Metabolomic Approaches to Study Nutritional Aspects in Cereal Crops

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Abstract

Owing to the increasing global population and food demand, cereals are the important source of food supply in the world. Due to global warming and other stresses, the overall nutritional quality and quantity have been severely affected in cereal crops. Various research studies have been conducted for cereal crop improvement but nutrient deficiency remains a major problem to the growing population, especially in developing countries. Hence, more comprehensive scientific tools like multi-omics will provide novel platforms to identify the high-yielding cultivars with improved nutritional quality which is important for food security. In the modern agricultural system, the development of nutrition-rich germplasm is necessary to cope with nutrient deficiencies. Among omics tools "Metabolomics", is a high-throughput and cost-effective approach in improving the nutritional quality in cereals through large-scale metabolic profiling and identifying the complex metabolic pathways. In addition, the integration of metabolomics with other modern techniques like genomics, proteomics, transcriptomics, and phenomics has witnessed an incredible technology not only the nutrition improvement but also in identifying contrasting nutritional genotypes. In this chapter, we have highlighted the application of metabolomics,

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tools, and database used in metabolomics, and analytical techniques for metabolic profiling focusing on nutritional aspects in cereal crops.

5.1 Introduction

Metabolomics is the complete evaluation of metabolites present endogenously from an experimental sample that uses a range of analytical platforms like NMR (nuclear magnetic resonance), MS (mass spectroscopy), GC (gas chromatography), HPLC (high-performance liquid chromatography), UPLC (ultraperformance liquid chromatography), and CE-MS (capillary electronics-mass spectrometry) providing different categories of small compounds counting carbohydrate, lipids, vitamins, and amino and organic acids. The primary metabolites play an important role in plant growth and development as a plant biomolecule or intermediate byproduct, while secondary metabolite takes part in influencing the former (primary metabolite) indirectly. Taking into account the complexity of metabolites in the plant system and the absence of a complete database of all plant metabolites, plant biologists are focusing on a single experimental tool that can isolate every single compound in the plant system. Metabolomics is one of the most important disciplines in high-throughput studies, which has been used for gene identification in several studies. The *Arabidopsis thaliana* has been the most comprehensively investigated because of the availability of huge genomic resources. Moreover, metabolomics has also been used broadly to identify gene functions in other non-model crops. Among different food crops, cereals serve as the main source of nutrition globally due to their seeds being enriched with essential fatty acids, carbohydrates, and macro and microelements for producing energy. Total food grain production of cereals like rice, wheat, barley, millets, and ragi was predicted to be 324 million tonnes (MT) in India (FAOSTAT 2021). The South Asian countries were the second-largest rice and wheat producer across the world during 2002–2020 (production volume of cereals India FY 2002–2020, Statista Research Department, 2020). Among cereals, metabolomics in rice has been used by many research groups to investigate the diversity of metabolites. Additionally, metabolomic research in maize has helped biologists to identify the superior genotypes with improved nutritional value. The metabolomic study has been conducted in several maize and rice varieties and their natural hybrids. The details of a total number of genes, their metabolic pathway, enzymatic reactions, transporters, and the protein features in different crops have been described in Table 5.1.

5.2 Application of Metabolomics in Crops

A metabolomic study identifies the low-molecular-weight molecules or metabolites within the biological systems.It is a novel approach that focuses on the dynamic nature and composition of biochemical within the living system. Intracellular metabolite contents, which are the primary product of protein-mediated cellular processes,

Table 5.1 Details of metabolomic profiling performed in different cereal crops showing different metabolic pathways using Plant Metabolic Network (PMN) database (https://plantcyc.org/)

	T. urartu	T. aestivum	O. brachyantha	O. punctata	O. longistaminata	O. meridionalis	O. barthii	O. glaberrima	O. sativa subspecies spontanea	O. sativa (Japonica)	O. rufipogon	Zea mays	Hordeum vulgare	Sorghum bicolor
Total genes	7185	24,489	6226	6232	7030	5604	6397	6511	6602	13,043	6555	8924	8933	7038
Pathways	431	446	435	431	444	426	440	442	443	545	442	499	464	439
Enzymatic reactions	2824	2980	2784	2765	2816	2722	2832	2785	2812	3547	2842	3089	3167	2805
Transporters	307	1388	247	322	239	376	332	238	348	547	380	416	3730	406
Compounds	2190	2336	2144	2135	2191	2139	2178	2151	2157	2859	2194	2468	2504	2160
GO terms	3	3	3	3	3	3	3	3	3	26	3	72	3	3
Transport reactions	84	84	67	70	70	53	71	65	74	90	72	95	95	79
Polypeptides	7186	30,682	6227	8371	7031	8608	7994	6512	9055	14,682	8833	27,682	57,690	9924
Protein complexes	0	0	0	0	0	0	0	0	0	5	0	13	0	1
Enzymes	7186	30,681	6226	8370	7029	8607	7993	6511	9054	13,043	8831	8695	57,688	9923
tRNA	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Protein features	0	0	0	0	0	0	0	0	0	0	0	1	0	0

