5 The Contribution of Metabolomics to Systems Biology: Current Applications Bridging Genotype and Phenotype in Plant Science

Marina C. M. Martins, Valeria Mafra, Carolina C. Monte-Bello, and Camila Caldana

Abstract

Metabolomics is a valuable approach used to acquire comprehensive information about the set of metabolites in a cell or tissue, enabling a functional screen of the cellular activities in biological systems. Although metabolomics provides a more immediate and dynamic picture of phenotypes in comparison to the other omics, it is also the most complicated to measure because no single analytical technology can capture the extraordinary complexity of metabolite diversity in terms of structure and physical properties. Metabolomics has been extensively employed for a wide range of applications in plant science, which will be described in detail in this chapter. Among them, metabolomics is used for discriminating patterns of plant responses to genetic and environmental perturbations, as diagnostics and prediction tool to elucidate the function of genes for important and complex agronomic traits in crop species, and flux measurements are used to dissect the structure and regulatory properties of metabolic networks.

Keywords

Networks · Flux · Metabolites · Phenotype · Metabolism

M. C. M. Martins (✉)
Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil
e-mail: martins.marina@usp.br

V. Mafra
Instituto Federal de Educação, Ciência e Tecnologia do Norte de Minas Gerais, Januária, Brazil

C. C. Monte-Bello
Universidade Estadual de Campinas, Campinas, Brazil

Laboratório Nacional de Ciência e Tecnologia do Bioetanol, Centro Nacional de Pesquisa em Energia e Materiais, Campinas, Brazil

Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany
e-mail: cassano@mpimp-golm.mpg.de

C. Caldana
Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany
e-mail: caldana@mpimp-golm.mpg.de

5.1 Introduction and Overview of Plant Metabolomics

Metabolism is a complex, dynamic and highly integrated network of pathways driving the processes of assimilation, transport and chemical modification of small molecules. Its ultimate function is to maximize growth, survival and reproduction. Metabolites are organic compounds with low molecular weight (<1500 Da), and their properties and functionality dictate the chemistry of life (Fiehn et al. 2000; Fiehn 2002;

F. V. Winck (ed.), *Advances in Plant Omics and Systems Biology Approaches*, Advances in Experimental Medicine and Biology 1346, https://doi.org/10.1007/978-3-030-80352-0_5

Bino et al. 2004; Hall 2006). In the plant kingdom, more than 200,000 metabolites have been estimated (Dixon and Strack 2003; Afendi et al. 2012). These molecules are extremely diverse in their chemical structure and physical properties (e.g. polarity, volatility, size, and stability) and have a wide range of relative concentrations (Bino et al. 2004; Saito and Matsuda 2010; Jorge et al. 2016). In addition, plants possess a remarkable degree of compartmentation within their cells (Lunn 2007; Sweetlove and Fernie 2013), and the physical separation of metabolic pathways enables incompatible reactions to occur simultaneously within one cell and also prevents metabolic imbalances. Altogether, these features make the study of plant metabolism particularly challenging (Fiehn et al. 2000; Fiehn 2002; Saito and Matsuda 2010).

Traditionally, metabolites were grouped as 'primary' and 'secondary'. Primary metabolites are compounds that play essential roles in basic cell metabolism (e.g. amino acids, nucleotides, sugars, and lipids) and are required for proper growth and development. These substances are directly involved in the processes of photosynthesis, respiration, nutrient assimilation, and synthesis of macromolecules (Sulpice and McKeown 2015). Besides, plants have the ability to synthesize other compounds termed as 'secondary' or specialized metabolites, which were initially thought to be functionless end products of metabolism with no major significance for plant life (Pichersky and Gang 2000; Bourgaud et al. 2001). Also, unlike primary metabolites, which are found spread throughout the plant kingdom, secondary metabolites are often restricted to particular plant groups (Moore et al. 2014). However, many studies have demonstrated that secondary metabolites play crucial protective roles in the adaptation and survival of plants in different ecological niches and environmental conditions such as defence against herbivores, pests and pathogens (Nakabayashi and Saito 2015; Tenenboim and Brotman 2016). Plant secondary metabolites are extremely heterogeneous and diverse in functions, but can be divided into three major groups according to their biosynthetic pathways: terpenes and steroids, phenolics, and alkaloids (Harborne 1999). Although many metabolites were initially discovered through the study of discrete pathways, the metabolism operates as a systemic integrated network (Sweetlove et al. 2008; Stitt et al. 2010a) and efforts have been directed to increase the understanding of metabolic networks at a systems level.

The functions of metabolites are also correlated with their abundance, which reflects the balance between their rates of synthesis and degradation (Last et al. 2007). Examples include metabolites such as sucrose that is the major product of photosynthesis, the systemic form of transport sugar, and a signal molecule that responds to internal and external environmental cues altering development and stress acclimation (Rolland et al. 2006; Ruan 2014), compared to intermediates of the glycolytic pathway with low steady-state concentration and fast turnover rates (Lunn et al. 2014). Interestingly, some metabolites found in extremely low concentrations also play roles as critical signalling molecules modulating plant growth, development, and physiology. The levels of the sugar trehalose-6-phosphate (Tre6P), the intermediate of trehalose synthesis, act as a sensor of sucrose availability (Lunn et al. 2006, 2014; Yadav et al. 2014) impacting embryogenesis, leaf growth and flowering (Lunn et al. 2014).

Metabolite levels not only fluctuate according to the diel cycle, developmental stage, and environmental stimuli but also diverge between different organs, tissues and cells in a multicellular organism. It has been assumed that higher plants have about 40 distinct cell-types (e.g. trichomes, guard, xylem fibres, and parenchyma cells) (Martin et al. 2001; Misra et al. 2014) that exhibit different morphologies and play specialized functions in different plant organs. For example, a single leaf contains up to 15 different cell types (Martin et al. 2001), and each of them may display highly contrasting metabolite pools posing a difficult task to unbiased identify and quantify these small molecules (Bino et al. 2004; Hall and Hardy 2012).

Unlike DNA, transcripts, and proteins, metabolites are at the endpoint of the flow of genetic information and are considered as the nearest

molecular readout of the phenotype–genotype relationship in a biological system (Fiehn 2002; Hall 2006). With the advent of post-genomic era, functional genomic studies have been greatly accelerated with technological advances in data generation from multiple levels (i.e. DNA sequences, transcripts, proteins and metabolites) using new 'omics' tools. While the well-established technologies for high-throughput DNA sequencing, gene expression analysis (transcriptomics), and protein profiling (proteomics) have been routinely adopted and explored in the last decades, metabolomics has developed into a powerful and complementary analytical technology for plant functional genomics in both basic and applied research (Fiehn 2002; Bino et al. 2004; Hall 2006; Roessner-Tunali 2007).

By definition, metabolomics aims an unbiased identification and quantification of all metabolites in a complex biological sample in a particular experimental condition (Fiehn et al. 2000; Fiehn 2002; Bino et al. 2004; Hall 2006). This well-accepted definition has some relevant questions. First, similar to other omics, metabolomics could be assayed in any level of complexity, such as a whole organism, a specific organ, tissue, a suspension cultured cell lines or even a single cell or cellular compartment depending on the biological question in investigation (Fiehn 2002). This aspect reveals the great potential of plant metabolomics to dissect the physiological state in a specific biological context, exploring all the richness and complexity of these small molecules (Hall 2006). Also, compared to transcriptomics and proteomics, metabolomics has the great advantage to be assayed independently from the availability of previous genome or transcriptome data, opening new perspectives to use plant metabolomics to gain more insights about the regulation of metabolism on complex traits in non-model and crops species (Hall 2006; Watanabe et al. 2018). Furthermore, metabolomics is the only instrumental tool allowing the true measurement of fluxes, essential to a comprehensive understanding of metabolism (Nikoloski et al. 2015; Allen 2016; Freund and Hegeman 2017).

