of plants with specific properties using integrated omics (metabolomics and transcriptomics) and MAB can provide new directions for future research to enhance HS tolerance in plants to achieve a goal of "zero hunger".

Keywords Abiotic stress · Bioinformatics · Crop improvement · Metabolites · Metabolomics-assisted breeding · mQTL · mGWAS · Omics · Systems biology · Extreme temperature · Zero hunger

Introduction

Metabolomics has become an excellent scientific field for the past two decades (Fernie et al. 2004), even though this technique has been executed since the 1970s (Jellum 1977). In early 2003, metabolomics was introduced as an essential tool for metabolites profiling, systems biology, and it was also linked with genome-wide metabolome modelling (Weckwerth 2003). In recent years, massive advancement has been made in the "omics" technologies, i.e., genomics, transcriptomics, proteomics, metabolomics, and phenomics. The data

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based on the omics tools have increased the ratability and the speed of the growing breeding scheme in order to develop the climate-resilient and nutrient-rich genotypes, which are vital for securing food security (Alseekh and Fernie 2018). Metabolomics is used as a critical tool to obtain data for systems biology, functional genomics, and omics approaches (Fig. 1) (Saito and Matsuda 2010; Zampieri et al. 2017). The biochemical phenotype of tissues or cells can be correctly defined by studying the metabolome components determined by gene expression. An organismic biochemical status can be checked by quantitative/qualitative analysis of cellular metabolites, which can be further used to check genes function (Weckwerth 2003; Dos Santos et al. 2017).

The changes in mRNA are compulsory for protein synthesis during transcription, but levels of protein should be strongly correlated with increased levels of mRNA (Selbach et al. 2008). Translated proteins do not need to be always active; thus, considering these reasons, alterations in the proteome level do not correlate to changes in the biochemical phenotype. In proteome and transcriptome profiling, proteins and mRNA are identified via databases or sequence similarity. When the database information is not available, the results of the analysis are limited. In order to understand any biological sample, metabolite profiling is beneficial when the database information is not available for transcriptome and proteome analysis (Weckwerth 2008, 2011). Since the chemical alteration of metabolites happens in any cellular metabolism, quantitative and qualitative metabolomics profiling of various organs, cells, and tissue are considered as an important target for analytical metabolomic fields (Durand et al. 2010; Templer et al. 2017). Hence, metabolites are a known product of cellular functions, and their levels are critically linked with plants' reactions to genetic manipulation and environmental responses. Therefore, metabolomics studies are used for the identification and measurement of primary and secondary/specialized metabolites in plants used in biological processes. Primary metabolites are the major components of the reproduction of plants and their normal growth, whereas specialized metabolites are used to provide strength to pants in harsh conditions. Primary metabolites

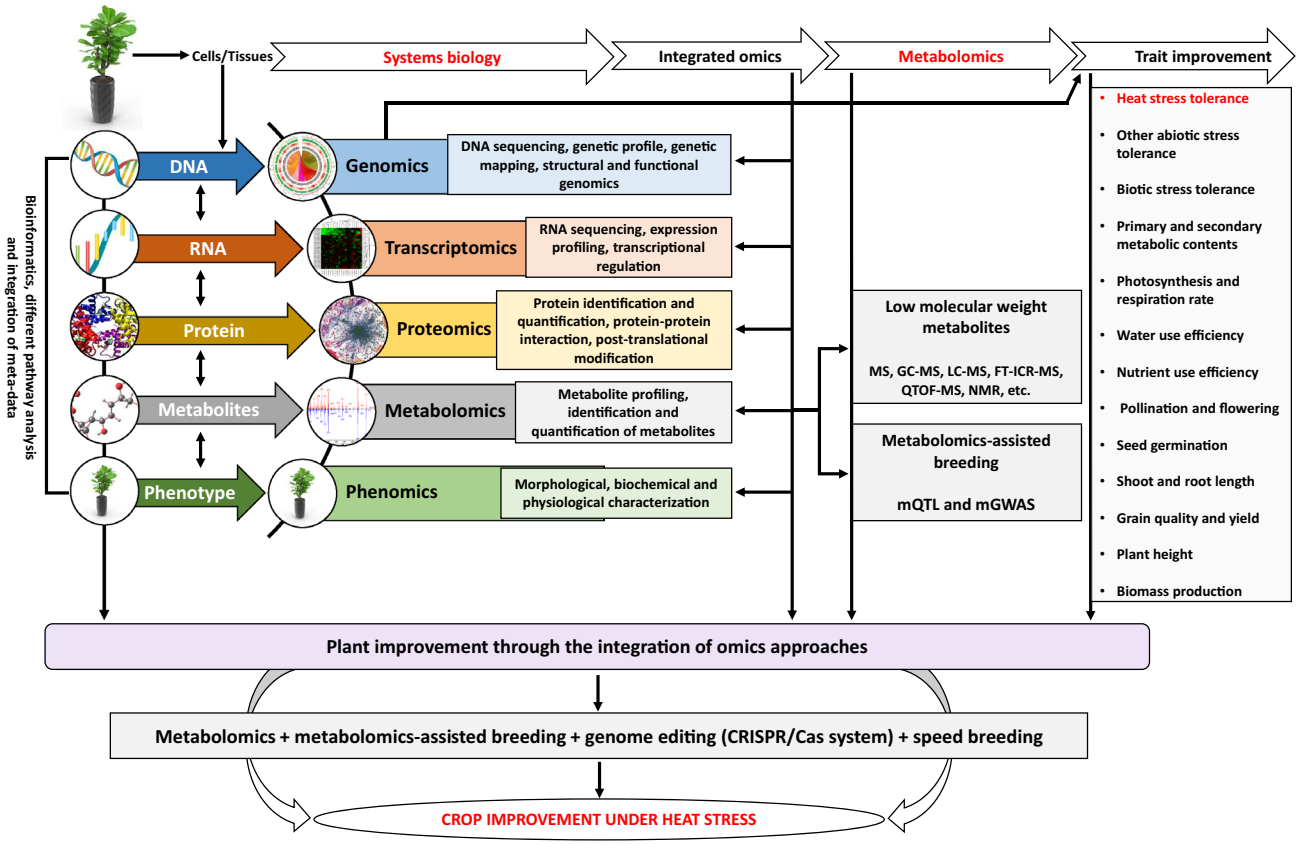


Fig. 1 An overview of the integration of omics, mainly metabolomics for crop improvement under heat stress. Central dogma showing the movement of biological information from genomics to phenomics to get the required phenotype under heat stress. Notably, omics, mainly metabolomics or/and genomics tools, improved several traits via the flow of biological information. Interestingly, the exploitation of metabolomics, metabolomics-assisted breeding platforms, genome and metabolic engineering using the CRISPR/Cas system, and the speed breeding on a large scale can help to improve the overall plant health under heat stress conditions and can help to feed billions worldwide

are limited, conserved in the structure, and are found widely in the whole plant kingdom, whereas specialized metabolites level varies over the plant kingdom (Alseekh and Fernie 2018; Fang and Luo 2019). Therefore, the information on the biologically active metabolites, mainly specialized metabolites, is essential.

Plant's metabolic networks are very complicated and consist of many biochemical steps, including the metabolomic network of primary and specialized metabolites pretentious by the different plant stressful environments. Hence, it is essential to quantify and identify changes in metabolite composition through proper analytical methods. Nowadays, various integrated technologies are used, such as nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), capillary electrophoresis-MS (CE-MS), liquid chromatography-MS (LC–MS), gas chromatography-MS (GC–MS), and Fourier transform ion cyclotron resonance-MS (FT-ICR-MS) (Kim et al. 2010; Vinaixa et al. 2016; Peukert et al. 2016; Mitchell et al. 2018). With the advancement of MS-imaging techniques, including matrix-assisted laser desorption (MALDI) and desorption electrospray (DESI) ionization platforms combined with high-resolution MS, it is feasible to conduct in-situ metabolome analysis (Blanksby and Mitchell 2010). Therefore, for liquid phase separations, ultrahigh performance liquid chromatography (UPLC) and high-performance-LC (HPLC) are used for metabolite analysis in various applications; these are a robust analytical method that permits the discovery of plant metabolites when it is integrated with another technique like MS (Theodoridis et al. 2012; Khan et al. 2017). A recent method for multi-component analysis is the metabolic profiling that is employed for the examination of acids and urinary drugs coupled with GC/MS. For metabolite profiling, GC based techniques like NMR and HPLC are used and exceptionally important in the research field (Görling et al. 2016). Nowadays, metabolites and cellular proteins are widely analyzed by modern mass spectrometry, a rare trend before. Systems biology and functional genomics use genome-scale molecular analysis to get the desired phenotype (Weckwerth 2011; Aebersold and Mann 2016; Chaturvedi et al. 2016; Ghatak et al. 2017).

Modern analytical technologies provide the basis to study biological systems. In plants, gene expression is changed by various stress responses that can alter qualitative status in the metabolite pool; therefore, metabolite identification becomes more difficult (Sweetlove et al. 2014; Razzaq et al. 2019). Plants face both biotic and abiotic stresses and are sessile organisms that have to cope with these conditions. Due to climate changes, among abiotic stresses, heat stress (HS, > 25 °C) is considered the most threatening factor affecting the growth and productivity of several plant species (Hasanuzzaman et al. 2013; Raza et al. 2019, 2020a). In the past few years, significant researches have been conducted to exploit the HS effect on the metabolome profile of numerous plants (Templer et al. 2017; Thomason et al. 2018; Lawas et al. 2019; Dhatt et al. 2019). Omics approaches such as transcriptomics, proteomics, metabolomics, bioinformatics, and high-throughput DNA sequencing have aided functional analysis of regulatory networks that control plant abiotic stress responses. Such research has noticeably augmented our knowledge of comprehensive plant systems in responses and adaptation to a variety of stress conditions (Urano et al. 2010). Mainly, metabolomics plays an essential role in the genetic improvement of various crops under HS. Different institutes and various commercial branches are all working towards the advancement of metabolomics. However, the measurement of metabolites is critical in plant molecular and/or physiological responses to HS and to elucidate the function of genes in functional genomics and systems biology. This review highlights the state-of-the-art plant metabolomics and its application in functional genomics and systems biology for the genetic improvement of crops under HS.