could accurately represent cellular physiological changes, especially when coupled with other molecular profiling datasets. This information could be used to construct complex molecular networking by integration of different RNA expression, DNA variation, DNA–protein binding, protein–metabolite interaction, and protein–protein interaction data with metabolite concentration to elucidate cellular regulation within the plant system (Zhu et al. 2012). Investigators can implement gene information onto supplementary sets of data to gain a far more widespread understanding of the disease or any other traits of interest using a multi-omics/systems-level approach, which combines data from the genome (genomics), RNA transcription (transcriptomics), proteins/peptides (proteomics), and metabolites (metabolomics) (Romero et al. 2006). Metabolomics stands out significantly in the sense of environment-gene interaction, mutant characterization, marker recognition, and drug discovery (Razzaq et al. 2019). Metabolomic strategies have the potency to use system biology platform to understand natural product pathways and novel pathways regulated by transcription factors beneficial for elevating trait enhancement in agricultural food and industrial product (Dixon et al. 2006).

Plants can produce over 20,000 metabolites that are involved in diverse resistance and stress tolerance responses or have a specific function in permitting distinct ecological niches to be adapted, as well as contributing to the color, taste, perfume, and scent of fruits and flowers (Oksman-Caldentey et al. 2004; Fiehn 2002; Bino et al. 2004). The customs of agricultural varieties vary from obsolete foods to those

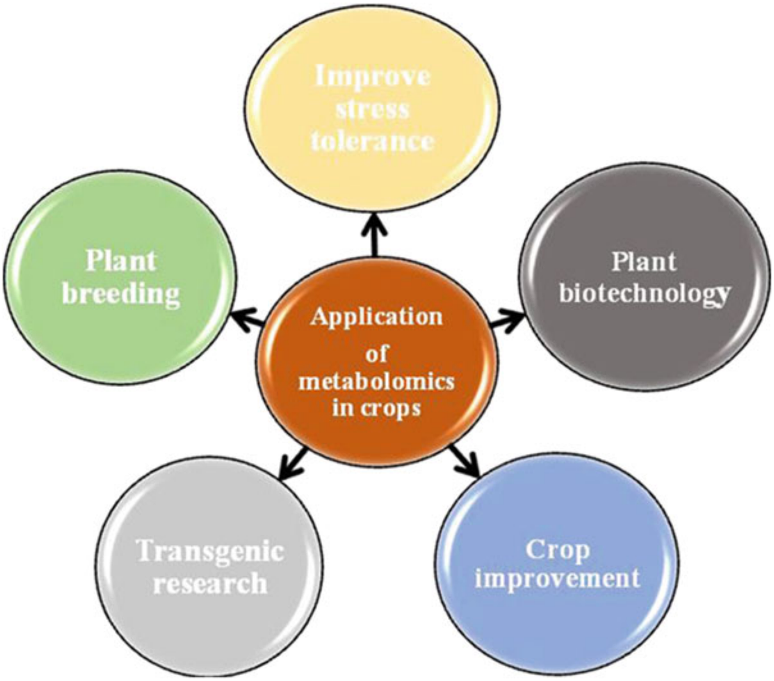

Fig. 5.1 Applications of metabolomics in agriculture crops for improving quality traits

with certain valuable characteristics, such as nutritional values, and industrial goods derived from fibers, latex, packaging material, polymers, and certain essential chemical fuels (Abbas and Cheryan 2002). The goal of the metabolomic approach in agriculture is to evaluate the biochemistry of metabolites and applying this information for food and environmental safety (Dixon et al. 2006) (Fig. 5.1).