Second, due to the dynamic nature of the metabolism, metabolomics experiments must be carefully planned and designed with respect to harvesting (e.g. sample type and size, pooling or not, replication, time scale) and sample preparation in order to obtain reliable and biologically relevant results. Some excellent literature are available to guide beginners in the field (Jenkins et al. 2004; Fiehn et al. 2007; Biais et al. 2012; Gibon and Rolin 2012; Hall and Hardy 2012; Lu et al. 2017).

Third, metabolomics is predicted to take a real picture of the total metabolic activities taking place in plants in a given experimental condition. However, practically, due to the myriad of metabolites with different physico-chemical properties, particularly for plants, no analytical method is able to simultaneously cover all the metabolites from a single extract (Fiehn et al. 2000; Bino et al. 2004; Hall 2006; Roessner-Tunali 2007; Hall and Hardy 2012). In this context, the field of plant metabolomics has greatly advanced with the development of multiple analytical approaches in parallel with the continuous increase in the plant metabolite databases to facilitate metabolite identification.

Basically, there are two general approaches to assess the overall metabolome: targeted and untargeted analyses (Fiehn 2002; Fernie 2003; Goodacre et al. 2004; Last et al. 2007). Targeted metabolomics allows an unbiased detection and quantification of a predefined set of known metabolites, usually applied to screen for selected compounds belonging to specific metabolic classes (Sawada et al. 2009; Cajka and Fiehn 2014). In contrast, untargeted metabolomics combines comprehensiveness with robustness to detect and quantify known and unidentified components. Untargeted analysis has been applied to large-scale profiling studies aiming at the identification of metabolite patterns or 'fingerprints' to discriminate different plant species/cultivars and in response to perturbations without the need of a formal metabolite identification (Keurentjes et al. 2006; Steinfath et al. 2010). In both targeted and untargeted strategies, coverage of detected metabolites and their obtained level of structural

information and quantification (e.g. absolute, relative or semi-quantitative) rely on the purpose of the study that will influence the choice of the most appropriate metabolite extraction and analytical platform (Hall 2006; Saito and Matsuda 2010; Lei et al. 2011). A combination of targeted and untargeted metabolomics methods has been often used in the last years as a complementary strategy to address different biological questions in the same study (Last et al. 2007; Farag et al. 2012; Cajka and Fiehn 2014).

Due to the inherent complexity of plant metabolism, no 'silver bullet' or single technology is currently available to cover the full metabolome from a single sample. Technological developments in analytical methods to analyse highly complex mixtures have led to the establishment of two leading platforms applied to plant metabolomics research, namely nuclear magnetic resonance (NMR) spectroscopy and mass-spectrometry (MS), which can be coupled to gas (GC-MS, GC-NMR) or liquid (LC-MS or LC-NMR) chromatographic separation methods to improve resolution (Last et al. 2007; Kim et al. 2011; Tenenboim and Brotman 2016; Jorge et al. 2016). NMR-based metabolomics enables accurate quantification of abundant metabolites and resolution of chemical structures with a high reproducibility and relatively short time (Verpoorte et al. 2007; Kim and Verpoorte 2010; Schripsema 2010; Kim et al. 2011; Markley et al. 2017). Also, a great advantage is its simple sample preparation as metabolites can be measured directly from crude plant extracts or in vivo (Markley et al. 2017). However, the major drawbacks in NMR reside in its poor sensitivity and dynamic range of detection compared to MS, as well as problems related with superimposed spectrum signals that hamper the structural elucidation process, limiting the numbers of metabolites truly resolved (Kim et al. 2011; Markley et al. 2017).

Unlike NMR, MS is by far the primary detection method of metabolomics, due to its higher sensitivity, accuracy, and speed to detect and identify a wide range of metabolites (Last et al. 2007; Gika et al. 2014; Aretz and Meierhofer 2016; Haggarty and Burgess 2017). GC-MS has emerged as the gold-standard MS-based method for plant metabolite analysis due to numerous advantages compared to other analytical instruments such as robust quantification of hundreds of naturally volatile metabolites (e.g. alcohols, esters and monoterpenes) as well as non-volatile and polar metabolites (mainly primary metabolites), which can be converted into volatile and thermally stable compounds through derivatization (Hall 2006). Furthermore, GC-MS has a superior reproducibility and high chromatographic resolution over other analytical instruments (Fernie 2003; Jorge et al. 2016) and allows the development of metabolite libraries (Schauer et al. 2005; Kopka et al. 2005; Kind et al. 2009). Compared to GC-MS, LC-MS is a most versatile technique able to detect a broader range of compounds, being the preferred method of choice for targeted and untargeted analysis of secondary metabolites (Allwood and Goodacre 2010) or specific metabolite classes like phosphorylated compounds, which are less stable during the derivatization process required for GC-MS analysis (Hall 2006). Dedicated literature concerning pros and cons for each technology is available (Ward et al. 2007; Gika et al. 2014; Engskog et al. 2016; Aretz and Meierhofer 2016; Haggarty and Burgess 2017; Lu et al. 2017).

The choice of the analytical platform is a compromise and will be highly dependent on the biological question and availability of instruments or methods. However, metabolome coverage has greatly benefited from multiple analytical approaches (Marshall and Powers 2017) in parallel with continuous increasing in the plant metabolite databases to facilitate metabolite identification.

5.2 Applications of Metabolomics in Plant Sciences

Throughout the substantial advances in metabolomics, this technology has been extensively used as a cornerstone in systems biology to elucidate the link between genotype–phenotype in plants (Aretz and Meierhofer 2016). Deciphering bio-

synthetic pathways, their regulation and interactions are essential for understanding how plants respond to different sorts of perturbations (developmental, genetic or environmental). This is crucial for functional genomics, metabolic engineering, and synthetic biology approaches aiming at the accumulation of specific products (e.g. pharmacologically relevant metabolites) as well as plants with higher vigour and biomass for food and fuels. In this section, we will illustrate some of the broad potential applicability of metabolomics in plant science.

5.2.1 Pattern Recognition and Discrimination

Due to their autotrophic nature, plants are dependent on the light period to perform photosynthesis and usually accumulate carbon reserves to support growth and metabolic activity during the night (Smith and Stitt 2007; Stitt et al. 2010b). Time-resolved measurements of the metabolome along the diurnal cycle have been investigated in several species from algae to higher plants (Bénard et al. 2015; Hirth et al. 2017), showing that the amplitude and timing of metabolic changes vary. Primary metabolite and lipid profiling in synchronized growing cells of *Chlamydomonas reinhardtii* revealed interesting patterns along light and dark cycle: (1) most amino acids peak after 4 h of light coinciding with the commitment point of the cell cycle and (2) the turnover of membrane lipids (MGDG, SQDG and DGTS) is very distinctive from storage lipids (TAG) (Jüppner et al. 2017). In addition, these authors identified some new lipid species for this model microalgae and pinpointed metabolic signatures that can be used as biomarkers for several phases of the cell cycle. Metabolic profiling in the CAM species Agave indicated some differences along the diel cycle in comparison to *Arabidopsis*, not only in malate and fumarate, organic acids related to the nocturnal CO_2 fixation in CAM, but also in ascorbic acid known to play a role in redox signalling (Abraham et al. 2016).

