The Plant Cell, Vol. 26: 3847–3866, October 2014, www.plantcell.org

REVIEW

Plant Metabolic Modeling: Achieving New Insight into Metabolism and Metabolic Engineering

Kambiz Baghalian,[a,b,c,1] Mohammad-Reza Hajirezaei,[a] and Falk Schreiber[b,d]

[a] Leibniz Institute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, Germany
[b] Institute of Computer Science, Martin Luther University Halle-Wittenberg, 06120 Halle, Germany
[c] College of Agriculture and Natural Resources, Islamic Azad University-Karaj Branch, Karaj 31485-313, Iran
[d] Faculty of IT, Monash University, Clayton, VIC 3800, Australia

Models are used to represent aspects of the real world for specific purposes, and mathematical models have opened up new approaches in studying the behavior and complexity of biological systems. However, modeling is often time-consuming and requires significant computational resources for data development, data analysis, and simulation. Computational modeling has been successfully applied as an aid for metabolic engineering in microorganisms. But such model-based approaches have only recently been extended to plant metabolic engineering, mainly due to greater pathway complexity in plants and their highly compartmentalized cellular structure. Recent progress in plant systems biology and bioinformatics has begun to disentangle this complexity and facilitate the creation of efficient plant metabolic models. This review highlights several aspects of plant metabolic modeling in the context of understanding, predicting and modifying complex plant metabolism. We discuss opportunities for engineering photosynthetic carbon metabolism, sucrose synthesis, and the tricarboxylic acid cycle in leaves and oil synthesis in seeds and the application of metabolic modeling to the study of plant acclimation to the environment. The aim of the review is to offer a current perspective for plant biologists without requiring specialized knowledge of bioinformatics or systems biology.

INTRODUCTION

Plant metabolism represents an enormous source of bioactive compounds with pharmaceutical and biotechnological importance. Currently, most commercialized metabolites are extracted from their native plant sources or are semisynthesized from metabolic intermediates. However, the natural manufacturing process has been overshadowed by low yield and numerous technical difficulties (Xu et al., 2013b).

Nevertheless, the use of plant metabolites for industrial purposes is growing, increasing the relevance of metabolic engineering, an approach which seeks to redesign natural pathways via the up- or downregulation of native genes or the introduction of transgenes (Jarboe et al., 2010). While engineering prokaryotic metabolism is a well-developed technology, its status in plants lags behind (Morgan and Rhodes, 2002; Toya and Shimizu, 2013; Xu et al., 2013b), mainly because of the extremely large diversity of metabolites present within plants compared with other organisms (Fernie et al., 2011). Such diversity implies a highly complex network of metabolic pathways, requiring sophisticated over-arching control by supercoordinated gene-metabolic interaction networks (Aharoni and Galili, 2011). An additional complication not faced in the prokaryotic cell is the number of distinct compartments present, some of which make plants more complicated than animal systems (large vacuoles and metabolically active plastids) (Heinig et al., 2013; Sweetlove and Fernie, 2013). Plants are also heterogeneous at the organ level, with recognizable energy sources and sinks and a well-defined transport system (Stitt et al., 2010).

Physiological complexities aside, the conventional paradigm of first identifying the relevant gene targets may need to be rethought. From the experimental point of view, the dominant idea was and partially still is that in every metabolic pathway there is a reaction or reactions that limit flux, mainly governed by irreversible enzymes, which play a key role in the regulation of metabolism (Morandini, 2009, 2013). However, the experimental evidence is incompatible with this assumption. For instance, genetic studies using gene dosage mutants or reverse genetics have shown that pathway flux is often unaffected by small changes in the level of enzymes catalyzing irreversible reactions (Flint et al., 1981; Morandini, 2009). Other studies have also predicted that manipulating a single enzyme is likely to give disappointing results (Small and Kacser, 1993). In summary, it is possible to imagine conditions where only a single reaction affects the rate of a pathway, although experimental studies show that this is not the usual case. In fact, attempts to analyze the kinetics of multireaction systems have shown that more than one enzyme can affect the rate of a pathway (Fell, 1997), and efforts to replace the concept of the rate-limiting step have led to introduction of a new approach known as metabolic control analysis (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; Heinrich et al., 1977).

It is also important to distinguish the difference between "flux control" and "flux regulation," which are distinct properties of complex metabolic systems. Flux control is defined as the extent to which a change in enzyme activity alters the flux through a pathway, whereas flux regulation refers to the response of

[1] Address correspondence to baghalian@ipk-gatersleben.de.
www.plantcell.org/cgi/doi/10.1105/tpc.114.130328

a metabolic process to an external perturbation (Fell, 1997). To minimize the effect of external perturbations, regulatory enzymes can regulate intermediate concentrations or control flux, and in this sense both concepts are connected to metabolic homoeostasis. These two concepts play a key role in linking the knowledge of molecular details to a system-level understanding. Therefore, an increased understanding of them could promote systemic approaches toward metabolic engineering.