5.3 Different Applications of Metabolomics in Crop Production

Metabolomics is an important tool having a role in various aspects of crop improvement from classical breeding to transgenic research, fruit maturation processes, resistance to adverse environmental factors, stress-related issues, and pathogen attacks, for sustainable agriculture. Some applications of plant metabolomics are the following:

5.3.1 Safety Assessment of Genetically Modified (GM) Crops

Food security is one of the most pressing challenges for the world's rising population. Modern agricultural biotechnologies, such as genetic modification, may provide a solution by allowing for increased productivity, more efficient use of natural resources, and lower environmental impacts. New crop varieties with altered genetic elements, on the other hand, may be submitted to safety studies before marketing in order to meet the regulatory criteria (Simó et al. 2014). Due to GM crop production, a huge understanding in economic potential effecting qualitative trait like tolerance to herbicide, resistance to insects, faster or delayed ripening, high level of antioxidant and nutrients, etc., has been performed. Metabolomics therefore provides more scope for the study of GM crops by enabling the id-entification of both intended and unintended effects (such as silencing off-target genes through RNA inference in the case of RNAi-based GM) that may occur in GM crops due to metabolic genetic modification. Some observable changes have been identified in the metabolite of some important crops such as rice, soybean, peas, wheat, potato, barley, and so on due to transgenic modification (Chang et al. 2012). In order to combine the authentic impact of genetic modification, metabolomic studies compare GM crops with their non-GM counterpart line using various cultural conditions (Zhou et al. 2009). The significant metabolic difference between GM and non-GM foods will be considered as healthy metabolic alteration and provide a deeper understanding on GM food composition (Harrigan et al. 2010).

5.3.2 Plant Improvement by Metabolomic Engineering

Plant metabolic pathway engineering combined with current technologies will be advantageous to humanity (food and medicines) as plants are capable to produce varied chemical compounds (Oksman-Caldentey and Saito 2005). Metabolic engineering of large datasets and logical metabolic pathway models through a huge-scale processing and mining of multiple omics data can help to improve the performance of engineered plants (Farre et al. 2015). For instance, plant metabolic engineering has been used to improve vintages of endogenous sugars such as higher-level sugars and simple sugar compounds by discovering sugar biosynthesis and accumulation pathways (Patrick et al. 2013).

5.3.3 Metabolomic Crop Improvement

Crop breeding rely on phenotypic and genomic assortment by genetic markers. However, this causes a great hurdle due to marker effects for selecting complex traits that are commonly different among population. This can be resolve using metabolomic approach combined with other omics technologies, which provide detailed information of crop plants that are performed in larger-scale environment. This mQTL and mGWAS data enables us to examine the existence of quantitative characteristics of interest (Langridge and Fleury 2011). Thus, plant metabolic technologies may contribute to the creation of a more logical models linked to precise metabolite or pathways associated with yield or quality characteristics by providing information on the number of metabolites defined that are also correlated with agronomically significant characteristics (Carreno-Quintero et al. 2013). Remarkably, continuous efforts illuminating the metabolic response to different stresses infer that breeding supported by metabolomics may also be helpful in obtaining more stress-resistant crops (Fernie and Schauer 2009).

5.3.4 Ecological Metabolomics

It deals with the study of plant biochemical relation of plant through discrete temporal (habitat lifespan to the generation time) and spatial (distance between habitat patches) framework. This method helps us to determine the interaction of abiotic factors with intra–interspecific linkages between two trophic levels and multiple impacts. In response to environmental factors, it encounters the effect of abiotic and biotic stressors on any biological activity by metabolite recognition. Biochemical network results from variance in the concentration of multiple metabolites that clarify the phenotype and physiological responses due to changes in the environment (Garcia-Cela et al. 2018).

5.3.5 Biological Control

In agriculture, biopesticides have many advantages, but still their usage is minimal due to unreliable manner, quality, and shelf-life and environmental restrictions (Babalola 2010). We need novel approach like metabolomics, which defines the need for stimuli or gene expression to synthesize metabolites that have already been discovered. Therefore, combination of molecular approach (gene sequencing and detection) and metabolomics can assist in the discovery of novel metabolites and reliable biopesticides for agricultural use (Mishra and Arora 2018).