Plants synthesize a plethora of value-added natural products with multiple applications to pharmaceutical, cosmetic, food, and agrochemical industries. Considering these bioactive molecules, the diversity and characterization of compound classes have been explored with metabolomics not only in model species (Li et al. 2016), but also in citrus (Wang et al. 2017), peach (Monti et al. 2016), yam (Price et al. 2017), pine (Meijón et al. 2016), wild grassland plants (French et al. 2018) and medicinal species (for review see Rai et al. 2017). In an outstanding study, the analysis of 17-hydroxygeranyllinalool diterpene glycosides in 35 solanaceous species identified 105 novel metabolites restricted to genera *Nicotiana*, *Capsicum*, and *Lycium*, indicating the potential of metabolomics to differentiate among species (Heiling et al. 2016). This work give evidence that MS metabolomics can be employed to evaluate phylogenetic occurrence of many secondary metabolic pathways.

With respect to the production of renewable fuels, algal biodiesel holds considerable promise to meet future energy demands. Microalgae have much faster growth rates than crops and are able to accumulate enormous amounts of lipids (from 20% to 40% of dry weight), mainly in the form of TAGs (Scranton et al. 2015; Wase et al. 2017). A vast collection of recent literature using metabolomics to identify lipid species in microalgae and evaluate factors influencing lipid accumulation is available (Yao et al. 2015; Bromke et al. 2015; Chen et al. 2017; Matich et al. 2018; Piligaev et al. 2018; Yang et al. 2018). A current challenge is to promote TAG accumulation and storage without penalties on biomass. GC-MS analysis of lipids and primary metabolites was utilized to test the effect of selected molecules from a high-throughput chemical genetics screening aiming to identify lipid-activating compounds in *C. reinhardtii* (Wase et al. 2017). These authors verified distinct metabolic response to five compounds that promoted TAG accumulation, four of them without decreasing galactolipids and their efficacy was also proved in three other algal species.

This example illustrates the value of metabolomics in assessing the response of plants to chemicals.

Metabolomics is also incredibly useful to recognize a wide range of other patterns that were not mentioned in this section, such as metabolic responses along plant development (Wang et al. 2016; Czedik-Eysenberg et al. 2016; Watanabe et al. 2018) and under stressful conditions that restrict growth (Obata and Fernie 2012; Arbona and Gomez-Cadenas 2016; Jorge et al. 2016). The latter has a huge impact on agriculture due to the identification of markers for increased stress tolerance.

5.2.2 Functional Genomics

Mutants and transgenic lines are excellent tools to determine gene function in plant morphology, biochemistry, and physiology. Metabolomics is very powerful to distinguish among genotypes even in the absence of growth phenotypes (Fukushima et al. 2014b), boosting functional readouts in comparison to classical chemical or genetic screens evaluating growth responses. Therefore, it is routinely employed for characterizing mutants and genetically modified (GM) lines.

Arabidopsis thaliana was the first plant genome to be completely sequenced, and although there are vast genetic resources for this species, only about 12% of gene function assignments were based on in vivo characterization (Rhee and Mutwil 2014). T-DNA sequence-indexed mutant collections have enabled allele coverage for most *Arabidopsis* genes (O'Malley et al. 2015), serving as basis for both forward and reverse genetic strategies. Metabolomics has been employed to determine the metabolomes of several lines containing T-DNA insertions in genes of unknown functions. A combination of various analytical platforms (including LC-MS, CE-MS, UHPLC-QTOF-MS, and GC–TOF-MS) was used to analyse 69 mutants, ensuring detection of important metabolic alterations and creation of a public database (Quanbeck et al. 2012). In a recent work, Monne et al. (2018) have biochemically characterized the properties of recombinant mitochondrial carriers previously thought to be uncoupling proteins 1 and 2, and detected their ability to transport amino acids. GC-MS metabolite profiling in T-DNA insertion mutants confirmed massive changes in organic and amino acids, enabling to assign a new function for these proteins as aspartate and glutamate transporters.

The combination of multiple analytical platforms revealed minimal or no clear metabolic differences between conventional and GM lines of tomato (Kusano et al. 2011) and soybean (Kusano et al. 2015), respectively, showing that metabolomics is also valuable to analyse risk assessment of GM crops.

5.2.3 Metabolomics as a Prediction Tool

Improving crop productivity has been a major issue concerning growing world population and climate change (White et al. 2016; van der Kooi et al. 2016; Shih et al. 2016; Altieri and Nicholls 2017; Frieler et al. 2017). As the composite of metabolic reactions represent the outcome of determinant genes generating the phenotype, metabolomics has contributed to improve the understanding of the genetic architecture and the key elements underlying biological functions and agronomic traits (Kumar et al. 2017). Attributes such as quality, shelf life, biomass production, yield, and resistance to diseases are controlled by multiple genes, and their genomic regions are known as quantitative trait loci (QTLs) (Collard et al. 2005). QTL mapping reveals the localization of loci, enabling the identification of coregulated compounds in naturally variable phenotypes (Keurentjes et al. 2006), with specific impact on crop breeding. However, many traits are controlled by a large number of QTLs (Bernardo 2008; Xu and Crouch 2008), which also have strong interactions with the environment. Metabolomics has greatly assisted genetic analyses to clarify the relationship between genetic and biochemical bases of plant metabolism (Fernie and Tohge 2017), serving as a tool to increase breeding efficiency. The pioneer works

on metabolite-based QTL (mQTL) were performed with *Arabidopsis* (Meyer et al. 2007, 2010; Lisec et al. 2008, 2009) and tomato (Schauer et al. 2006), aiming to predict biomass production. These works opened new perspectives for using metabolites as biomarkers for accurate estimation of plant performance based on parental information (for review see Fernandez et al. 2016), and since then, several studies in rice (Matsuda et al. 2012; Dan et al. 2016), potato (Sprenger et al. 2017), tomato (Quadrana et al. 2014; Toubiana et al. 2015), wheat (Hill et al. 2015), and other crops have been conducted. In general, those works provide hints on heritable mechanisms affecting the levels of metabolites, show that various mQTLs have a strong influence on metabolite levels and pinpoint mQTL hotspots, suggesting that modification of small genomic regions could control the metabolic status. Depending on the density of the genetic map, it is even possible to identify candidate genes involved in particular pathways. Gong et al. (2013) successfully assigned the function of genes to many mQTLs related to flavonoid metabolism and other mQTLs of unknown functions in rice. Moreover, they performed functional characterization of three candidate genes confirming their relationship to the accumulation of the corresponding metabolites and could also reconstruct some metabolic pathways.

High-throughput genotyping technologies have revolutionized genome-wide association studies (GWAS), another method suitable for mapping the loci responsible for natural variations in a phenotype of interest. GWAS focus on the identification of significantly associated genetic polymorphisms in a large population and has some advantages in comparison to traditional QTL mapping (Korte and Farlow 2013). Metabolomics has also been combined with GWAS originating high-resolution maps of genomic regions related with metabolite variation (Luo 2015; Fernie and Tohge 2017). A comprehensive study of maize kernel metabolism combined metabolomics analysis by LC-MS/MS and GWAS in an association panel in different locations (Wen et al. 2014). The results made it possible to verify and update the annotation of many maize genes through the identification of novel metabolites and genes involved in the formation of phenolamides and flavonoids, and also to explore biomarkers for kernel weight. Other few recent examples are (1) evaluation of metabolites in maize roots and identification and validation of a terpene synthase gene that plays a role in antifungal defence (Ding et al. 2017) and (2) discovery of candidate genes contributing to steroidal glycoalkaloid and flavonoid metabolism in tomato fruit along domestication, with some of the genes annotated and characterized (Zhu et al. 2018).