Plant metabolomics: an overview

Metabolome has been defined as the last receiver of the flow of biological information to get the desired phenotype (Fig. 1). However, a metabolite is generally described as a molecule with a size of < 1.5 kDa (Wakayama et al. 2015). From the last decade, plant metabolomics has emerged as a highly recommended and widely used approach from an exclusively hypothetical idea. Plant metabolomics studies have been usually adapted to study the metabolites from different crop plants. Due to the untargeted nature of many metabolomic techniques, the study will deliver an extensive indication of both the primary and specialized metabolites (van Dam and van der Meijden 2018). In 1993, a considerable amount of estimated metabolites was ranged between 100,000–200,000 (Mazza and Miniati 1993). Approximately 2 lac metabolites are present in plants, out of which seven to fifteen thousand are present in individual species (Fernie et al. 2004), and three to five thousand are existing in plant leaves (Kim et al. 2010). Nevertheless, the number of estimated metabolites seems to increase with the advancement in analytical tools (Last et al. 2007).

Except for simple identification, selective metabolite profiling used to find results for biological characteristics of plants, which includes (1) ecotypes for taxonomic or biochemical information and fingerprinting of species, (2) check the response of metabolites under physical stimuli and exogenous chemicals, (3) learn the symbiotic association and developmental process, metabolite content comparison of transgenic and wild type plants (Sumner et al. 2003; Templer et al. 2017; Alseekh and Fernie 2018). In

all the above-mentioned studies, metabolome profiles were combined with other omics tools for comprehensive understanding, such as complex regulatory networks that control global gene expression, protein alteration, and metabolite configuration under stress conditions (Weckwerth 2011). To study the chemical configuration of different plant species, metabolite profiling is integrated with the markers (Schauer et al. 2006). As metabolomics plays an essential role in plant research except for individual cell-metabolome analysis for root hairs, pollen tissues, a trichome, guard cells were also studied (Nägele et al. 2017). Findings showed that metabolome analysis could be convenient for a single cell type, and it may vary from cell to cell. The phenotype of any plant depends on the metabolite concentration and synthesis in various organs of plants at different developmental stages; thus, the nature of metabolites is relevant to tissues/organs characteristics (Roldan et al. 2014). Due to considerable differences in biochemical pathways at the cellular and sub-cellular levels in crops, the application of various metabolomics techniques with different protocols, notably augmented. In short, metabolomics can be helpful for the detection of novel gene functions and clarification about the governing metabolisms in a metabolome network.

Current analytical techniques for plant metabolomics research

Metabolomics has developed as an outstanding scientific field; however, a single analytical technique is not adequate to detect and quantify the metabolites (Weckwerth, 2003; Templer et al. 2017). Presently, various metabolomic techniques are being applied in plant metabolomics research, as discussed in the introduction. Out of these, GC, MS, NMR, and HPLC dominate the metabolite tools. Two basic techniques, MS and NMR, are used in modern metabolomics, including the generation of metabolomics data. Interestingly, NMR is preferred to MS because of its high capacity in detecting protein binding sites, direct binding of target proteins, physical properties of ligands, and uncovered protein structure coupled with ligands. Metabolite exposure reliant on NMR uses magnetic properties of various nuclei of atoms. The different applications, such as metabolite profiling and fingerprinting, metabolic flux, and atomic structural details of different biological samples, are integrated with NMR. Owing to the non-destructive nature of NMR with a smaller molecular weight is widely used to detect metabolites (Eisenreich and Bacher 2007; Kim et al. 2010). Hence, this technique is so sensitive, and it has a low abundance of biomarkers that causes its limited use. Except for NMR, the MS technique has the best sensitivity, and researchers can get an extensive range of metabolome data. This technique would help researchers to detect molecules and metabolic biomarkers that can rebuild metabolic networks and pathways. Different ionization methods such as matrix-assisted laser desorption/ionization (MALDI-TOF), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI) were accurately detected by MS (Issaq et al. 2009). To get accurate results, MS is coupled with various techniques such as field asymmetric waveform ion mobility spectrometry (FAIMS), CE, GC, FT-ICR, and LC. Figure 2 indicates the comparison of frequently working analytical techniques in plant metabolomics research.

Notably, MS has obtained a progressively vital role in the field of metabolomics and proteomics due to the significant progress that has been made in instrument technologies. The frequently used technique for untargeted analysis is GC–MS (Rohloff 2015). Sample derivatization was done by the GC–MS technique, making the compound volatile; however, some compounds are left as underivatized during analysis. GC–MS has been recognized as a high-throughput analytical technology with a high rate of sensitivity for metabolic profiling. GCxGC-TOF–MS enhanced the output through the segregation of co-eluting peaks (Hurtado et al. 2017). Higher mass primary and specialized metabolites (< 1500 Da) are detected by targeted and untargeted techniques facilitated by LC–MS that uses ESI and APCI (Turner et al. 2016). Identification of several metabolites increases peak resolution, and mass accuracy was done in a short time with the help of UPLC coupled with QTOF-MS (Chawla and Ranjan 2016). In targeted and untargeted metabolomics analysis, high-resolution separation of metabolites is mainly done by CE-MS (Ramautar et al. 2019). FT-ICR-MS is driven by high-resolution mass analysis that provides extensive and reliable detection of metabolites. It is also coupled with separation techniques to settle complex matrices, and ion separation was also done by this technique (Ghaste et al. 2016; Nakabayashi et al. 2016; Lopes et al. 2017).

Data produced from the above-mentioned techniques are processed by Met-Align, PlantMAT, MET-XAlign, MET-COFEA, XCMS, and ChromaTOF, etc. (Table 1). Statistical analysis of identified metabolites is followed by using a combination of (1) univariate analysis (Student *t* test; ANOVA; Mann–Whitney *U* test; Benjamini–Hochberg false discovery rate correction; Kruskal Wallis 44%), and (2) multivariate analysis (principal component analysis (PCA); partial least squares discriminant analysis (PLS-DA); orthogonal partial least squares (O-PLS); high-content screening (HCA); heatmap, correlation analysis, neural networks, genetic algorithms, and random forest methods. Currently, several different software and online tools are available for metabolome analysis, like MetaboAnalyst, MetaboliteDetector, MetaMapR, MetExplore, Cytoscape, g:Profiler, Gene-set enrichment analysis (GSEA), Metabolite-set enrichment analysis (MSEA), EnrichmentMAP, Workflow4Metabolomics

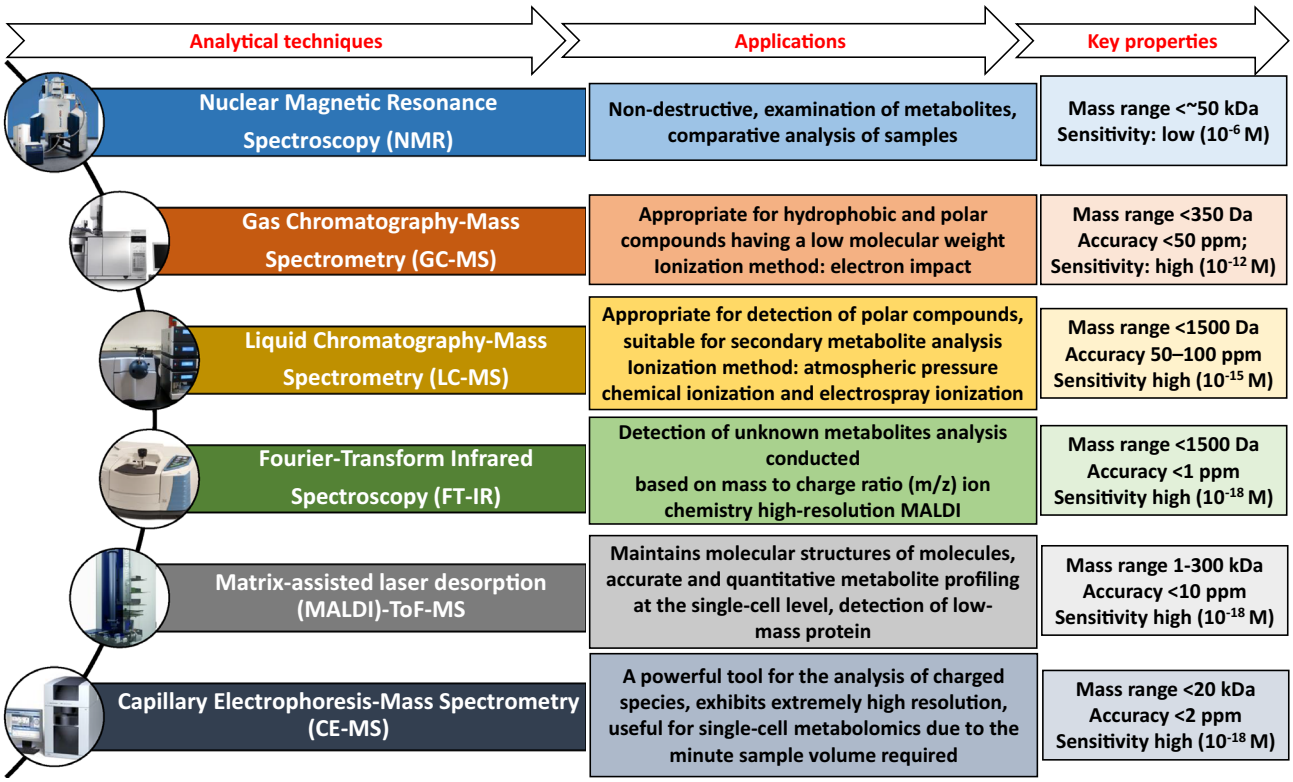


Fig. 2 Comparison of frequently working analytical techniques in plant metabolomics research

(W4M), and different statistical analysis tools, etc. (Hiller et al. 2009; Chong et al. 2018; Reimand et al. 2019; Giacomoni et al. 2014; Weber et al. 2017). A list of accessible online databases/tools and software for the analysis, data processing, statistical analysis, biomathematical modelling, and functional interpretation of metabolomics data is shown in Table 1. Notably, enlisted tools/software are suitable to analyze and identify the different metabolites related to various agronomic parameters.