5.3.6 Metabolomic-Assisted Breeding

For improvement of crop quality, we need the most probable, faster, cheaper, and modern breeding technique other than the standard breeding methods (Gao 2018). Metabolomics is the rightful platform to develop new crop varieties that can overcome the environmental changes, pests, and diseases and maintain their health status without change in its metabolome caused by various environmental factors like season, time, and temperature. The quality of end products involves the use of metabolomics in crop breeding, genetic modification, and biomarker discovery to meet the demand of overgrowing populations (Khakimov et al. 2014). Linkages between quantitative trait loci (QTLs) and phenotype function are a part of metabolic and transduction pathways. Metabolomics was used to classify the methylation qualitative trait loci (mQTLs) and to assess the difference in metabolic adaptation to heat and drought stress (Beckles and Roessner 2012; Templer et al. 2017). These findings revealed an important feature of mQTLs located on genes encoding the pathway of enzymes that generate antioxidant metabolites. For breeders to breed cultivators with abiotic stress tolerance, this can be a useful source. The metabolomic method is known to be a useful tool for plants to deal with environmental stresses (Rouphael et al. 2016). In conjunction with other omics techniques, metabolomic-assisted breeding would thus dramatically increase the accuracy and efficiency required for future breeding (Christ et al. 2018). Metabolomics is an emerging omics tool strategy, which has now been widely used for crop improvement. It is essential for the tolerance of abiotic stress, pathogen resistance, robust ecotype, metabolic-assisted crop reproduction (Shulaev et al. 2008). To understand traditional biological pathways and explorer secret networks that regulate crop growth and development, current metabolomic approaches are demoralized (Deborde et al. 2017). Several metabolome extraction methods and their analysis techniques have been used to assess the complex nature of metabolite and diverse chemical composition (Wishart 2011). Integration of modern plant genomic tools (GBS, genome-wide genetic variants and whole-genome sequencing) with metabolomics reveals exciting horizon for crop improvement (Zivy et al. 2015). In addition, metabolomic tool performs metabolic profiling of biofluid and various cell tissues to reflect the entire physiological composition of the cell (Yang et al. 2018).

The metabolome comprises a huge number of different chemical and physical composition such as pka, stability, molecular weight, size, polarity, and solubility (Villas-Bôas et al. 2007). A variety of analytical technologies were applied for separation, detection, and quantification of these chemicals. Metabolite content in agriculture is related to several different processes, such as fruit maturation, resistance to adverse environmental condition, stress tolerance, and pathogen infection. To analyze these compounds, various analytical techniques are used. For instance, liquid chromatography combine with mass spectrometry can be used to investigate a

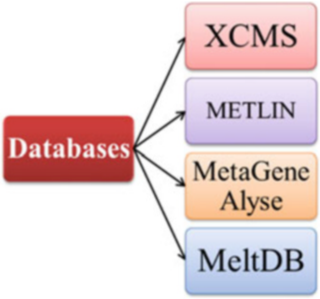

Fig. 5.2 Different databases or platformsavailable online that are useful inmetabolomic profiling for improving important food crop traits in agriculture. (**a**) XCMS (https://xcmsonline.scripps.edu), (**b**) METLIN (https://metlin.scripps.edu), (**c**) MetaGeneAlyse (http://metagenealyse.mpimp-golm.mpg.de/), and (**d**) MeltDB (https://meltdb.cebitec.uni-bielefeld.de)

wide range of compound like vitamins, coenzyme, carbohydrates, amino acids, and many more (Carreno-Quintero et al. 2013).

5.4 Tools and Databases Used in Metabolomics

Computational informatics is needed for metabolomic processes as a result of advancement in modern analytical and technological tools (Wishart 2007). To support metabolomic data mining, data evaluation, and data interpretation, we need to design online-based program. Hence, we have discussed some important tools and databases for metabolic processes (Gardinassi et al. 2017) (Fig. 5.2).

XCMS It is a bioinformatics platform available online (https://xcmsonline.scripps.edu) that enable direct access to raw data and facilitate data processing and statistical analysis. Often due to limited space, it is unfit (Tautenhahn et al. 2012). This software has been recognized for data transfer by LC-MS, which decreases data processing time and increases online system performance (Montenegro-Burke et al. 2017).

METLIN It is an online database used in plants for metabolic profiling of stress response. For metabolite profiling and for data mining, annotation, and processing, this database is the most accurate. It retrieves the results of experiments with LC-MS, FT-IR, and MS by allowing its operators to position queries in the database via the program system (Smith et al. 2005).

MetaGeneAlyse It is an online method (http://metagenealyse.mpimp-golm.mpg.de/) for routine clustering technique implementation, i.e., ICA (independent component analysis) and k-mean. It is also useful for PLS-DA, pathway enrichment analysis, and t-test, in addition to this (Daub et al. 2003).