Metabolomics has also been employed solely to investigate the relationship between biochemical characteristics and geographic origins, genotypic characteristics and morphological traits in seeds of 100 cultivars of *japonica* and *indica* rice (Hu et al. 2014). Non-targeted UHPLC-MS/MS and GC-MS revealed opposite abundance of some metabolites (e.g. asparagine and alanine) between *japonica* and *indica* cultivars, suggesting different strategies for nitrogen utilization in rice seeds. Few significantly different metabolite and morphological trait correlations between the two subgroups indicated that they tend to be subspecies-specific (Hu et al. 2014). Another study in a panel of sorghum breeding lines determined associations between metabolites in leaves and morpho-physiological traits, revealing that chlorogenic and shikimic acids are related to photosynthesis, initial plant growth, and final biomass (Turner et al. 2016). Together, the abovementioned studies are examples of the building bases for ameliorating agronomic traits in crops.

5.2.4 Flux Analysis

Although steady-state measurements of metabolites are very valuable for giving a general overview of metabolic alterations in response to a defined perturbation, they do not provide detailed information about flux distributions. Therefore, conventional metabolomics and flux analysis are complementary approaches for characterizing the plant metabolic network. Metabolic reactions are catalysed by enzymes

and depend on the concentration of substrate and end products. On another hand, metabolites can regulate enzyme activity at several levels, from allosteric to transcriptional regulation (Wegner et al. 2015). A large number of metabolites are intermediates of branched and circular metabolic pathways, and frequently metabolite levels and enzyme activities have only poor correlations with transcripts or proteins (Gibon et al. 2004; Piques et al. 2009; Stitt and Gibon 2014), which also do not correlate with fluxes (Fernie and Stitt 2012; Schwender et al. 2014). Those findings place posttranslational modifications of enzymes as regulatory events integrating signalling, gene expression, and metabolism (Grabsztunowicz et al. 2017; O'Leary and Plaxton 2017).

Fluxes are challenging to determine because no simple methodology is able to follow the dynamic rate of metabolite interconversions or the intracellular activity of multiple enzymes (Kruger and Ratcliffe 2015). Flux analyses make it possible to determine metabolic pathways that are actively operating and how their activity is coordinated with additional pathways to establish a balanced network (Nikoloski et al. 2015). This information can be used to estimate optimal configuration for a network and fluxes for the production of interesting end-products (Farre et al. 2014). The measurement of metabolome-wide fluxes is an emerging field contributing to a more integrated output of cellular function (Salon et al. 2017).

The use of isotope labelling with radioactive or stable isotopes is a classical biochemical technique for measuring intracellular fluxes (Freund and Hegeman 2017) and is known as metabolic flux analysis (MFA). Briefly, MFA consists of monitoring the redistribution of the labelled compound in a large number of metabolites using MS or NMR, building a model of the network, fitting the model to the MS or NMR data in order to obtain a set of fluxes, and extensive statistics to evaluate the reliability of the estimated flux (Kruger et al. 2012; Kruger and Ratcliffe 2015; Allen et al. 2015; Salon et al. 2017). MS enables resolving fragments or complete isotopic composition of a metabolite, whereas NMR allows to measure positional labelling information. It is worthwhile mentioning that some elements must be taken into consideration when performing this sort of experiment, involving the labelling magnitude of the precursor substrate molecule through the system, the size of the metabolite pool and the conversion rate of the precursor substrate into the metabolite (Nikoloski et al. 2015). In the last years, various protocols to perform MFA in plants have been described (Cocuron and Alonso 2014; Heise et al. 2014; Tivendale et al. 2016; Dethloff et al. 2017; Obata et al. 2017; Acket et al. 2017). Stable isotope-labelling experiments with ^{13}C-pyruvate, ^{13}C-glutamate and ^{15}N-ammonium were used to evaluate a switch of the tricarboxylic acid cycle to a noncyclic operation mode under hypoxia in soybean (António et al. 2016). The monitoring of label redistribution with GC-TOF-MS showed that metabolic alterations were independent from the supply of isotope-labelled substrate and accumulation of alanine, GABA, and succinate occur due to activation of alanine metabolism and GABA shunt.

The other approach typically used to estimate fluxes is a constraint model combining genomic information and biochemical data to predict metabolic fluxes through the network, namely flux balance analysis (FBA). As FBA demands fewer measurements, it is often easier to implement than MFA (Kruger and Ratcliffe 2015). FBA is frequently employed to predict fluxes to maximize biomass production or minimize energy consumption (Colombie et al. 2015; Yuan et al. 2016), and substantial progress in plant metabolic modelling has been achieved in recent years (Shi and Schwender 2016). The power of FBA prediction was confirmed comparing flux profiles between guard and mesophyll leaf cells. Modelling predicted a C4-like metabolism in guard cells (due to higher anaplerotic CO_2 fixation into oxaloacetate) and higher fluxes through sucrose synthesis as a result of a futile cycle, which could be confirmed with a ^{13}C-labelling experiment using isolated mesophyll and guard cells (Robaina-Estévez et al. 2017). This study demonstrates the application of FBA to investigate different cellular types.

5.2.5 Integration with Other Omics

The integration of metabolomics with other high-throughput technologies permits a more holistic view of biological phenomena, as exemplified by the mQTL and GWAS studies above mentioned. Another case is the investigation of transcripts and metabolites in duckweed, the smallest and fastest growing aquatic flowering plants, aimed at elucidating the phenotype of starch accumulation under nitrogen starvation. Duckweeds are able to accumulate impressive amounts of starch, evidencing their potential for bioethanol production (Xu et al. 2011; Cui and Cheng 2015; Fujita et al. 2016). RNASeq analysis hypothesized more partitioning into starch due to the up-regulation of enzymes involved in gluconeogenesis and down-regulation of glycolysis, as well as alterations in genes coding for enzymes of starch and sucrose synthesis (Yu et al. 2017). Metabolite profiling by LC-MS/MS confirmed higher ADP-glucose and lower UDP-glucose amounts, substrates for starch and sucrose synthesis, respectively, and enzymatic activity of the enzymes producing these substrates was also in agreement with transcript and metabolic data. Only due to the integration of the different information levels, it was possible to confirm that the increased starch content was a consequence of increased output from gluconeogenesis and TCA pathways (Yu et al. 2017).

By combining photosynthetic rate, measurements of metabolites, transcripts and proteins, polysome loading and growth analysis, it was possible to achieve a systemic response of metabolism and growth after a shift to higher irradiance in the non-saturating range for photosynthesis in the algal *C. reinhardtii* (Mettler et al. 2014). This temporal analysis revealed an initial increase in photosynthesis prior to stimulation of growth to match increased carbon fixation, and higher metabolic fluxes leading to accumulation of metabolic intermediates and starch. Transcriptional and posttranscriptional regulation were found to be important after primary changes in metabolites, leading to alterations in the abundance of particular proteins, which also brought about subsequently changes in the levels of metabolites. This is an outstanding work showing that the different levels of information present very distinct temporal kinetics, and are orchestrated to ensure fast readjustment of metabolism in a fluctuating light environment.

Usually, the integration of data from two system-levels is primarily made on simple correlations methods (Rajasundaram and Selbig 2016). However, several statistical methods and tools are available for network visualization, pathway analyses, genome-scale metabolic reconstruction and integration of multidimensional data (Rohn et al. 2012; Bartel et al. 2013; Fukushima et al. 2014a; Villaveces et al. 2015; Bersanelli et al. 2016; Sajitz-Hermstein et al. 2016; Schwahn et al. 2017; Therrien-Laperrière et al. 2017; Robaina-Estevez and Nikoloski 2017; Basu et al. 2017).