Furthermore, metabolic pathways are highly branched and most of the time multiple enzymes contribute to flux control through a pathway (Peterhansel et al., 2008; Morandini, 2013). Significant interventions in flux are therefore likely to require the manipulation of not one, but several sites in the network (Stitt et al., 2010; Kruger and Ratcliffe, 2012). Thus, the development of a successful engineering experiment will require not just a determination of which gene/s are the optimal candidates (Lee et al., 2011), but also the gaining of an improved understanding of the regulatory circuits governing cellular metabolism (Curien et al., 2009). Advances in metabolic modeling are now beginning to address these issues (Yuan et al., 2008; Stitt et al., 2010).

The aim of this review is to highlight the increasing importance of modeling in understanding metabolism and the role it can play in plant metabolic engineering. The initial section discusses the potential of metabolic modeling in the context of plant systems biology, and an outline is given of the systems workflow required for plant metabolic modeling. In subsequent sections, the relevant mathematical modeling approaches are discussed, and an explanation is offered of the software tools available for simulation purposes. We then review the opportunities opened up by recent improvements in modeling; finally, a description of the insights provided by modeling into the metabolic engineering of plant central metabolism is given, along with an outline of the challenges that still lie ahead.

SYSTEMS BIOLOGICAL APPROACH TOWARD UNDERSTANDING PLANT METABOLISM

Systems biology has been defined as the study of the interactions between genes, metabolites, proteins, and regulatory elements and seeks to elaborate integrative models and/or networks (Yuan et al., 2008). Its overriding focus is to take a holistic view of the structure and dynamics of organisms, rather than to pursue the arguably more conventional reductionist approach. Systems biology has helped in summarizing huge disparate data sets and testing their consistency (Xu et al., 2013b). Efforts to adopt this approach for plant studies have led to the concept of the "in silico plant" (Raikhel and Coruzzi, 2003). In fact, significant improvements in the available resources for systems biology have made it more realistic to take a systemic approach toward studying not just model plants (*Arabidopsis thaliana*) but some economically important crop plants such as rice (*Oryza sativa*) (Chandran and Jung, 2014).

Some recent achievements of the plant systems biology approach have been reviewed by Keurentjes et al. (2011). The applications include analyses of the abiotic stress response (Cramer et al., 2011), of the host/pathogen interaction (Elena et al., 2011; Pritchard and Birch, 2011), of nitrogen nutrition (Gutiérrez, 2012), and of general metabolism (Sweetlove et al., 2003).

At the simplest level, a metabolic model captures the connectivity of the metabolic network of the target organism. Depending on the type of model and the data that have been embedded, a metabolic model can quantify the flux yield, predict alternative routes through which fluxes can move, and suggest possible novel routes (Pitkänen et al., 2010). Constructing a metabolic model using a systems biological approach requires the four steps described in the following paragraphs.

1. Structural Establishment of the Metabolic Network

Literature sources or online databases (Table 1) provide much of the information required for establishing the structure of a metabolic network. A "pathway mapping" tool developed for the KEGG database produces a graphical display of the location of various metabolites in the relevant metabolic network (Fiehn et al., 2011). The ever-expanding volume of omics data requires the elaboration of new databases and associated analytical tools to allow for the efficient archiving, access, and sharing of data (Gutiérrez et al., 2005). Few such plant-centered databases are in existence, and many of the necessary tools are still under development (Go, 2010; Schreiber et al., 2012). Bioinformatics efforts have been intensified to address this gap and examples are the Golm Metabolome Database, which provides access to custom mass spectral libraries, metabolite profiling experiments, as well as additional information and tools (Kopka et al., 2005); MetaCrop, which is a manually curated resource of high-quality data devoted to crop plant metabolism (Schreiber et al., 2012); and the Plant Metabolic Network, which contains links to plant metabolic pathway databases (Zhang et al., 2010).

Some of the currently available databases are species-specific, but others are more general (Table 1). Prominent among the former are MoTo DB, a metabolic database for tomato (*Solanum lycopersicum*) (Grennan, 2009), and AraCyc, a biochemical pathway database for *Arabidopsis* (Mueller et al., 2003).

Nevertheless, there are usually gaps in which neither the literature nor databases can provide the required information. This is especially common when it comes to inter/intracompartmental transport reactions along with their related transport systems, as information in this area remains, for the most part, uncharacterized.