Key steps and workflow for plant metabolome analysis

Plant metabolomes have chemically diverse and multifaceted structures. Wide range metabolic pictures and vast identification of metabolomes can be made with the help of analytical and metabolic strategies as well as analytical procedures and extraction protocols (Gorrochategui et al. 2016; Christ et al. 2018; Wolfender et al. 2018). The metabolomic analysis is based on four major steps: (1) design of the experiment, (2) preparation of the samples, (3) data acquisition by using analytical procedures, and (4) identification of compounds and data extraction with the help of statistical analysis. Finally, these steps are used to interpret biological data, results validation, and submission to public repositories. These steps are necessary and interlinked, as shown in Fig. 3, followed by various sub-steps to propose biochemical strategies (Gorrochategui et al. 2016; Wolfender et al. 2018).

Metabolite identification is made by sample preparation, i.e., a critical step that plays an essential role in identification. Sample preparation consists of many steps, like selection, harvesting, drying procedure, and metabolite extraction. The researcher selects plant material based on experimental design. In order to not affect the results with unwanted material, every step should be performed with care. Contamination, sample degradation, use of enzyme inhibitors, organic solvents, acids could also affect the metabolome results (Kim et al. 2015; Kim and Verpoorte 2010).

Plants metabolites are as complex in their structure as in polarity, stability, solubility, quantity, volatility, and size (Riedelsheimer et al. 2012). Various metabolite extraction methods are used for plants, but it all depends on various factors like a solvent´s physicochemical properties and biochemical composition. Commonly used methods are sonication, superficial fluid extraction solvent, and solid-phase extraction (Kim and Verpoorte 2010; Vilkhu et al. 2008). Nevertheless, no technique can identify all kinds of metabolites with high results. Sample analysis can be done by advanced methods by which ultra-complex metabolites can also be measured (Salem et al. 2017). Analytical platforms'

Table 1 List of bioinformatics databases/tools available for the data analysis, data processing, statistical analysis, biomathematical modelling, and functional interpretation/annotation of metabolome data

Name	URL	Function
Databases		
KomicMarket	http://webs2.kazusa.or.jp/komicmarket/	Annotations of the metabolite identified by MS in metabolomics studies
MetFusion	http://mgerlich.github.io/MetFusion/	Integrated compound identification from MS and NMR data
MetaboAnalyst	http://www.metaboanalyst.ca/	Statistical, functional, and integrative analysis of metabolomics data
MetaboSearch	http://omics.georgetown.edu/metabosearch.html	Mass-based metabolite identification using multiple databases
MZedDB	http://maltese.dbs.aber.ac.uk:8888/hrmet/index.html	The annotation of high-resolution MS metabolomics data
METLIN	https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage	Metabolite detection and annotation
MyCompoundID	http://www.mycompoundid.org/mycompoundid_IsoMS/	Identification of compounds of interest-based on chemical properties of a molecule by MS
MetExplore	https://metexplore.toulouse.inra.fr/index.html/	Metabolic network curation and exploration
MS-DIAL	http://prime.psc.riken.jp/compms/msdial/main.html	Untargeted metabolomics data processing of multiple instruments (GC/MS, GC/MS/MS, LC/MS, and LC/MS/MS)
MAGMa	https://www.emetabolomics.org/	Automatic chemical annotation of accurate multi-stage MS spectral data
MetaGeneAlyse	http://metagenealyse.mpimp-golm.mpg.de/	High-throughput analysis of integrated transcriptional and metabolic data
metaP-server	http://metabolomics.helmholtz-muenchen.de/metap2/	Data analysis for the processing of metabolomics experiments
MetNetDB	http://www.metnetdb.org/MetNet_overview.html	Provide information on metabolic and regulatory networks and interactions in *Arabidopsis*
MetiTree	http://www.metitree.nl/	Organizing and processing high-resolution multi-stage MS metabolomics data
MetaMapp	http://metamapp.fiehnlab.ucdavis.edu/	Mapping and visualization of metabolomics data by chemical and biochemical relationships
mzMatch	http://mzmatch.sourceforge.net/	Data processing of metabolomics LC/MS data
SoyMetDB	http://soymetdb.org/	Storage, integration, and mining the soybean metabolomics data in a well-organized way allowing the visualization of the expressions across various experiments
The Golm metabolome database (GMD)	http://gmd.mpimp-golm.mpg.de/Default.aspx	Search and dissemination of reference mass spectra from biologically active metabolites quantified via GC/MS
TagFinder	http://www-en.mpimp-golm.mpg.de/03-research/researchGroups/01-dept1/Root_Metabolism/smp/TagFinder/index.html	Quantitative analysis of GC–MS-based metabolome profiling
XCMS	https://xcmsonline.scripps.edu/landing_page.php?pgcontent=mainPage	Statistical and pathway data analysis
Tools/Software		
AnalyzerPro	https://www.spectralworks.com/products/analyzerpro/	Processing of low- and high-resolution LC–MS and GC–MS data
ChormaTOF	https://www.leco.com/product/chromatof-software	Acquiring, processing, and reporting the MS data system to support multi-dimensional GCxGC data fully
COVAIN	http://www.univie.ac.at/mosys/software.html	Uni- and multi-variate statistics, time-series, and correlation network analysis

Table 1 (continued)

Name	URL	Function
FEMTO	https://homepage.univie.ac.at/thomas.naegele/Software/Software_1.html	Functional interpretation of metabolomic data in the context of metabolic network information
iMet-Q	http://ms.iis.sinica.edu.tw/comics/Software_iMet-Q.html	Quantification and processing of metabolites in LC/MS data
MeltDB	https://meltdb.cebitec.uni-bielefeld.de/cgi-bin/login.cgi	Analysis and integration of metabolomics data
MET-IDEA	http://bioinfo.noble.org/gateway/index.php?option=com_wrapper&Itemid=57	Data extraction tool for MS-based metabolomics
MET-COFEI	http://bioinfo.noble.org/manuscript-support/met-cofei/	GC–MS data processing platform for metabolite compound feature extraction and annotation
MarVis-Suite	http://marvis.gobics.de/	Visualization, filtering, clustering, and functional analysis of data sets
MET-COFEA	http://bioinfo.noble.org/manuscript-support/met-cofea/	LC–MS data processing platform for metabolite compound feature extraction and annotation
MET-XAlign	http://bioinfo.noble.org/manuscript-support/met-xalign/	Metabolite cross-alignment tool for LC/MS-based comparative metabolomics analysis
MetaboliteDetector	https://md.tu-bs.de/	Targeted and non-targeted GC/MS based metabolome analysis
MeltDB 2.0	https://meltdb.cebitec.uni-bielefeld.de/cgi-bin/login.cgi	Analysis and integration of metabolomics data
MetMask	http://metmask.sourceforge.net/	Metabolite identification and analysis
MetAlign	https://www.wur.nl/en/show/MetAlign-1.htm	The pre-processing and comparison of full scan nominal or accurate mass LC–MS and GC–MS data
MSClust	http://www.metalign.nl	Metabolite identification and analysis of GC–MS and LC–MS datasets derived from untargeted profiling
MZmine 2	http://mzmine.sourceforge.net/	Processing, visualizing and analyzing MS-based metabolomics data
R packages		
CAMERA	https://bioconductor.org/packages/release/bioc/html/CAMERA.html	Compound spectra extraction and annotation of LC/MS data sets
FELLA	https://github.com/b2slab/FELLA	Network-based enrichment of affected metabolites
Lilikoi	https://github.com/lanagarmire/lilikoi	Personalized pathway-based classification modeling using metabolomics data
MAIT	http://b2slab.upc.edu/software-and-tutorials/metabolite-automatic-identification-toolkit/	Peak detection for metabolomic LC/MS data sets
MI-Pack	https://github.com/Viant-Metabolomics/MI-Pack	Metabolite identification in mass spectra by integrating accurate masses and metabolic pathways
MetabR	http://metabr.r-forge.r-project.org/	Statistical analysis of metabolomic data
RAMClustR	https://github.com/cbroeckl/RAMClustR	Spectral-matching-based annotation for metabolomics data
MetaboAnalystR	https://github.com/xia-lab/MetaboAnalystR	Metabolomics data analysis and interpretation
MetabNet	https://sourceforge.net/projects/metabnet/	Metabolic association analysis of high-resolution metabolomics data
MetaboDiff	http://github.com/andreasmock/MetaboDiff/	Differential metabolomic analysis
MetID	https://www.bioconductor.org/packages/release/bioc/html/MetID.html	Network-based prioritization of putative metabolite IDs detected by LC–MS
metabox	https://github.com/kwanjeeraw/metabox	Data analysis, interpretation, and integrative exploration

Table 1 (continued)

Name	URL	Function
Omu	https://cran.r-project.org/web/packages/omu/vignettes/Omu_vignette.html	Rapid analysis of metabolomics data sets
XCMS	https://github.com/sneumann/xcms	Framework for processing and visualization of chromatographically separated and single-spectra mass spectral data
Pathway-related databases/tools		
AraCyc	https://www.arabidopsis.org/biocyc/	Visualizing biochemical pathways of *Arabidopsis*
AraPath	http://bioinformatics.sdstate.edu/arapath/	Pathway analysis in *Arabidopsis*
BioSilico	http://biosilico.kaist.ac.kr/	Search and analysis of metabolic pathways
BioCyc	http://biocyc.org/	Integration of sequenced genomes with predicted metabolic pathways for thousands of organisms
KaPPA-View4	http://kpv.kazusa.or.jp/	A metabolic pathway viewer database for representation and analysis of correlation networks of gene co-expression and metabolite co-accumulation and omics data
KEGG PATHWAY	https://www.genome.jp/kegg/pathway.html	Represent knowledge of the molecular interaction, reaction, and relation networks
MetaCyc	http://metacyc.org/	Provide information on primary and secondary metabolism, as well as associated metabolites, reactions, enzymes, and genes of experimentally elucidated metabolic pathways from all domains of life
MapMan	http://mapman.gabipd.org/	Displays large datasets onto diagrams of metabolic pathways or other processes
MetaCrop	http://metacrop.ipk-gatersleben.de	Summarizes diverse information about metabolic pathways in crop plants and allows automatic export of information for the creation of detailed metabolic models
MetPA	http://metpa.metabolomics.ca	Pathway analysis and visualization together with advanced statistical enrichment analysis
MINE	http://minedatabase.mcs.anl.gov/	Provide in silico network expansion of computationally predicted metabolites for untargeted metabolomics
Mummichog	https://shuzhao-li.github.io/mummichog.org/	Pathway and network analysis for metabolomic data
Pathvisio	http://www.pathvisio.org/	Drawing, editing, and analyzing biological pathways
UniPathway	http://www.unipathway.org/	Exploration and annotation of metabolic pathways
VANTED	https://immersive-analytics.infotech.monash.edu/vanted/	Interpretation of biochemical data by analyzing and visualizing them in the context of the underlying metabolic pathways or other networks