The use of biological networks for integrative analysis offers new directions to identify how large networks are coregulated. More recently, integrative approaches were shown to provide systemic views of plant defence against insects (Barah and Bones 2015), secondary wall formation (Li et al. 2016), structure and regulation of metabolic pathways (Tohge et al. 2015), hormone signalling (Yoshida et al. 2015), and single cells (Colomé-Tatché and Theis 2018). The integration of multi-omics data has expanded the mechanistic comprehension of plant metabolism and function.

5.3 Final Considerations and Future Perspectives

Since the appearance of metabolomics almost two decades ago, higher resolution analytical platforms and their use in combination have enabled the detection of hundreds of metabolic features within a complex biological sample. However, a significant portion of these detected peaks usually cannot be identified, hindering the accomplishment of a complete metabolome. The elucidation of new metabolites is still very laborious and remains an enormous challenge. Serial combination of columns in tandem and column switching are means to improve metabolome coverage. In addition to the technological advances, efforts in sharing reference compounds and organization of metabolite spectral signa-

tures in public libraries, as well as standardization of protocols to report metabolite data will definitely increase identification confidence and take a leap forward in the use of metabolomics as discovery tool.

Another bottleneck in metabolomics is highly compartmentalization of plant metabolism with a range of biochemical steps in a single pathway taking place in different cellular organelles and/or being catalysed by isoforms of enzymes at different subcellular locations. Strategies to track spatial distribution of metabolites and proteins include isolation or organelles, fractionation techniques, immunohistochemistry and the powerful flux analyses, which has increased the understanding about how metabolic pathways are integrated. These approaches together with natural variation might unravel crucial metabolic modules contributing for efficient manipulation of plant metabolism via metabolic engineering.

There is a growing interest in using metabolomics for a wide range of biological targets, and although it has still some limitations, metabolomics use alone or combined with other omics technologies is revolutionizing plant biology and crop breeding providing new insights into genetic regulation of metabolism, cellular function and the structure of metabolic networks.

References

Abraham PE, Yin H, Borland AM et al (2016) Transcript, protein and metabolite temporal dynamics in the CAM plant Agave. Nat Plants 2:16178

Acket S, Degournay A, Merlier F, Thomasset B (2017) 13C labeling analysis of sugars by high resolution-mass spectrometry for metabolic flux analysis. Anal Biochem 527:45–48

Afendi FM, Okada T, Yamazaki M et al (2012) KNApSAcK family databases: integrated metabolite–plant species databases for multifaceted plant research. Plant Cell Physiol 53:e1

Allen DK (2016) Quantifying plant phenotypes with isotopic labeling & metabolic flux analysis. Curr Opin Biotechnol 37:45–52

Allen DK, Bates PD, Tjellstrom H (2015) Tracking the metabolic pulse of plant lipid production with isotopic labeling and flux analyses: past, present and future. Prog Lipid Res 58:97–120

Allwood JW, Goodacre R (2010) An introduction to liquid chromatography mass spectrometry instrumentation applied in plant metabolomic analyses. Phytochem Anal 21:33–47

Altieri MA, Nicholls CI (2017) The adaptation and mitigation potential of traditional agriculture in a changing climate. Clim Chang 140:33–45

António C, Päpke C, Rocha M et al (2016) Regulation of primary metabolism in response to low oxygen availability as revealed by carbon and nitrogen isotope redistribution. Plant Physiol 170:43–56

Arbona V, Gomez-Cadenas A (2016) Metabolomics of disease resistance in crops. Curr Issues Mol Biol 19:13–30

Aretz I, Meierhofer D (2016) Advantages and pitfalls of mass spectrometry based metabolome profiling in systems biology. Int J Mol Sci 17:632

Barah P, Bones AM (2015) Multidimensional approaches for studying plant defence against insects: from ecology to omics and synthetic biology. J Exp Bot 66:479–493

Bartel J, Krumsiek J, Theis FJ (2013) Statistical methods for the analysis of high-throughput metabolomics data. Comput Struct Biotechnol J 4:e201301009

Basu S, Duren W, Evans CR et al (2017) Sparse network modeling and metscape-based visualization methods for the analysis of large-scale metabolomics data. Bioinformatics 33:1545–1553

Bénard C, Bernillon S, Biais B et al (2015) Metabolomic profiling in tomato reveals diel compositional changes in fruit affected by source–sink relationships. J Exp Bot 66:3391–3404

Bernardo R (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Sci 48:1649–1664

Bersanelli M, Mosca E, Remondini D et al (2016) Methods for the integration of multi-omics data: mathematical aspects. BMC Bioinformatics 17:S15

Biais B, Bernillon S, Deborde C et al (2012) Precautions for harvest, sampling, storage, and transport of crop plant metabolomics samples. In: Hardy NW, Hall RD (eds) Plant metabolomics. Methods in molecular biology (methods and protocols). Humana Press, Totowa, NJ, pp 51–63

Bino RJ, Hall RD, Fiehn O et al (2004) Potential of metabolomics as a functional genomics tool. Trends Plant Sci 9:418–425

Bourgaud F, Gravot A, Milesi S, Gontier E (2001) Production of plant secondary metabolites: a historical perspective. Plant Sci 161:839–851

Bromke MA, Sabir JS, Alfassi FA et al (2015) Metabolomic profiling of 13 diatom cultures and their adaptation to nitrate-limited growth conditions. PLoS One 10:e0138965

Cajka T, Fiehn O (2014) Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. Trends Anal Chem 61:192–206

Chen B, Wan C, Mehmood MA et al (2017) Manipulating environmental stresses and stress tolerance of microal-