Table 5.2 Various metabolomic-profiling tools used in analysis workflow, data preprocessing, metabolite annotation, data-post processing, and statistical analysis

	Tool	Operating system (OS)	Software type/ interface	Language	Website	**Referece**
Analysis workflow	Workflow4 metabolomics	Unix/Linux, Mac OS, Windows	Graphical user interface/galaxy-based	Galaxy-based	http://workflow4metabolomics.org	Giacomoni et al. (2014)
	Galaxy-M	-do-	Galaxy-based	R Package, Python, MATLAB	https://github.com/Viant-Metabolomics/Galaxy-M	Davidson et al. (2016)
	XCMS online	-do-	Web user interface	R package	https://xcmsonline.scripps.edu/landing_page.php?pgcontent=mainPage	Tautenhahn et al. (2012)
	Metabo Analyst 3.0	-do-	-do-	Java, R Package	http://www.metaboanalyst.ca	Xia et al. (2009)
	MAVEN	-do-	Graphical user interface	C++	https://maven.apache.org/	Clasquin et al. (2012)
	MAIT	-do-	Command line interface/R Package	R package	https://www.bioconductor.org/packages/release/bioc/html/MAIT.html	Fernández-Albert et al. (2014)
	MZmine 2	-do-	Graphical user interface	Java, R package	http://mzmine.github.io/	Pluskal et al. (2010)
Data pre processing	XCMS	Command line interface	Unix/Linux, Mac OS, Windows	R Package, C ++	http://bioconductor.org/packages/release/bioc/html/xcms.html	Smith et al. (2005)
	MetAlign	Command line interface, graphical user interface	Windows 7 (32 And 64 bit)/XP/NT/2000	Visual C++	http://www.wageningenur.nl/en/show/MetAlign-1.html	Lommen and Kools (2012)
	MS-DIAL	-do-	Windows XP/vista/ 7/8	C	http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/index.html	Tsugawa et al. 2015

(continued)

Table 5.2 (continued)

	Tool	Operating system (OS)	Software type/ interface	Language	Website	**Referece**
	mzMatch	Command line Interface	Unix/Linux, Mac OS, Windows	R Package, Java	http://mzmatch.sourceforge.net/index.php	Daly et al. 2014
	AMDIS	-do-	Windows GUI	–	http://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:amdis	Meyer et al. (2010)
	Metabolite Detector	-do-	Unix/Linux, Mac OS, Windows	C++	http://md.tu-bs.de	Hiller et al. 2009
	MET-IDEA	-do-	Windows	.NET	http://bioinfo.noble.org/download	
	MeltDB	Web user Interface	Unix/Linux, Mac OS, Windows	Perl, Java, R Package	https://meltdb.cebitec.uni-bielefeld.de/cgi-bin/login.cgi	Kessler et al. (2013)
	metaMS	-do-	-do-	R Package	http://bioconductor.org/packages/release/bioc/html/metaMS.html	Wehrens et al. (2014)
	MSeasy	-do-	-do-	-do-	https://cran.r-project.org/web/packages/MSeasy/index.html	Nicolè et al. (2012)
	SpectConnect	Web user interface	-do-	Python, C	http://spectconnect.mit.edu	Styczynski et al. (2007)
	rNMR	-do-	-do-	R Package	http://rnmr.nmrfam.wisc.edu	Lewis et al. (2009)
Metabolite annotation	CAMERA	Command line interface	Unix/Linux, Mac OS, Windows	-do-	http://bioconductor.org/packages/release/bioc/html/CAMERA.html	Mahieu et al. (2016)
	BATMAN	-do-	-do-	R Package, C ++, MATLAB	http://batman.r-forge.r-project.org	Draper et al. (2009)
	Bayesil	-do-	-do-	R Package	http://bayesil.ca	Ravanbakhsh et al. (2015)
	CFM-ID	Web user nterface	-do-	Ruby, Java, MySQL	https://sourceforge.net/projects/cfm-id/	Allen et al. (2014)
	COLMAR	-do-	-do-	–	http://spin.ccic.ohio-state.edu/index.php/colmar	Zhang et al. (2009)

	FingerID	-do-	-do-	MATLAB, Python	https://github.com/icdishb/fingerid	Heinonen et al. (2012)
	MAGMa	-do-	-do-	-do-		Ridder et al. (2013)
	MetaboMiner	Command line interface	-do-	Java	http://wishart.biology.ualberta.ca/metabominer	Xia et al. (2008)
	MetAssign-mz Match	Graphical user Interface	-do-	Java, C, MATLAB	http://mzmatch.sourceforge.net/index.php	Daly et al. (2014)
	MetFrag	Web user interface, Command line Interface	-do-	Java, R package	http://c-ruttkies.github.io/MetFrag	Ruttkies et al. (2016)
	MI-PACK	Command line Interface	-do-	Python, R package	http://www.biosciences-labs.bham.ac.uk/viant/mipack	
	MyCompound ID	Web user Interface	-do-	Java	http://www.mycompoundid.org	Li et al. (2013)
	MZedDB	-do-	-do-	MySQL, Perl, PHP, R package	http://maltese.dbs.aber.ac.uk:8888/hrmet/index.html	Draper et al. (2009)
	ProbMetab	Command line Interface	-do-	R package	http://labpib.fmrp.usp.br/methods/probmetab	Silva et al. (2014)
	PUTMEDID-LCMS	-do-	-do-	Beanshell (Java)	http://www.mcisb.org/resources/putmedid.html	Brown et al. (2011)
	Rdisop	-do-	-do-	R package	http://bioconductor.org/packages/release/bioc/html/Rdisop.html.	Bioconductor - Rdisop (2016)
	SIRIUS	Command line interface, graphical user interface	-do-	Java	https://bio.informatik.uni-jena.de/software/sirius	Bocker et al. (2009)
	SpinAssign	Web user interface	-do-	Javascript, HTML, PHP, MySQL	http://prime.psc.riken.jp/?action=nmr_search	Chikayama et al. (2010)