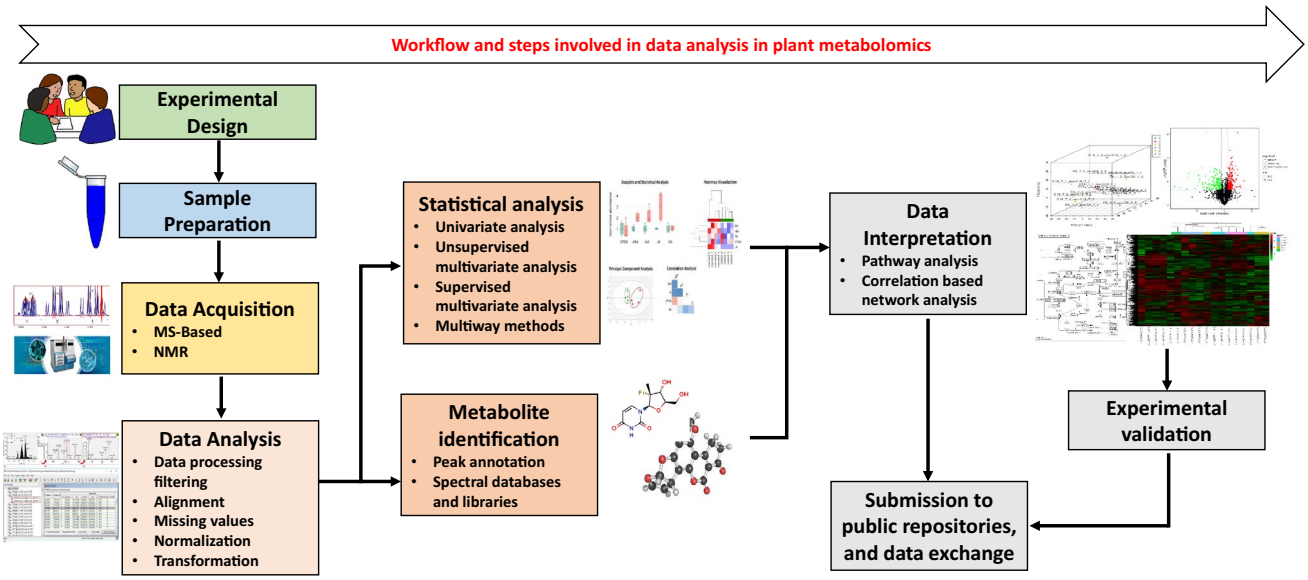


Fig. 3 Schematic presentation of the workflow and steps involved in the high-throughput data analysis in plant metabolomics. There are four major steps in the metabolome analysis, i.e., experimental design, sample preparation, data acquisition, and data analysis produced by any analytical approach, which ultimately leads to identifying metabolites, interpretation of the biological data, results validation, and submission to public sources

ranges were described in Fig. 2, having their selectivity, sensitivity, and limitations. A platform can be chosen based on metabolites, their class, concentration level, physical and chemical properties (Allwood and Goodacre 2010). A new method discovered that syndicates separation of metabolites, DNA, long RNAs, small RNAs, and proteins could be possible from a single sample. This procedure is used to understand the inter-relation structure (Weckwerth et al. 2004; Valledor et al. 2014; Wang et al. 2016a). The molecular dynamics structure of a cellular organization is the outcome of biochemical regulation. So, there is a need to know about biochemical regulation to get insight the covariance data about structures (Nägele et al. 2014; Wang et al. 2016b).

Presently, various approaches/methods are available for metabolomics analysis (Fig. 4). Metabolomics can be classified based on the data quality and the number of identified metabolites. Mainly, metabolites are classified into three classes. First-class is the metabolite-targeted investigation dealing with the discovery and the exact quantification of each targeted metabolite or small group of targeted metabolites. The second class is defined as metabolite profiling, which provides information about the detection, documentation, and estimated evaluations of a large set of targeted metabolites integrated with specific biochemical pathways. The third class is described as metabolite fingerprinting. This class can be applied for the complete metabolite comparison lacking the knowledge of metabolite identification. Generally, a spectral study is used for the metabolome fingerprinting. Metabolite variations have been observed in the whole chromatographic design variations without the prior information of the examined metabolites. Hence, the identification of the metabolites is not required because the deducible theory does not manage this method; consequently, it is the open method for innovative discoveries (Carraro et al. 2009).

Metabolomic studies in plant stress response

Productive molecular breeding that is dependent on the comprehensive and helpful molecular mechanisms responsible for plant development, that acquire through the systems biology approaches along with metabolomics, under normal and stress conditions. In nature, unfavourable climatic conditions mainly consist of multiple factors in which every single stress is accompanied primarily by or followed by other stress (Kráľová et al. 2012). To understand the role of single stress, a precise fluctuating technique was developed, and plants were exposed to individual stress to unravel the systems biology (Nakabayashi and Saito 2015; Bowne et al. 2018; Razzaq et al. 2019). In-plants stress physiology and biochemistry, the metabolomic studies are becoming increasingly common (Fig. 5). The subsequent section covers a review of the applications of metabolomics to explore the plant responses to HS.

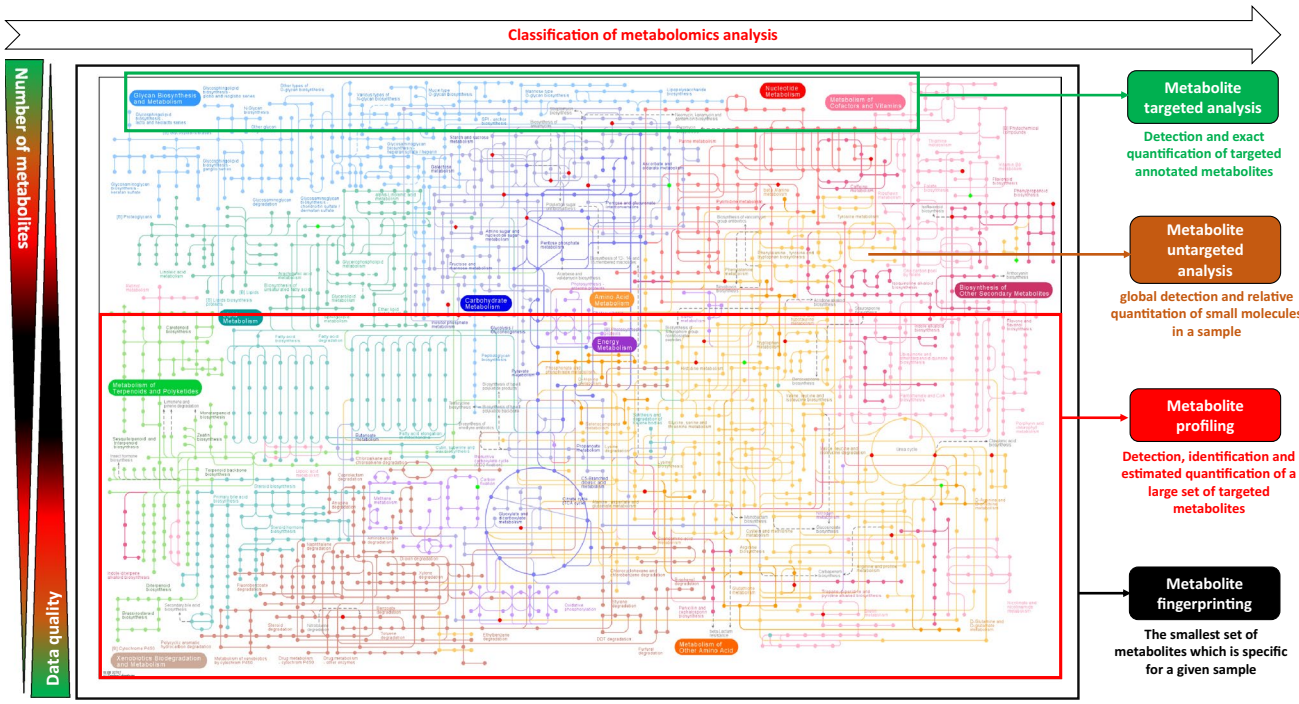


Fig. 4 Classification of metabolomics analysis. The level of an identified metabolite can supply the knowledge about the biochemical position in response to the environment and genetic manipulation, even at a single gene level. With the advancement in the "omics" approaches, we can find out what genes and proteins are being expressed and present, and now what patterns and amount of many cellular metabolites are present. Metabolomics seeks for the detection, identification, and quantification of low molecular weight metabolites to get useful data in a biological scheme. The quality of obtained data and the number of metabolites may vary with the analysis, plant, and cell/tissue type