gae for enhanced production of lipids and value-added products–a review. Bioresour Technol 244:1198–1206
Cocuron J-C, Alonso AP (2014) Liquid chromatography tandem mass spectrometry for measuring $^{(13)}$C-labeling in intermediates of the glycolysis and pentose phosphate pathway. Methods Mol Biol 1090:131–142
Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169–196
Colombie S, Nazaret C, Benard C et al (2015) Modelling central metabolic fluxes by constraint-based optimization reveals metabolic reprogramming of developing Solanum lycopersicum (tomato) fruit. Plant J 81:24–39
Colomé-Tatché M, Theis FJ (2018) Statistical single cell multi-omics integration. Curr Opin Syst Biol 7:54–59
Cui W, Cheng JJ (2015) Growing duckweed for biofuel production: a review. Plant Biol 17:16–23
Czedik-Eysenberg A, Arrivault S, Lohse MA et al (2016) The interplay between carbon availability and growth in different zones of the growing maize leaf. Plant Physiol 172:943–967
Dan Z, Hu J, Zhou W et al (2016) Metabolic prediction of important agronomic traits in hybrid rice (Oryza sativa L.). Sci Rep 6:21732
Dethloff F, Orf I, Kopka J (2017) Rapid in situ $^{(13)}$C tracing of sucrose utilization in Arabidopsis sink and source leaves. Plant Methods 13:87
Ding Y, Huffaker A, Kollner TG et al (2017) Selinene volatiles are essential precursors for maize defense promoting fungal pathogen resistance. Plant Physiol 175:1455–1468
Dixon RA, Strack D (2003) Phytochemistry meets genome analysis, and beyond. Phytochemistry 62:815–816
Engskog MKR, Haglöf J, Arvidsson T, Pettersson C (2016) LC–MS based global metabolite profiling: the necessity of high data quality. Metabolomics 12:114
Farag MA, Porzel A, Wessjohann LA (2012) Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC-MS, LC-MS and 1D NMR techniques. Phytochemistry 76:60–72
Farre G, Blancquaert D, Capell T et al (2014) Engineering complex metabolic pathways in plants. Annu Rev Plant Biol 65:187–223
Fernandez O, Urrutia M, Bernillon S et al (2016) Fortune telling: metabolic markers of plant performance. Metabolomics 12:158
Fernie AR (2003) Metabolome characterization in plant system analysis. Funct Plant Biol 30:111–120
Fernie AR, Stitt M (2012) On the discordance of metabolomics with proteomics and transcriptomics: coping with increasing complexity in logic, chemistry, and network interactions scientific correspondence. Plant Physiol 158:1139–1145
Fernie AR, Tohge T (2017) The genetics of plant metabolism. Annu Rev Genet 51:287–310
Fiehn O (2002) Functional genomics. In: Functional genomics. Springer Netherlands, Dordrecht, pp 155–171
Fiehn O, Kopka J, Dörmann P et al (2000) Metabolite profiling for plant functional genomics. Nat Biotechnol 18:1157–1161
Fiehn O, Robertson D, Griffin J et al (2007) The metabolomics standards initiative (MSI). Metabolomics 3:175–178. https://doi.org/10.1007/s11306-007-0070-6
French KE, Harvey J, McCullagh JSO (2018) Targeted and untargeted metabolic profiling of wild grassland plants identifies antibiotic and anthelmintic compounds targeting pathogen physiology, metabolism and reproduction. Sci Rep 8:1695
Freund DM, Hegeman AD (2017) Recent advances in stable isotope-enabled mass spectrometry-based plant metabolomics. Curr Opin Biotechnol 43:41–48
Frieler K, Schauberger B, Arneth A et al (2017) Understanding the weather signal in national crop-yield variability. Earths Fut 5:605–616
Fujita T, Nakao E, Takeuchi M et al (2016) Characterization of starch-accumulating duckweeds, Wolffia globosa, as renewable carbon source for bioethanol production. Biocatal Agric Biotechnol 6:123–127
Fukushima A, Kanaya S, Nishida K (2014a) Integrated network analysis and effective tools in plant systems biology. Front Plant Sci 5:598
Fukushima A, Kusano M, Mejia RF et al (2014b) Metabolomic characterization of knockout mutants in Arabidopsis: development of a metabolite profiling database for knockout mutants in Arabidopsis. Plant Physiol 165:948–961
Gibon Y, Rolin D (2012) Aspects of experimental design for plant metabolomics experiments and guidelines for growth of plant material. Methods Mol Biol 860:13–30
Gibon Y, Blaesing OE, Hannemann J et al (2004) A robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. Plant Cell 16:3304–3325
Gika HG, Wilson ID, Theodoridis GA (2014) LC-MS-based holistic metabolic profiling. Problems, limitations, advantages, and future perspectives. J Chromatogr B, Anal Technol Biomed Life Sci 966:1–6
Gong L, Chen W, Gao Y et al (2013) Genetic analysis of the metabolome exemplified using a rice population. Proc Natl Acad Sci U S A 110:20320–20325
Goodacre R, Vaidyanathan S, Dunn WB et al (2004) Metabolomics by numbers: acquiring and understanding global metabolite data. Trends Biotechnol 22:245–252
Grabsztunowicz M, Koskela MM, Mulo P (2017) Post-translational modifications in regulation of chloroplast function: recent advances. Front Plant Sci 8:240
Haggarty J, Burgess KEV (2017) Recent advances in liquid and gas chromatography methodology for extending coverage of the metabolome. Curr Opin Biotechnol 43:77–85

Hall RD (2006) Plant metabolomics: from holistic hope, to hype, to hot topic. New Phytol 169:453–468
Hall RD, Hardy NW (2012) Practical applications of metabolomics in plant biology. In: Methods in molecular biology. Humana Press, Totowa, NJ, pp 1–10
Harborne JB (1999) Classes and functions of secondary products from plants. In: Chemicals from plants. World Scientific; Imperial College Press, Singapore; London, pp 1–25
Heiling S, Khanal S, Barsch A et al (2016) Using the knowns to discover the unknowns: MS-based dereplication uncovers structural diversity in 17-hydroxygeranyllinalool diterpene glycoside production in the Solanaceae. Plant J 85:561–577
Heise R, Arrivault S, Szecowka M et al (2014) Flux profiling of photosynthetic carbon metabolism in intact plants. Nat Protoc 9:1803–1824
Hill CB, Taylor JD, Edwards J et al (2015) Detection of QTL for metabolic and agronomic traits in wheat with adjustments for variation at genetic loci that affect plant phenology. Plant Sci 233:143–154
Hirth M, Liverani S, Mahlow S et al (2017) Metabolic profiling identifies trehalose as an abundant and diurnally fluctuating metabolite in the microalga Ostreococcus tauri. Metabolomics 13:68
Hu C, Shi J, Quan S et al (2014) Metabolic variation between japonica and indica rice cultivars as revealed by non-targeted metabolomics. Sci Rep 4:5067
Jenkins H, Hardy N, Beckmann M et al (2004) A proposed framework for the description of plant metabolomics experiments and their results. Nat Biotechnol 22:1601–1606
Jorge TF, Rodrigues JA, Caldana C et al (2016) Mass spectrometry-based plant metabolomics: metabolite responses to abiotic stress. Mass Spectrom Rev 35:620–649
Jüppner J, Mubeen U, Leisse A et al (2017) Dynamics of lipids and metabolites during the cell cycle of Chlamydomonas reinhardtii. Plant J 92:331–343
Keurentjes JJB, Fu J, de Vos CHR et al (2006) The genetics of plant metabolism. Nat Genet 38:842–849
Kim HK, Verpoorte R (2010) Sample preparation for plant metabolomics. Phytochem Anal 21:4–13
Kim HK, Choi YH, Verpoorte R (2011) NMR-based plant metabolomics: where do we stand, where do we go? Trends Biotechnol 29:267–275
Kind T, Wohlgemuth G, Lee DY et al (2009) FiehnLib: mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. Anal Chem 81:10038–10048
van der Kooi CJ, Reich M, Löw M et al (2016) Growth and yield stimulation under elevated CO2 and drought: a meta-analysis on crops. Environ Exp Bot 122:150–157
Kopka J, Schauer N, Krueger S et al (2005) GMD@CSB.DB: the Golm metabolome database. Bioinformatics 21:1635–1638
Korte A, Farlow A (2013) The advantages and limitations of trait analysis with GWAS: a review. Plant Methods 9:29
Kruger NJ, Ratcliffe RG (2015) Fluxes through plant metabolic networks: measurements, predictions, insights and challenges. Biochem J 465:27–38
Kruger NJ, Masakapalli SK, Ratcliffe RG (2012) Strategies for investigating the plant metabolic network with steady-state metabolic flux analysis: lessons from an Arabidopsis cell culture and other systems. J Exp Bot 63:2309–2323
Kumar R, Bohra A, Pandey AK et al (2017) Metabolomics for plant improvement: status and prospects. Front Plant Sci 8:1302
Kusano M, Redestig H, Hirai T et al (2011) Covering chemical diversity of genetically-modified tomatoes using metabolomics for objective substantial equivalence assessment. PLoS One 6:e16989
Kusano M, Baxter I, Fukushima A et al (2015) Assessing metabolomic and chemical diversity of a soybean lineage representing 35 years of breeding. Metabolomics 11:261–270
Last RL, Jones AD, Shachar-Hill Y (2007) Towards the plant metabolome and beyond. Nat Rev Mol Cell Biol 8:167–174
Lei Z, Huhman DV, Sumner LW (2011) Mass spectrometry strategies in metabolomics. J Biol Chem 286:25435–25442
Li Z, Omranian N, Neumetzler L et al (2016) A transcriptional and metabolic framework for secondary wall formation in Arabidopsis. Plant Physiol 172:1334–1351
Lisec J, Meyer RC, Steinfath M et al (2008) Identification of metabolic and biomass QTL in Arabidopsis thaliana in a parallel analysis of RIL and IL populations. Plant J 53:960–972
Lisec J, Steinfath M, Meyer RC et al (2009) Identification of heterotic metabolite QTL in Arabidopsis thaliana RIL and IL populations. Plant J 59:777–788
Lu W, Su X, Klein MS et al (2017) Metabolite measurement: pitfalls to avoid and practices to follow. Annu Rev Biochem 86:277–304
Lunn JE (2007) Compartmentation in plant metabolism. J Exp Bot 58:35–47
Lunn JE, Feil R, Hendriks JHM et al (2006) Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. Biochem J 397:139–148
Lunn JE, Delorge I, Figueroa CM et al (2014) Trehalose metabolism in plants. Plant J 79:544–567
Luo J (2015) Metabolite-based genome-wide association studies in plants. Curr Opin Plant Biol 24:31–38
Markley JL, Brüschweiler R, Edison AS et al (2017) The future of NMR-based metabolomics. Curr Opin Biotechnol 43:34–40. https://doi.org/10.1016/J.COPBIO.2016.08.001
Marshall DD, Powers R (2017) Beyond the paradigm: combining mass spectrometry and nuclear magnetic resonance for metabolomics. Prog Nucl Magn Reson Spectrosc 100:1–16
Martin C, Bhatt K, Baumann K (2001) Shaping in plant cells. Curr Opin Plant Biol 4:540–549