(continued)

Table 5.2 (continued)

	Tool	Operating system (OS)	Software type/ interface	Language	Website	**Referece**
Data post-processing	batchCorr	Unix/Linux, Mac OS, Windows	Command line interface	R Package	https://gitlab.com/CarlBrunius/batchCorr	Brunius et al. (2016)
	Crmn	-do-	-do-	-do-	https://cran.r-project.org/web/packages/crmn/	Redestig et al. (2009)
	EigenMS	-do-	-do-	R/MATLAB	https://sourceforge.net/projects/eigenms	Karpievitch et al. (2014)
	KMDA	-do-	-do-	R Package	https://cran.r-project.org/web/packages/KMDA/	Zhan et al. (2015)
	Metabolomics	-do-	-do-	-do-	https://cran.r-project.org/web/packages/metabolomics/	De Livera et al. (2012)
	Metabomxtr	-do-	-do-	-do-	https://www.bioconductor.org/packages/release/bioc/html/metabomxtr.html	Nodzenski et al. (2014)
	Metabnorm	-do-	-do-	-do-	https://sourceforge.net/projects/metabnorm	Jauhiainen et al. (2014)
	MetabR	-do-	Graphical user interface	-do-	http://metabr.r-forge.r-project.org/	Ernest et al. (2012)
	MetNorm	-do-	Command line interface/graphical user interface	-do-	https://cran.r-project.org/web/packages/MetNorm/	De Livera et al. (2015)
	MSPrep	-do-	Command line interface	-do-	https://sourceforge.net/projects/msprep/	Hughes et al. (2014)
Statistical analysis	Ionwinze	Windows (32 bit)	-do-	R Package/C ++	https://sourceforge.net/projects/ionwinze	Kokubun and D'Costa (2013)

	Metabol Analyze	Unix/Linux, Mac OS, Windows	-do-	R Package	https://cran.r-project.org/web/packages/MetabolAnalyze	Nyamundanda et al. (2010)
	Metabolomics	-do-	-do-	-do-	https://cran.r-project.org/web/packages/metabolomics/	De Livera et al. (2012)
	MetaboLyzer	-do-	-do-	R Package/ Python	https://sites.google.com/a/georgetown.edu/fornace-lab-informatics/home/metabolyzer	Mak et al. (2014)
	Muma	-do-	-do-	R Package	https://cran.r-project.org/web/packages/muma/	Gaude et al. (2013)
	Ropls	-do-	-do-	-do-	https://www.bioconductor.org/packages/release/bioc/html/ropls.html	Thévenot et al. (2015)
	mOTL.NMR	-do-	-do-	-do-	https://www.bioconductor.org/packages/release/bioc/html/mQTL.NMR	Hedjazi et al. (2015)

MeltDB It is a web-based platform (https://meltdb.cebitec.uni-bielefeld.de) for data assessment, processing, and statistical analysis used in plant metabolomics (Kessler et al. 2013). Other than these, many more databases have been used to analyze and compare different metabolites such as MetPA (http://metpa.metabolomics.ca), MSEA (http://www.msea.ca), iMet-Q (http://ms.iis.sinica.edu.tw/comics/Software_iMet-Q.html), MS-Dial (http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/), and MetAlign (www.metalign.nl). (Xia and Wishart 2010; Kessler et al. 2013; Chang et al. 2016; Lommen and Kools 2012; Tsugawa et al. 2015). The detail of various metabolomic tools at different stages has been given in Table 5.2.

5.5 Cutting-Edge/High-Throughput Analytical Techniques in Metabolomic Analysis

Not only is one method used in metabolomics to examine all the metabolites present in a metabolome, but a lot of different technologies are required to manage data blocks (Fig. 5.3).