The role of metabolomics in HS tolerance and adaptation

Plants require ideal temperatures for better productivity. Temperature variations can severely harm and terminate the plant's developmental progressions. Notably, HS overwhelmingly suppresses plant growth and development by harmfully disturbing main metabolic advances like photosynthesis, primary and/or secondary metabolisms, lipid and hormonal signaling (Raza et al. 2019, 2020b; Youldash et al. 2020; Sabagh et al. 2020). Likewise, extended HS can harm root length, plant height, grain quality, and biomass production amongst most field crops (Kilasi et al. 2018; Hütsch et al. 2019; Youldash et al. 2020). Another antagonistic effect of HS is the adverse effect on the plant root system, which offers support, nutrient and water uptake, and transportation to other plant organs, causing interrupted pollination, flowering, and root growth (Valdés-López et al. 2016; Sehgal et al. 2017). Extended HS can induce cellular oxidative injury due to ROS production (Raza et al. 2020b; Youldash et al. 2020). Plants own several adaptive, escaping, and/or acclimation mechanisms to deal with HS conditions. Additionally, main tolerance mechanisms that employ ion transporters, proteins, osmoprotectants, antioxidants, and other influences elaborated in signaling cascades and transcriptional regulations are triggered to balance stress-induced biochemical and physiological amendments (Hasanuzzaman et al. 2013). Plant responses to HS differ with the temperature fluctuation, extent, and plant type. Elevated HS causes cellular damage/cell death within minutes, which may cause a shattering failure of the cellular body. Further, HS differentially affects the constancy of numerous proteins, membranes, RNA species, and cytoskeleton assemblies and modifies the productivity of enzymatic responses in the cell (Hasanuzzaman et al. 2013, 2020). Consequently, information about how plants adapt, respond, and tolerate HS conditions is vital for the development of plant productivity under changing climatic conditions. To reduce the adverse impact of HS, reviewing how the plants have advanced stress tolerance, surviving mechanisms will bring new ideas and lead to ground-breaking approaches in breeding for climate-resilient crops. In this line, one of the rapidly emerging systems biology approaches, named "metabolomics" played a very vital role and helped to reveal the mechanisms responsible for metabolites-mediated phenotypic effects under HS. Thus, recently many investigations have been performed to unravel the metabolic responses of several plant species under HS (Table 2).

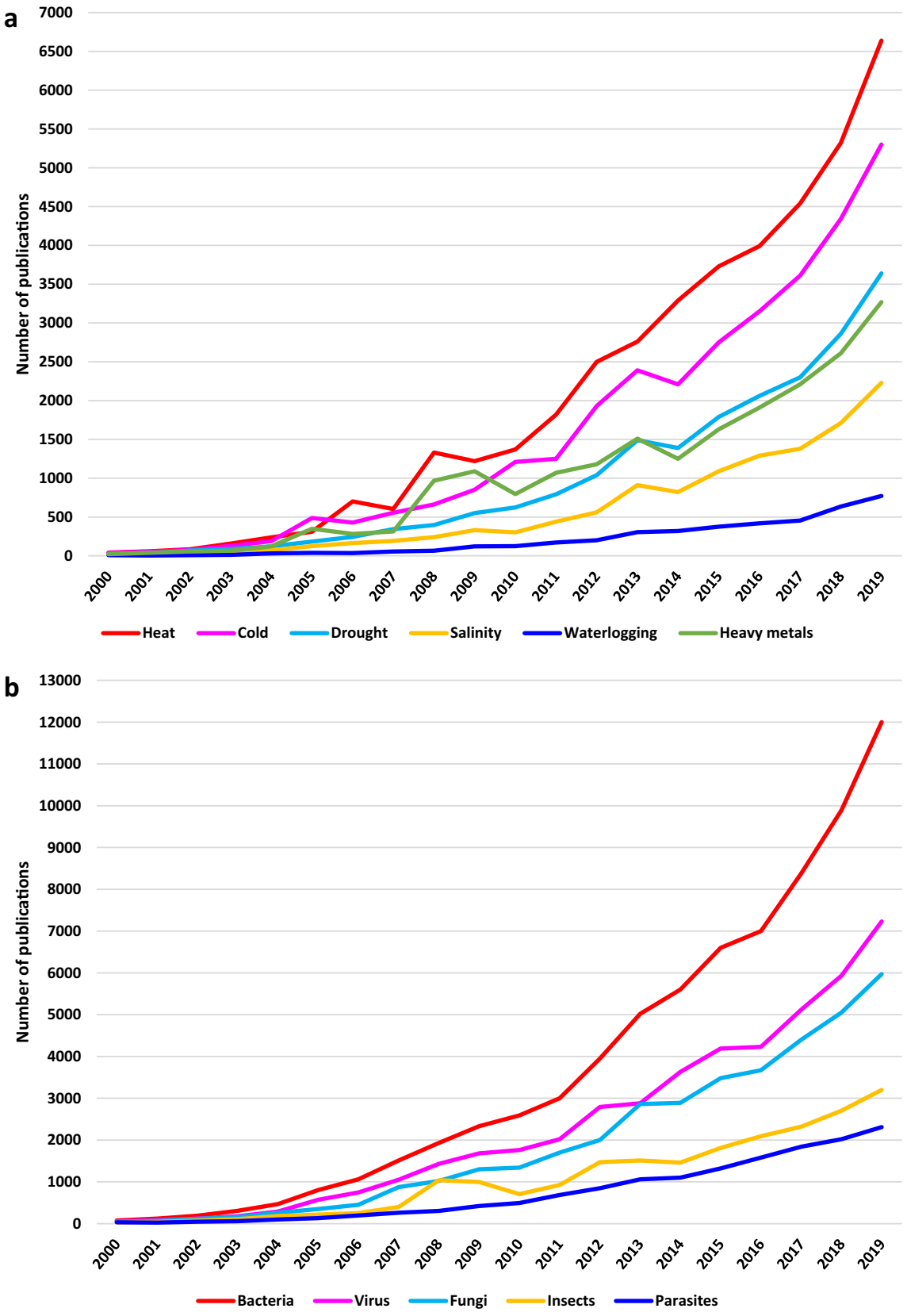
Mainly, HS causes metabolic redeployment on the way to homeostasis, sustaining vital metabolism, and producing

metabolites with HS-defensive and signaling features. For example, untargeted metabolome profiling of soybean (*Glycine max* L.) leaf was achieved under HS. In response to HS, several differently produced metabolites (DPMs) (carbohydrates, lipids, amino acids, peptides, cofactors, nucleotides, and secondary metabolites) were detected in leaves. Numerous DPMs (ribose, deoxyribose, gluconate, xylose, xylitol, lysine, alanine, methionine, and isoleucine) involved in cellular processes pathways, e.g., glycolysis, the pentose phosphate pathway, TCA cycle, and starch biosynthesis, were affected by HS. In short, the up-regulation of sugar and nitrogen metabolisms can significantly help to cope with the HS (Das et al. 2017). Moreover, Thomason et al. (2018) reported the untargeted LC–MS based metabolome analysis of wheat (*Triticum aestivum* L.) plants under post-anthesis HS. Among several DPMs, L-tryptophan and pipecolate were significantly up-regulated and exhibited a negative association with yield-related traits under HS. Likewise, two metabolites (Drummondol and anthranilate) were downregulated and positively associated yield traits under HS. Furthermore, the biosynthesis of aminoacyl-tRNA and secondary metabolites were significantly affected by HS. The study emphasized that numerous DPMs are distinguishing the heat-stressed genotypes from controls, and this might be used as possible biomarkers for genetic advancement investigations (Thomason et al. 2018). In tomato (*Solanum lycopersicum* L.) pollen, several putatively notorious secondary metabolites went to three major sets, i.e., alkaloids, flavonoids, and polyamines, in response to HS (Paupière et al. 2017). Moreover, Wang et al. (2018a) performed the metabolome analysis of wheat at the grain filling phase and identified 98 DPMs (60 decreased and 38 increased) induced by HS. Carbohydrate-related metabolic contents were significantly reduced, whereas amino acids and starch biosynthesis-related contents were increased under HS (Wang et al. 2018a). Similarly, Qu et al. (2018) reported that some vital compounds such as malate, valine, isoleucine, glucose, starch, sucrose, proline, glycine, and serine were effectively produced in response to CO_2 and HS in maize (*Zea mays* L.) plants (Qu et al., 2018).

In another study, HS influencing rice (*Oryza sativa* L.) seed was observed by metabolic profiling (Dhatt et al. 2019). Masses of sugars (sucrose, glucose, fructose), tricarboxylic acid (TCA) cycle, and starch biosynthesis were strongly linked with the HS tolerance in rice. In another cluster of genes, the physical deterioration of starch granules, modification of mature seed, and accumulation of aspartate under HS were observed (Dhatt et al. 2019). Moreover, under HS, Lawas et al. (2019) used three rice cultivars to perform a GC–MS based metabolome analysis of rice organs at several developmental stages, i.e., flag leaves, flowering spikelets, and developing seeds. In the flag leaves, the identified metabolites (> 50%) at the flowering phase were expressively different in the two cultivars. In the flowering spikelets, the up-regulation of the polyols, Myo-inositol, and glycerol were observed in the heat-tolerant cultivar (N22). In the developing seeds, putrescine level was up-regulated in N22; some other metabolites, e.g., vanillic acid, arbutin, arabitol, 4-hydroxy-benzoic acid, and hydroquinone were up-regulated in Dular (heat sensitive) cultivar, and only erythritol and Myo-inositol were up-regulated in Anjali cultivar. Further, during the developmental stages of flag leaves, nine DPMs were expressed in all three cultivars. These DPMs were then well-thought-out to be precise to the overall response to HS (Lawas et al. 2019).