Matich EK, Ghafari M, Camgoz E et al (2018) Time-series lipidomic analysis of the oleaginous green microalga species Ettlia oleoabundans under nutrient stress. Biotechnol Biofuels 11:29

Matsuda F, Okazaki Y, Oikawa A et al (2012) Dissection of genotype-phenotype associations in rice grains using metabolome quantitative trait loci analysis. Plant J 70:624–636

Meijón M, Feito I, Oravec M et al (2016) Exploring natural variation of Pinus pinaster Aiton using metabolomics: is it possible to identify the region of origin of a pine from its metabolites? Mol Ecol 25:959–976

Mettler T, Mühlhaus T, Hemme D et al (2014) Systems analysis of the response of photosynthesis, metabolism, and growth to an increase in irradiance in the photosynthetic model organism Chlamydomonas reinhardtii. Plant Cell 26:2310–2350

Meyer RC, Steinfath M, Lisec J et al (2007) The metabolic signature related to high plant growth rate in Arabidopsis thaliana. Proc Natl Acad Sci 104:4759–4764

Meyer RC, Kusterer B, Lisec J et al (2010) QTL analysis of early stage heterosis for biomass in Arabidopsis. Theor Appl Genet 120:227–237

Misra BB, Assmann SM, Chen S (2014) Plant single-cell and single-cell-type metabolomics. Trends Plant Sci 19:637–646

Monne M, Daddabbo L, Gagneul D et al (2018) Uncoupling proteins 1 and 2 (UCP1 and UCP2) from Arabidopsis thaliana are mitochondrial transporters of aspartate, glutamate and dicarboxylates. J Biol Chem 293:4213

Monti LL, Bustamante CA, Osorio S et al (2016) Metabolic profiling of a range of peach fruit varieties reveals high metabolic diversity and commonalities and differences during ripening. Food Chem 190:879–888

Moore BD, Andrew RL, Külheim C, Foley WJ (2014) Explaining intraspecific diversity in plant secondary metabolites in an ecological context. New Phytol 201:733–750

Nakabayashi R, Saito K (2015) Integrated metabolomics for abiotic stress responses in plants. Curr Opin Plant Biol 24:10–16

Nikoloski Z, Perez-Storey R, Sweetlove LJ (2015) Inference and prediction of metabolic network fluxes. Plant Physiol 169:1443–1455

O'Leary BM, Plaxton WC (2017) Mechanisms and functions of post-translational enzyme modifications in the organization and control of plant respiratory metabolism. In: Tcherkez G, Ghashghaie J (eds) Plant respiration: metabolic fluxes and carbon balance. Springer International Publishing, Cham, pp 261–284

O'Malley RC, Barragan CC, Ecker JR (2015) A user's guide to the Arabidopsis T-DNA insertional mutant collections. Methods Mol Biol 1284:323–342

Obata T, Fernie AR (2012) The use of metabolomics to dissect plant responses to abiotic stresses. Cell Mol Life Sci 69:3225–3243

Obata T, Rosado-Souza L, Fernie AR (2017) Coupling radiotracer experiments with chemical fractionation for the estimation of respiratory fluxes. Methods Mol Biol 1670:17–30

Pichersky E, Gang DR (2000) Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends Plant Sci 5:439–445

Piligaev AV, Sorokina KN, Shashkov MV, Parmon VN (2018) Screening and comparative metabolic profiling of high lipid content microalgae strains for application in wastewater treatment. Bioresour Technol 250:538–547

Piques M, Schulze WX, Höhne M et al (2009) Ribosome and transcript copy numbers, polysome occupancy and enzyme dynamics in Arabidopsis. Mol Syst Biol 5:314

Price EJ, Bhattacharjee R, Lopez-Montes A, Fraser PD (2017) Metabolite profiling of yam (Dioscorea spp.) accessions for use in crop improvement programmes. Metabolomics 13:144

Quadrana L, Almeida J, Asis R et al (2014) Natural occurring epialleles determine vitamin E accumulation in tomato fruits. Nat Commun 5:3027

Quanbeck SM, Brachova L, Campbell AA et al (2012) Metabolomics as a hypothesis-generating functional genomics tool for the annotation of Arabidopsis thaliana genes of "unknown function". Front Plant Sci 3:15

Rai A, Saito K, Yamazaki M (2017) Integrated omics analysis of specialized metabolism in medicinal plants. Plant J 90:764–787

Rajasundaram D, Selbig J (2016) More effort - more results: recent advances in integrative "omics" data analysis. Curr Opin Plant Biol 30:57–61

Rhee SY, Mutwil M (2014) Towards revealing the functions of all genes in plants. Trends Plant Sci 19:212–221

Robaina-Estevez S, Nikoloski Z (2017) On the effects of alternative optima in context-specific metabolic model predictions. PLoS Comput Biol 13:e1005568

Robaina-Estévez S, Daloso DM, Zhang Y et al (2017) Resolving the central metabolism of Arabidopsis guard cells. Sci Rep 7:8307

Roessner-Tunali U (2007) Uncovering the plant metabolome: current and future challenges. Springer Netherlands, Dordrecht

Rohn H, Junker A, Hartmann A et al (2012) VANTED v2: a framework for systems biology applications. BMC Syst Biol 6:139

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel m. Annu Rev Plant Biol 57:675–709

Ruan Y-L (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol 65:33–67

Saito K, Matsuda F (2010) Metabolomics for functional genomics, systems biology, and biotechnology. Annu Rev Plant Biol 61:463–489