1. Nuclear magnetic resonance spectroscopy (NMR).
    - It provides rapid, highly reproducible, and nondestructive high-throughput method (Wishart 2019).
    - It provides structural and functional information of biomolecules and also is capable to analyze solid, liquid, and gas samples (Gouilleux et al. 2018).
    - In order to achieve biochemical measurement, it increased the sensitivity and spectral resolution of analytical assays on metabolomic samples.
    - Help in examining food quality and standardization of phytomedical preparations (Ward et al. 2007).
    - Detection in one single study of various groups of metabolites with different chemical and physical properties (Ward et al. 2007).
2. Liquid chromatography mass spectrometry (LC-MS).
    - It detects mass spectrometric of metabolites.
    - Separation of various metabolites based on different mobile phase and stationary phase portioning coefficients (Khakimov et al. 2014).
    - It is best suitable for detection of polar compound and secondary metabolite analysis like vitamins and flavonoids.
    - It utilizes the source of electroscopy ionization (ESI) to analyze metabolites of high molecular weight.
    - It allows direct probing of metabolites in any sample without derivatization (Wang et al. 2017).
3. Gas chromatography-mass spectroscopy (GC-MS).
    - This method is suitable for hydrophobic and polar compound with high resolving power and sensitivity.
    - Electron impact method is used.
    - It is used to classify thermally volatile and unstable compounds and has great power of separation and reproducibility (Jorge et al. 2016).

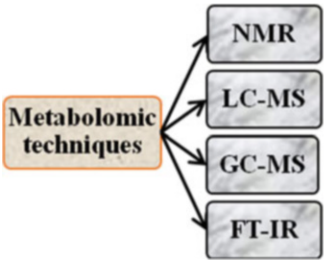

Fig. 5.3 High-throughput metabolomic technique used for metabolic profiling or quantification of metabolomes present in a host system. (**a**) Nuclear magnetic resonance spectroscopy (NMR), (**b**) liquid chromatography mass spectrometry (LC-MS), (**c**) gas chromatography mass spectroscopy (GC-MS), and (**d**) Fourier transform infrared spectroscopy (FT-IR)

- GC-MS metabolomic approach is used to display the effects of drought and heat on metabolite distribution of cultivators at different developmental stages (Lawas et al. 2019).

4. Fourier transform–infrared spectroscopy (FT-IR).
   - It allows the study of unknown metabolites to be identified based on the ratio of mass to charge.
   - In plant science, this approach has also been introduced as a metabolic fingerprinting technique (Kaderbhai et al. 2003).
   - It provides the most reliable information about data.
   - It allows characterization and separation of mixed sample (Vasmatkar et al. 2019).

5.6 Metabolomic Approaches to Improve Nutritional Quality in Major Crops

Cereals or foodomics such as wheat, rice, barley, corn, rye, and oat are agriculturally important food crops that are grown all over the world. These are the largest part of crops having important role in human utilization basically for health beneficial factors. The important characteristics of cereal crops include the carbohydrates, fats, essential and nonessential amino acids, dietary fibers, and micronutrients. Some primary and secondary metabolites often have a major impact on the health and nutritional implications. In an experimental research comparing hypercholesterolemic patients, whole grain barley consumption was shown to reduce the low-density lipoprotein (LDL) cholesterol. In addition, phenolic acids have been identified as essential texturizing agents in food preparation and key antioxidant component of cereals. The variation in phenolic content in different cereal crops has been shown in Fig. 5.4.

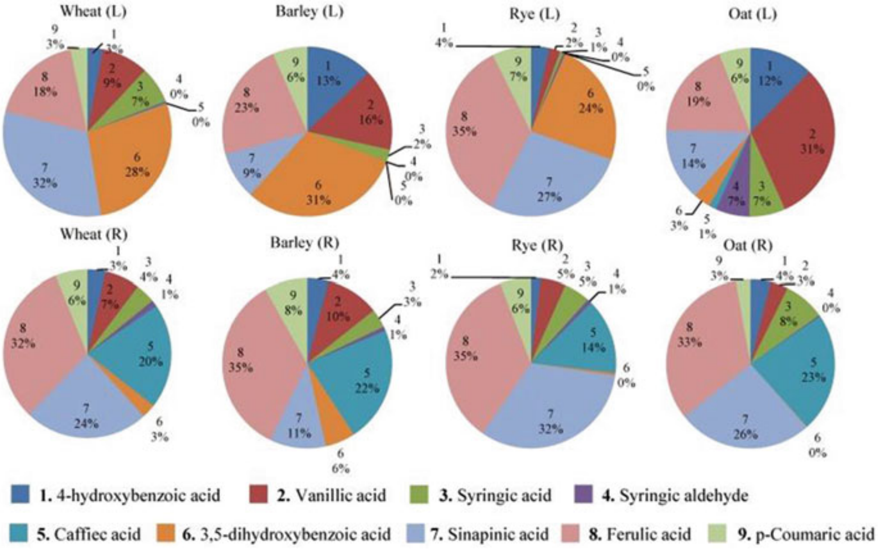

Fig. 5.4 Detailed pie-chart illustration showing percent variation of phenolic acid in different cereal crops including wheat, barley, rye, and oat