The metabolome profile of the *Arabidopsis thaliana* plant responded inversely toward different HS levels, i.e., control, prolong warming, and heat shock (Wang et al. 2020). Decreased stomatal conductance and suppressed TCA cycle were detected under prolong warming, while heat shock improved transpiration, glycolysis pathway but limits the biosynthesis of acetyl-coenzyme-A. Heat shock factors (HSFA1s), DREBs, and bZIPs were observed to be up-regulated under all stress levels (Wang et al. 2020). Recently, in a different study, the picoPPESI-MS approach was used to reveal the metabolites in response to HS-treated single pollen grains of heat-tolerant (N22) and heat-sensitive rice cultivars (Koshihikari). Overall, 106 DPMs were detected in both cultivars along with the variations in phosphatidylinositol (PI) (34:3) in mature pollen. More PI content was noticed in N22 pollen, but not for Koshihikari pollen. Interestingly, considerable low PI content was detected in the single mature pollen grains in both cultivars (Wada et al. 2020). Additionally, Liu and Lin (2020) evaluated the impact of HS on *Sargassum fusiforme* leaf using the GC–MS approach. Further, a robust variety of numerous metabolisms was detected, such as organic and amino acids, sugars/sugar alcohols, esters, and amines. These metabolisms were meaningfully augmented in 10 pathways, e.g., aminoacyl-tRNA biosynthesis; glycine, serine, and threonine metabolism; alanine, aspartate, and glutamate metabolism; valine, leucine, and isoleucine biosynthesis; cyanoamino acid metabolism; cysteine and methionine metabolism; arginine and proline metabolism; tyrosine metabolism; TCA cycle; and glucosinolate biosynthesis under HS. These metabolic pathways may be a way forward for the development of resistance and improve the flexibility of *Sargassum fusiforme* to HS (Liu and Lin 2020).

Recently, Wei et al. (2020) investigated the metabolic response of lettuce (*Lactuca sativa* L.) seed under HS via an untargeted metabolome profiling. The results showed that seeds of heat-tolerant (N106) and heat-sensitive (N62) cultivars employed diverse metabolic stratagems in response to HS throughout germination. Notably, 867 DPMs were observed between both cultivars. Particularly, N62 buds accumulated higher levels of organic and amino acids,


a
Number of publications
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Bacteria
Virus
Fungi
Insects
Parasites

◂**Fig. 5** The number of publications (including preprints from the last few years) per year related to plant metabolomics under both **a** abiotic and **b** biotic stresses from 2000 to 2019. Since the innovation of metabolomics in the 1970s, it got much attention after the 2000s in plant stress studies. Currently, there is a rapidly growing interest in the field of plant metabolomics. Notably, during the past 10 years, plant metabolomics has been altered from a morally theoretical idea into an extremely appreciated and extensively exploited field. From the available literature, it can be assumed that temperature stresses (heat and cold) significantly affecting agricultural productivity, and scientists are doing their best to develop heat tolerant crops using metabolomics approaches. Source: Google Scholar with custom range (Keywords (plant metabolomics + stress name such as drought, cold, heat, salinity, heavy metals, and waterlogging), (metabolomics + biotic stress name such as bacteria, virus, fungi, insects, and parasites) were used for pursuing the number of publications in Google Scholar

sugars, sterols, phenolic compounds, and terpenoids compared to N106 buds at 21 °C. N106 accumulated higher levels of amino and organic acids, sugars, sesquiterpene lactones, sterols, and fatty acids derivatives throughout the germination at 35 °C. These findings cover how to connect the metabolomics to additional external and interior influences, disturbing lettuce seed germination under HS (Wei et al. 2020). Numerous untargeted metabolomics researches reported that salicylic acid, ascorbic acid, phenolic secondary metabolites, and almost all antioxidant defense enzymes could lessen HS indicators in several plant species (Zhang et al. 2017; Mobin et al. 2017; Muhlemann et al. 2018; Salvi et al. 2018). In addition, Ihsan et al. (2019) documented that sulphur-comprising molecules, e.g., glutathione, play a crucial role in HS mitigations in several plant species.

Conclusively, several DPMs have been identified that are unique to only heat-tolerant cultivar in several plant species. For example, in rice, the polyols, Myo-inositol, putrescine and glycerol (Lawas et al. 2019); cysteine, serine, and threonine, cytokinin, uridine diphosphate glucose, coumaryl-alcohol, and α-L-rhamnose (Wada et al. 2020); in lettuce, amino and organic acids, sugars, sesquiterpene lactones, sterols, and fatty acids derivatives (Wei et al. 2020), in pepper, citrulline, serine, cysteine, glutamine, homocitrulline, alanine, and ornithine (Wang et al. 2019) were significantly up-regulated and/or accumulated in heat-tolerant cultivars. Additionally, some examples have also been documented in Table 2. Moreover, Fig. 6 shows the cluster of significantly up-regulated and/or accumulated metabolites and metabolic pathways in response to heat stress, identified in the above-cited studies. These DPMs can be used as a targeted biomarker for future investigations in the genetic improvement of heat-stressed plants. For this purpose, future researches should be emphasized on the metabolome profiling of heat-tolerant cultivars rather than or in comparison to the sensitive-cultivars.

All these studies showed that the differential expression and accumulation of sugars, amino acids, and carbohydrates related metabolites and metabolic/biosynthesis pathways play a major role in response to HS. Further investigation should be carried on for the genetic or metabolic engineering of such pathways, which can help plants to cope and adapt to the HS environment. Further, most of the studies were carried out in a controlled environment (growth rooms, incubators, chamber, etc.); therefore, future research should expand its experimental approach from the laboratory setting to the field environment where plants face multiple stresses at once. This may alter the metabolome profile compared to the indoor environment. This idea will help to identify the multiple stress responses, key metabolites, and pathways and can be used for future research plans.

Metabolomics-assisted breeding for HS tolerance

During the past few years, metabolomics has accomplished substantial progress in both software and instrumentation, providing an excellent opportunity to examine the complete metabolome profiling of numerous plant species. Metabolic applications have helped many investigation areas, particularly biotechnology, such as precise plant breeding and plant functional genomics (Rai and Saito 2016; Christ et al. 2018). Additionally, metabolome applications open new windows for translational metabolome investigations in the context of plant breeding. Current developments in post-genomic techniques have augmented the examination method. Combining plant metabolomics with additional high-throughput (HTP) platforms will decrease the time required for crop improvement with enhanced stress tolerance (Christ et al. 2018). Metabolomics has remarkable potential to deliver a complete investigation of many metabolic and phenotyping analysis of plants under stressful environments (Christ et al. 2018). Recent researches making an effort to combine metabolomics with other omics systems, such as epigenomic QTL (eQTL), proteomic QTL (pQTL), and metabolic QTL (mQTL), for the mapping for quantitative traits and dismembering genetic differences at the mRNA, protein, and metabolic stages (Fig. 7), due to the availability of all-inclusive datasets of several omics approaches. Interestingly, genome-wide association studies (GWAS) together with metabolomics (mGWAS), mQTLs, metabolome-wide association studies (MWAS), and genome-phenome wide association studies (GPWAS) are powerful platforms for the investigation of genetic differences related to metabolic characters in plants (Fang et al. 2019; Templer et al. 2017).

Understanding the metabolic systems directing the multifaceted machines in metabolomics have a significant impact on metabolomics-assisted breeding (MAB) in order to develop improved cultivars that can withstand numerous stresses. Besides, data attained from mQTL studies lead to further inclusive information about quantifiable genetics (Acuña-Galindo et al. 2015). Metabolomics cut down

Table 2 Summary of some recent metabolomics studies for plant health improvement under HS

Plant species	HS condition	Specific tissue	Approach	Data analysis	Key observations	References
Glycine max	43/35 °C	Leaf	GC/LC–MS	PCA	266 DPMs (differently produced metabolites) The majority of the detected DPMs regulate carbohydrate, amino acid, and peptide metabolism, and purine and pyrimidine biosynthesis Resulting in sugar, nitrogen and phytochemical metabolisms are of great significance under HS	Das et al. (2017)
Triticum aestivum	35:28 ± 0.08 °C	Flag leaves	LC-HRMS	PLS-DA, KEGG	64 DPMs L-tryptophan and pipecolate showed negative, whereas drummondol and anthranilate showed a positive correlation with yield-related traits Biosynthesis of aminoacyl-tRNA and secondary metabolites were greatly affected by HS	Thomason et al. (2018)
Triticum aestivum	37 °C	Grains	LC–MS/MS HPLC	Metaboanalyst and KEGG	98 DPMs Carbohydrate contents were decreased under HS Amino acids and starch biosynthesis contents were increased under HS	Wang et al. (2018a)
Oryza sativa	34.3 ± 0.50 °C	Flag leaves, flowering spikelets, and developing seeds	GC–MS	PCA	Different numbers of DPMs were expressed in three cultivars based on different organs During the developmental stages of flag leaves, nine DPMs (phenylalanine, threonine, raffinose, and others) were expressed in all three cultivars These DPMs can be used as a biomarker for the development of HS tolerant rice cultivars	Lawas et al. (2019)
Oryza sativa	28 ± 1 °C	Immature and mature seed	GC–MS analysis	PCA and HCA	19 DPMs Alteration in mature seed, structural deterioration of starch granules, accumulation of aspartate under High Nighttime (HNT) Decreased proline, lysine, citric acid, xylose, and glycolic acid during seed development, increased protein content, and sugar metabolism	Dhatt et al. (2019)

Table 2 (continued)

Plant species	HS condition	Specific tissue	Approach	Data analysis	Key observations	References
Populus tomentosa	45 °C	Leaves	GC/TOF–MS	KEGG	Increase antioxidant enzyme activities and concentration of H_2O_2, proline, raffinose, and melibiose Glycolysis was enhanced by transformed metabolites Significant increase in Va, Leu, Arg, and pro metabolic pathways	Ren et al. (2019)
Capsicum annuum	40 °C	Leafs	LC–MS/MS	PCA and KEGG	94 and 108 DPMs in heat-tolerant (17CL30) and heat-sensitive (05S180) cultivars Significantly enriched metabolites and pathways are belonging to amino and organic acids, flavonoids, and sugars The changes in several metabolites showed the complex mechanisms responsible for HS tolerance	Wang et al. (2019)
Oryza sativa	34 °C	Grains	picoPPESI-MS	Two-way ANOVA	106 DPMs Phosphatidylinositol contents with a 34:3 ratio were detected in mature pollen of both cultivars Organic acids, carbohydrates, amino acids, cell wall-related materials, and hormones-related DPMs are observed in the single pollen grains	Wada et al. (2020)
Arabidopsis thaliana	28 and 38 °C	Leaves	GC/TOF–MS analysis	OPLS-DA, PCA, KEGG, GO	34 DPMs Increase in production of ROS, rate of transpiration, and photosynthesis by a heat shock Increase the concentration of H_2O_2 and activities of antioxidant enzymes Upregulation of bZIPs, DREB, and HSFA	Wang et al. (2020)
Sargassum fusiforme	27 °C and 32 °C	Leafs	GC–MS	PCA, OPLS-DA, KEGG	31 DPMs HS decreases 14 metabolites related to sugars, amino acids, and carbohydrates, including phenylacetaldehyde, glucose-6-phosphate, p-benzoquinone, trehalose, etc. and increases the tryptophan contents under HS The most enriched pathways are aminoacyl-tRNA biosynthesis, alanine, aspartate, and glutamate metabolism, valine, leucine, and isoleucine biosynthesis, arginine and proline metabolism, tyrosine metabolism, TCA cycle, etc	Liu and Lin (2020)