Sajitz-Hermstein M, Topfer N, Kleessen S et al (2016) iReMet-flux: constraint-based approach for integrat-

ing relative metabolite levels into a stoichiometric metabolic models. Bioinformatics 32:i755–i762
Salon C, Avice J-C, Colombie S et al (2017) Fluxomics links cellular functional analyses to whole-plant phenotyping. J Exp Bot 68:2083–2098
Sawada Y, Akiyama K, Sakata A et al (2009) Widely targeted metabolomics based on large-scale MS/MS data for elucidating metabolite accumulation patterns in plants. Plant Cell Physiol 50:37–47
Schauer N, Steinhauser D, Strelkov S et al (2005) GC-MS libraries for the rapid identification of metabolites in complex biological samples. FEBS Lett 579:1332–1337
Schauer N, Semel Y, Roessner U et al (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nat Biotechnol 24:447–454
Schripsema J (2010) Application of NMR in plant metabolomics: techniques, problems and prospects. Phytochem Anal 21:14–21
Schwahn K, Beleggia R, Omranian N, Nikoloski Z (2017) Stoichiometric correlation analysis: principles of metabolic functionality from m data. Front Plant Sci 8:2152
Schwender J, König C, Klapperstück M et al (2014) Transcript abundance on its own cannot be used to infer fluxes in central metabolism. Front Plant Sci 5:668
Scranton MA, Ostrand JT, Fields FJ, Mayfield SP (2015) Chlamydomonas as a model for biofuels and bioproducts production. Plant J 82:523–531
Shi H, Schwender J (2016) Mathematical models of plant metabolism. Curr Opin Biotechnol 37:143–152
Shih PM, Liang Y, Loqué D (2016) Biotechnology and synthetic biology approaches for metabolic engineering of bioenergy crops. Plant J 87:103–117
Smith AM, Stitt M (2007) Coordination of carbon supply and plant growth. Plant Cell Environ 30:1126–1149. https://doi.org/10.1111/j.1365-3040.2007.01708.x
Sprenger H, Erban A, Seddig S et al (2017) Metabolite and transcript markers for the prediction of potato drought tolerance. Plant Biotechnol J 16:939
Steinfath M, Strehmel N, Peters R et al (2010) Discovering plant metabolic biomarkers for phenotype prediction using an untargeted approach. Plant Biotechnol J 8:900–911
Stitt M, Gibon Y (2014) Why measure enzyme activities in the era of systems biology? Trends Plant Sci 19:256–265
Stitt M, Sulpice R, Keurentjes J (2010a) Metabolic networks: how to identify key components in the regulation of metabolism and growth. Plant Physiol 152:428–444
Stitt M, Lunn J, Usadel B (2010b) Arabidopsis and primary photosynthetic metabolism - more than the icing on the cake. Plant J 61:1067–1091
Sulpice R, McKeown PC (2015) Moving toward a comprehensive map of central plant metabolism. Annu Rev Plant Biol 66:187–210
Sweetlove LJ, Fernie AR (2013) The spatial organization of metabolism within the plant cell. Annu Rev Plant Biol 64:723–746
Sweetlove LJ, Fell D, Fernie AR (2008) Getting to grips with the plant metabolic network. Biochem J 409:27–41
Tenenboim H, Brotman Y (2016) Omic relief for the biotically stressed: metabolomics of plant biotic interactions. Trends Plant Sci 21:781–791
Therrien-Laperrière S, Cherkaoui S, Boucher G et al (2017) PathQuant: a bioinformatic tool to quantitatively annotate the relationship between genes and metabolites through metabolic pathway mapping. FASEB J 31:769.3–769.3
Tivendale ND, Jewett EM, Hegeman AD, Cohen JD (2016) Extraction, purification, methylation and GC–MS analysis of short-chain carboxylic acids for metabolic flux analysis. J Chromatogr B, Anal Technol Biomed Life Sci 1028:165–174
Tohge T, Scossa F, Fernie AR (2015) Integrative approaches to enhance understanding of plant metabolic pathway structure and regulation. Plant Physiol 169:1499–1511
Toubiana D, Batushansky A, Tzfadia O et al (2015) Combined correlation-based network and mQTL analyses efficiently identified loci for branched-chain amino acid, serine to threonine, and proline metabolism in tomato seeds. Plant J 81:121–133
Turner MF, Heuberger AL, Kirkwood JS et al (2016) Non-targeted metabolomics in diverse sorghum breeding lines indicates primary and secondary metabolite profiles are associated with plant biomass accumulation and photosynthesis. Front Plant Sci 7:953
Verpoorte R, Choi YH, Kim HK (2007) NMR-based metabolomics at work in phytochemistry. Phytochem Rev 6:3–14
Villaveces JM, Koti P, Habermann BH (2015) Tools for visualization and analysis of molecular networks, pathways, and -omics data. Adv Appl Bioinforma Chem 8:11–22
Wang L, Nägele T, Doerfler H et al (2016) System level analysis of cacao seed ripening reveals a sequential interplay of primary and secondary metabolism leading to polyphenol accumulation and preparation of stress resistance. Plant J 87:318–332
Wang S, Yang C, Tu H et al (2017) Characterization and metabolic diversity of flavonoids in Citrus species. Sci Rep 7:10549
Ward JL, Baker JM, Beale MH (2007) Recent applications of NMR spectroscopy in plant metabolomics. FEBS J 274:1126–1131
Wase N, Tu B, Allen JW et al (2017) Identification and metabolite profiling of chemical activators of lipid accumulation in green algae. Plant Physiol 174:2146–2165
Watanabe M, Tohge T, Balazadeh S et al (2018) Comprehensive metabolomics studies of plant developmental senescence. In: Guo Y (ed) Plant senescence: methods and protocols. Springer, New York, NY, pp 339–358

Wegner A, Meiser J, Weindl D, Hiller K (2015) How metabolites modulate metabolic flux. Curr Opin Biotechnol 34:16–22

Wen W, Li D, Li X et al (2014) Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights. Nat Commun 5:3438

White AC, Rogers A, Rees M, Osborne CP (2016) How can we make plants grow faster? A source–sink perspective on growth rate. J Exp Bot 67:31–45

Xu Y, Crouch JH (2008) Marker-assisted selection in plant breeding: from publications to practice. Crop Sci 48:391–407

Xu J, Cui W, Cheng JJ, Stomp A-M (2011) Production of high-starch duckweed and its conversion to bioethanol. Biosyst Eng 110:67–72

Yadav UP, Ivakov A, Feil R et al (2014) The sucrose–trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signalling by Tre6P. J Exp Bot 65:1051–1068

Yang L, Chen J, Qin S et al (2018) Growth and lipid accumulation by different nutrients in the microalga Chlamydomonas reinhardtii. Biotechnol Biofuels 11:40

Yao L, Gerde JA, Lee S-L et al (2015) Microalgae lipid characterization. J Agric Food Chem 63:1773–1787

Yoshida T, Mogami J, Yamaguchi-Shinozaki K (2015) Omics approaches toward defining the comprehensive abscisic acid signaling network in plants. Plant Cell Physiol 56:1043–1052

Yu C, Zhao X, Qi G et al (2017) Integrated analysis of transcriptome and metabolites reveals an essential role of metabolic flux in starch accumulation under nitrogen starvation in duckweed. Biotechnol Biofuels 10:167

Yuan H, Cheung CYM, Hilbers PAJ, van Riel NAW (2016) Flux balance analysis of plant metabolism: the effect of biomass composition and model structure on model predictions. Front Plant Sci 7:537

Zhu G, Wang S, Huang Z et al (2018) Rewiring of the fruit metabolome in tomato breeding. Cell 172:249–261.e12