To date, several studies have been conducted in cereal crops including corn (count 99), rice and barley (count 103), oat (count 23), wheat (count 4), and rye (count 48). Previous research has established that utility of metabolomics in understanding the molecular mechanisms underlying diverse quality-related attributes. Metabolomics has emerged as a valuable talent for comprehensive characterization and synthesis of diverse metabolites in biological systems, and a number of plant science programs have been documented.

The primary and secondary metabolite compositions in the kernel as well as other aerial components were presented in cultivated rice with the aid of advanced techniques such as GC-MS, LC-MS, and (CE)-MS focusing on nutritionally essential bioactive compounds. Rice bran metabolite profiling was carried out to uncover 209 amino acids, including cofactors and vitamins, as well as other secondary metabolites, in order to improve the present understanding on chemicals delivered during dietary supplementation. Furthermore, certain wild rice species from North America (*Zizania palustris*) and China (*Zizania latifolia*) were compared for secondary metabolite levels, and it was found that the two differed particularly in anthocyanins and catechins among 357 metabolites studied. Similarly, comparative metabolomic profiling of giant vs regular embryo rice suggested the better quality of giant embryo-derived rice grains.

Wheat is the third-largest cereal crop in global production. Metabolic profiling has proven contrasting facts in numerous wheat organs. For example, a total of 118 and 56 metabolites have been identified in durum wheat roots; 111 in spring wheat roots, 53 metabolites in wheat phloem; followed by 51, 93, and

103 metabolites in leaf while 127 and 117 metabolites in spikelet and rachis of wheat, respectively. In the immature grain of bread wheat and durum wheat grain, a total of 74 and 42–50 metabolites were found, respectively (Zhen et al. 2016). Finally, a UPLC-TOF-MS was used to identify 935 ions on the mature grain of diverse genotypes of durum and bread wheat (Matthews et al. 2012).

Numerous metabolomic research have focused on deciphering the complex metabolic pathways involved in abiotic and biotic stress in corns along with the comparative metabolic profiling between genetically manipulated and nongenetically manipulated corn lines. Apart from these various studies targeting nutritional improvement (such as proteins, carbohydrates, fat) in corn along with an emphasis on anthocyanins, primary metabolites and mineral composition have also been presented. Out of 398 genetically distinct colored corn varieties from various regions, a total of 167 were chosen for producing anthocyanins. In addition to this, a total of 210 metabolites including primary metabolites (199), secondary metabolites (9), and phytohormones (2) have been identified in mature Chinese kernel lines. An integrated metabolic map from the identification of essential macronutrients and other important molecules was built having seven key pathways and 23 subpathways of corn kernel metabolism.

The metabolite patterns from three barley lines (*lys3.a*, *lys5.f*, and Bomi) from developing seeds were analyzed for alpha-glucan using GC-MS. Mutation in *lys3.a* and *lys5.f* led to an increase in lysine and ADP-glucose transporter gene production, respectively, regulating the carbohydrate, lipid, tricarboxylic acid cycle (TCA), shikimate–phenylpropanoid (SPP), and mevalonate pathways resulting in the improvement of new highly nutritive foods.

5.7 . Conclusions and Future Perspective

In conclusion, targeting nutritional metabolomics is an increasingly budding area to combine nourishment with multifarious metabolomic data to determine novel genetic markers. In order to incorporate, nutritional metabolomics with dietary supplements and observable traits, a broad range of softwares, repositories, and analytical tools are available. In addition, metabolomics can lead to the market problem analysis, consumer expectation, and food security. The holistic amalgamation of metabolomics with supplementary omic tools such as genomics, transcriptomics, proteomics, and phenomics may open a new opportunity for nutrition improvement as well as identification of nutrition-rich germplasm not only in cereal crops but also in other agriculturally important crops. The combination of metabolomics with high-throughput genotyping tools in the future would provide novel avenues to the plant breeders to develop highly nutritional cereals that adequately meet the expanding population's food requirement and provide food security.

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