Table 2 (continued)

Plant species	HS condition	Specific tissue	Approach	Data analysis	Key observations	References
Lactuca sativa	21, 28 and 35 °C	Seeds	GC/MS and UPLC-IMS-QTOF-MS	PCA	867 DPMs between the heat-tolerant (N106) and heat-sensitive (N62) genotypes N62 buds accumulated higher levels of organic and amino acids, sugars, sterols, phenolic compounds, and terpenoids at 21 °C N106 accumulated more elevated levels of amino and organic acids, sugars, sesquiterpene lactones, sterols, and fatty acids derivatives at 35 °C	Wei et al. (2020)

Abbreviations are defined in the main text

the gap between the genotype–phenotype and unlocks new prospects for metabolic dissevering, starting with the documentation of SNP markers or mQTL mapping examination to discover candidate genes. Further, metabolic markers play a vital approach for agronomic attribute detection and examining the genetic pathways linked with plant phenotype (Sweetlove et al. 2014).

For example, Templer et al. (2017) performed a metabolome profiling of 81 barley (*Hordeum vulgare* L.) accessions under a combination of drought and HS for the identification of mQTLs related to stress tolerance. A total of 57 metabolites were found to be linked with antioxidant defense metabolism under HS. Identified mQTLs related to the pathways (γ-tocopherol, glutathione, and succinate) generated antioxidant enzymatic metabolites in response to stress. These mQTLs-based antioxidant defense help barley to cope with a stressful environment (Templer et al. 2017). Previously, wheat mQTL analysis was performed under both drought and HS. The result shows that 234 QTLs were linked with HS and 66 mQTL distributed all over the wheat genome. Further, 43 mQTL were correlated with both stresses, while only 2 were specific for HS. A combination of 137 SNP markers for HG-related candidate genes recognized 50 SNPs inside mQTL and these genes elaborated in sugar metabolism, ROS scavenging, and ABA-induced stomatal opening and closing. Recognized mQTL and genes could be considered for future investigations and genetic advancement of wheat under HS (Acuña-Galindo et al. 2015). Rice mQTL investigation has been completed, and, among 12 chromosomes, numerous mQTLs have been perceived in flag leaf and evolving seeds (Gong et al. 2013). Previously, Feng et al. (2012) performed the metabolic and genetic analysis dependent on glucosinolate biosynthesis in rapeseed (*Brassica napus* L.). Notably, 105 mQTLs related to glucosinolate biosynthesis in rapeseed seed and leaves have been observed.

Another fascinating approach, mGWAS in plants, has arisen as an influential tool for advanced functional genomics in order to define the ordinary genetic foundation of numerous metabolic variations in the plant metabolome study (Fang et al. 2019). mGWAS has been effectively used to subordinate primary and/or secondary metabolites with mechanical genetic factor, elaborated in glucosinolate synthesis, amino acid and phenylpropanoid biosynthesis, flavonoid metabolism in *Arabidopsis thaliana* and other crop plants (Alseekh and Fernie 2018; Fang and Luo 2019). In a previous study, Wu et al. (2016) provided an enhanced discovery of the contributing genes for ninety-four primary metabolites in *A. thaliana* by assimilating quantitative genetics together with metabolite–transcript association-network investigation. In 2018, the same group of scientists, Wu et al. (2018), used an untargeted mGWAS approach for > 3000 LC–MS–detected semipolar metabolites from 309 *A.*

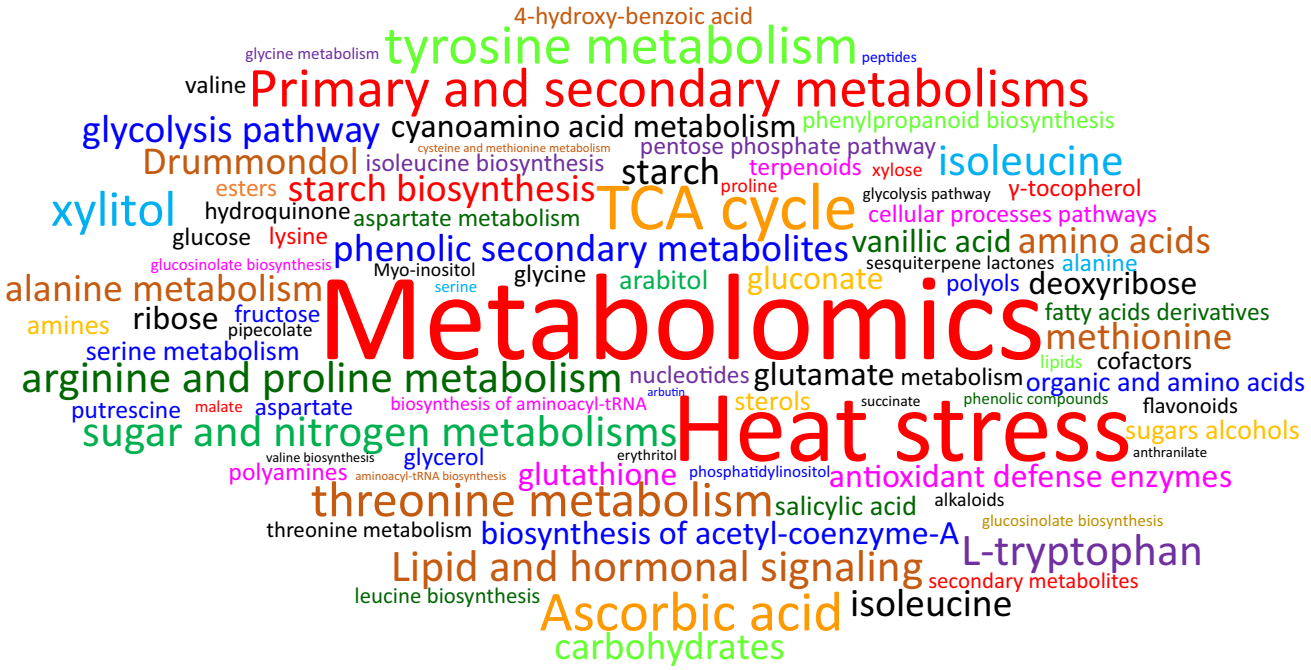

Fig. 6 A word-cloud illustration shows the keywords of significantly up-regulated and/or accumulated metabolites and metabolic pathways identified under heat stress in different plant species. Most of them were commonly identified in several studies, e.g., carbohydrate metabolism, amino acid metabolism, primary and secondary metabolisms, etc. and the metabolites involved in the biosynthesis and breakdown to other compounds

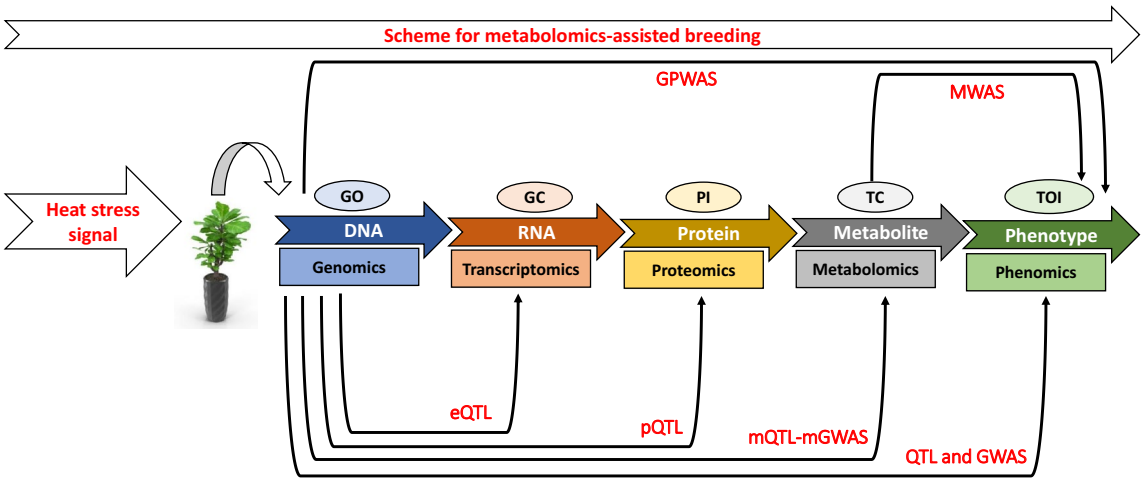

Fig. 7 Systematic model of MAB for gene expression together with molecular phenotype. Black arrows show that each molecular phenotype can be mapped onto the genome using QTL mapping and GWAS techniques. Nevertheless, MWAS does not demand precise knowledge to exploit the impacts of genetic deviant on metabolites. *MWAS* metabolome-wide association studies, *GPWAS* genome-phenome wide association studies, *GO* gene ontology, *GC* gene co-expression, *PI* protein interaction, *TC* trait correlation, *TOI* a trait of interest. Other abbreviations are explained in the text. Read the text for further information. Modified from Razzaq et al. (2019) (color figure online)

thaliana seedlings grownup in control and stress environments. They employed a statistical outline for 5 different metabolite modules and recognized 42 important attribute locus relations, advancing the documentation of 70 aspirant genes related to a stress response. In rice, the mGWAS study with 175 accessions effectively recognized 323 connotations between 143 SNPs and 89 metabolites. It showed that the metabolite contents are firmly connected with a not significant amount of robust QTLs (Matsuda et al. 2015). In tomato, mGWAS detected the 44 novel loci related to fruit metabolic characters (Sauvage et al. 2014). Moreover, an mGWAS investigation has been completed to recognize

biochemical and genetic differences in rice metabolisms. They recognized 36 genes related to unique metabolites controlling nutritional and physiological characters in rice plants (Chen et al. 2014).

In a different study, Muthuramalingam et al. (2018) conducted a global investigation on the genes regulating the threonine (Thr) metabolism using computational-mGWAS in rice under several abiotic stresses, including HS. The findings showed that 16 abiotic stress associated–Thr metabolite making genes (*ThrMPG*) modified metabolite contents and played a substantial role in defining both the physiological and nourishing status of rice plants. A set of 1373 and 1028 SNPs were associated with complex characters and genomic differences. Relative mapping of stress-*ThrMPG* exposed the chromosomal collinearity with C4 grasses. Additionally, the computational appearance design of these genes prophesied a different expression outlining diverse progressive matters. Analysis of protein interaction showed that abiotic stresses *ThrMPG* are multigenic. The outcomes provided an important source for a more functional examination of these candidate genes in response to several abiotic stresses for the genetic improvement of rice (Muthuramalingam et al. 2018).

Based on the available literature, only a very few groundbreaking studies have been carried out for the identification of HS-related genes and QTLs using metabolomic-mediated mQTL and mGWAS approaches. With focused-on key-locus documentation in many stresses for secondary metabolite levels, remains are lacking. Thus, future studies should be emphasized in the efficient utilization of these state-of-the-art approaches.

Persisting bottlenecks in metabolomic studies

Although tremendous advancement has been made in the field of metabolomics, several bottlenecks prerequisites remain to be solved to use metabolomics to its full potential. The elimination of these bottlenecks will help discover novel stages for crop improvement under HS, which sequentially will promise world food safety.

Metabolomic platforms have no such ability to change the detailed profile of tissues and cells. This is limited due to the dynamic range of instruments, chemical, and biological possessions of metabolites. The transcriptome and genome are composed of linear polymers of nucleotides, which have a similar chemical nature; due to this structure, a better analytical result would be expected. Proteome consists of a short number of amino acids, while it appears as more complex. The chemical nature of biopolymers is better defined, and the wide range of proteins are identified in a single analysis by various analytical technologies such as shotgun proteomics and 2DE gel electrophoresis as well as methylation and phosphorylation (Voelckel et al. 2017; Klose and Kobalz 1995). Chemical complexities are much higher in complex natural products, hydrophobic lipids, inorganic moiety, and hydrophilic carbohydrates. That is why metabolome profiling was so tricky due to the metabolome complexity and diversity. This hurdle could be overcome using new protocols and advanced technologies for metabolome (Weckwerth 2003; De Luca et al. 2012).

The coefficient of variation, which leads to an experimental approach, is known as analytical variation. According to the technology employed, this variance could be different. Quantitative variation gives rise to biological alteration at the metabolite level in plants grown under the same condition (Nägele et al. 2016; Nägele and Weckwerth 2012). Resolution is limited in metabolomics due to the biological variance. Biological variance can be reduced by pooling different samples together. Using statistical analysis, this strategy also reduces random variation, and it also leads to the dilution of various tissues that are very important for metabolite regulations. Interestingly, these variations can be minimized by various targeted analysis. Sampling could be problematic because of multiple parameters like growth stages, photosynthesis, and environmental stages; thus, various strategies are also inline to minimize these variations (Sumner et al. 2003; Kim et al. 2015).

Dynamic range is a critical challenge in metabolomics. Concentration boundaries that set-up for analytical determination are known as dynamic range. The sample matrix, competing, and interfering compounds all limited the dynamic range. For individual components, there is a different mass spectrometer that has a dynamic range of 10^4–10^6, and this could be limited because of other chemical compounds, e.g., secondary metabolites like flavonoids being interfered with primary metabolites (Sumner et al. 2003; Blum et al. 2018). Hence, high-level metabolites are so unique that they play a role in the differentiation of organisms at cellular states, tissues, and organs. Moreover, these are known as biomarkers. To diagnose various diseases like cancer and diabetes, selective profiling of biomarkers is essential. Due to the differential characteristics of highly targeted profiling, the previous detection should not be considered as metabolomics (Clifford et al. 2018). Another significant issue is salt concentration; low level leads to the problem in the profiling of various species, as well as affects the ionization efficiency of MS (Wang et al. 2018b). To improve the identification process and dynamic range, various analytical techniques have been progressed, as discussed in Sects. 2 and 3 (Sumner et al. 2003; Hall 2018).

Metabolomics data availability, legal issues, and benefits

Metabolomics is still covering overdue other omics approaches concerning data sharing and availability. Data sharing is being progressively obligatory by publishers

and has been indicated as a resolution to the duplication crisis. In the age of publicly available, reusable, and open science, materials should be made accessible to the scientific community. Progressing metabolomics examination by helping data sharing and availability restricted by the Nagoya Protocol on access and benefit-sharing should be a key target of metabolomics researchers (Watanabe 2015). The ideas of data sharing and open data are gradually imperative in science. Sharing data openly and freely is a vital way of cultivating reproducibility and screening that scientists are assured in their effort (McKiernan et al. 2016).

Interestingly, works with material shared in a repository also obtain extra citations than those lacking openly accessible data (Drachen et al. 2016). On the other hand, acknowledging the creative supplier upon reusing available data is imperative both as a recompense for data originators and to essay the attribution of examination discoveries (Piwowar and Vision 2013). Minimal submission and domain-definite databases to detention and distribute core data in metabolomics have risen in the 90s, afterwards forming a preliminary round of standardization exertions by the Metabolomics Standards Initiative (MSI) (Sansone et al. 2007; Deborde and Jacob 2014). Metabolomics data should acquiesce following the MSI guiding principles (Sansone et al. 2007). Currently, there are several publicly available metabolomics data repositories, such as MetaboLights (https://www.ebi.ac.uk/metabolights/), MetabolomeXchange (http://www.metabolomexchange.org/), OmicsDI (https://www.omicsdi.org/), Metabolomics Workbench (https://www.metabolomicsworkbench.org/), MetaPhen (https://www.metabolome-express.org/phenometer.php), MeRy-B (http://services.cbib.u-bordeaux.fr/MERYB/), GNPS (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp), Dryad (https://datadryad.org/), Figshare (https://figshare.com/), Zenodo (https://zenodo.org/), and SciLifeLab Data Repository (https://www.scilifelab.se/community-pages/systems-data/repository/).

The metabolomics field continues to progress original data standards and procedures as its evolutions. For instance, SPLASH (http://splash.fiehnlab.ucdavis.edu/), a hashed identifier for mass spectra, has been published (Wohlgemuth et al. 2016), which advances the exchange of mass spectra and agrees for attribution and duplicate discovery. In some specific databases, authors may submit data with domain-specific repositories and controlled access owing to personal privacy or academic issues by informing the consents and getting ethical agreements, etc. Currently and shortly, unlimited data availability can play a significant role in educating next-generation scientists in metabolomics and corresponding applications without spending much money and time on the original or/and initial data analysis and validation.

Conclusion and future directions

Metabolomics has gained an important place in plant biology exploration. The advancement of expertise from a single metabolite study to HTP examines producing several ways to diversify metabolites from a single drive, which has covered the system from the detection of improved models for metabolite systems and the documentation of robust biomarkers. There are enormous applications in plant research, such as identifying the candidate gene's functions (using integrated omics, i.e., transcriptomics and metabolomics) for investigating the entire biological apparatus in cells and dichotomizing the relationship between genotype–phenotype in response to several stresses, including HS. Numerous examples from the current scientific works demonstrate how metabolomics may produce novel data on the possessions of hybridization on plant and genotype-environment connections that could not have been so effortlessly gained with targeted investigations alone. In a rapidly changing climatic era, HS has become the most critical threat for crop production globally since it significantly affects the growth, development, and efficiency of plants. Nevertheless, the range to which this happens in particular climatic regions hangs on the possibility and period of HS and the daylight timing of HS. Therefore, under HS, plants modify themselves to adjust to the current stress by modulating genes, proteins, and metabolites regulation. It is vital to clarify the roles of recently acknowledged stress-responsive metabolites and genes to know the stress responses of plants. Metabolomics has a vast role in plant genetic breeding in which the HTP genotyping or sequencing techniques reliant on NGS tools together with metabolomics; hence, it has meaningfully decreased the diverse developmental period through MAB. Moreover, the combination of metabolomics, post-genomics, and several genetic techniques has also presented thrilling ways to investigate genetic procedures for plants in response to their metabolisms. A set of systems biology approaches can help to explore the multifaceted metabolic ways that oversee significant regulatory functions in plant metabolism. Furthermore, some persisting bottlenecks still require more attention for the precise documentation of compounds by different analytical techniques.

Crop production must be doubled by 2050 to guarantee future food security and to cope with the challenge of the "zero hunger" anticipated by the Food and Agriculture Organization (FAO), but several environmental stresses, mainly HS, are offsetting this goal. In the future, metabolomics can be employed for the identification of novel HS-responsive biomarkers to exploit plant metabolisms and to determine the mode and depth of stress. MAB, including

mQTL and mGWAS, can discover several ways in crop enhancement to produce high yielding, stress-resistant cultivars and generate climate-resilient ready-to-grow varieties. Moreover, single cell-based metabolomics research in plants should be employed to gain insight into each cell/tissue-mediated stress responses at the metabolic level. Additionally, metabolomics tools can be employed for metabolome profiling of genetically engineered plants that employ the most promising genome-editing (CRISPR/Cas) technique for risk evaluation and supervisory matters related to genetically engineered plants. Further, metabolic engineering of HS-related metabolic pathways and genes can open new windows for future research and the development of improved HS tolerant genotypes. Recently, speed breeding has emerged as a fascinating tool where plant metabolomics is prepared to see miracles for crop improvement under HS conditions. In the end, the combination of omics, genome editing, and speed breeding can do substantial wonders to feed millions worldwide.

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