2. Conversion from Reconstruction to Mathematical Model and Visualization

While step one provides a static view of the metabolic network, mathematical methods are required in order to process and integrate heterogeneous omics data and to build a comprehensive metabolic model (Kurata et al., 2007; Ghosh et al., 2011). Depending on the mathematical model chosen, the outcome of integrating mathematical formulations with available information can be used to simulate metabolism (Gómez-Galera et al., 2007; Stitt et al., 2010). Computational platforms have been developed to make the mathematical analysis and visualization convenient. In plants, however, the complexity of the metabolic network has held back the development of computer-aided synthesis methods (Mendes, 2002), but this sort of bioinformatics platform is quite widely used for modeling in microorganisms. Fortunately, the mathematical approaches are developing rapidly and efforts have been intensified to develop plant-specific

Table 1. Major Public Databases Containing Metabolic Information on Plants

Database	Comment
General	
Expasy	Provides access to databases and software tools in areas such as omics, phylogeny, systems biology, population genetics, etc.
Golm Metabolome Database	Enables exchange and presentation of metabolomic and related information for metabolite identification.
KEGG	An exclusive database covering a wide variety of organisms. These pathways are hyperlinked to metabolite and protein/enzyme information.
MetaCrop	Includes manually curated data of metabolic pathways in major crops. It allows automatic data export for creation metabolic models.
MetaCyc	A collection of model organism databases of metabolic pathways, including reactions, enzymes, genes, and substrate compounds.
PlantCyc	Includes manually curated or reviewed information about shared and unique metabolic pathways present in over 350 plant species.
Plant Reactome	A manual curated plant pathway database which hosts plant metabolic and regulatory pathways.
Pubchem	Database of chemical structures of small organic molecules. Linked with NIH PubMed/Entrez information.
SolCyc	Pathway Tools-based PGDBs for tomato, potato, tobacco, pepper, and petunia that were created at the Sol Genomics Network.
Wiki Pathways	An open, collaborative platform that enhances and complements ongoing efforts, such as KEGG, Reactome, and Pathway Commons.
Species-specific	
Arabidopsis Reactome	Initially developed to represent biological processes in *Arabidopsis*, but which has been extended to include information about other plant species.
AraCyc	Includes metabolic data for *Arabidopsis*. The pathways may be unique to *Arabidopsis* or shared with other organisms.
GRAMENE	Pathway databases for rice, maize, *Brachypodium*, and sorghum.
MedicCyc	Pathway database for *Medicago truncatula* containing more than 400 pathways with related genes, enzymes, and metabolites.
MoTo DB	A metabolic database for tomato
PoplarCyc	Includes metabolic data for the model tree *P. trichocarpa* and a few other related *Populus* species and hybrids.

platforms, allowing us to analyze and model plant metabolic pathways (Libourel and Shachar-Hill, 2008; Stitt et al., 2010). In subsequent sections, we shall discuss the different methods of modeling and the implications of computer aided platforms and toolboxes.

3. Validation

A valid model faithfully reflects the biologically realistic behavior of the metabolic network (Arnold and Nikoloski, 2011). Of course, errors can arise during the elaboration of the structure and/or during the embedding of quantitative data (Collakova et al., 2012). Any model therefore must be subjected to iterative hypothesis generation, followed by both wet and dry (in silico) experimental hypothesis testing (Klipp and Schaber, 2006). Lack of concordance between observed and predicted behavior requires reelaboration of the model to remove inconsistencies (Pitkänen et al., 2010). A model becomes acceptable as soon as the outcome of future experiments can be predicted (Wiechert, 2002). It is also an overriding priority to continuously update the validated model by reference to new findings. Such findings may include new locations for specific processes or the discovery of formerly unknown reactions (Kruger et al., 2012). An example in which a validated model was revised and updated occurred during a recent study by Wang et al. (2012). Using the validated model of C4GEM (de Oliveira Dal'Molin et al., 2010b) for their study, they realized that there were missing reactions in the xylose pathway and that the model could be improved by including them.

4. Analytical Investigation

A valid model can be regarded as a virtual laboratory, so predictions can be made much faster and more cheaply than by conducting the necessary wet lab experiments (Rohwer, 2012). Where the intention is to manipulate a given pathway to produce a specific outcome, applying the model can potentially generate a variety of alternative strategies, which can lead to a directed experimental validation (Copeland et al., 2012). However, we should bear in mind that the computational model represents an in silico prediction of what is supposed to occur in reality and it is the biologist who can determine appropriate experiments and the approach to be used.

To give an example of how modeling can contribute to experimental analysis, we refer to a study of photosynthesis (Zhu et al., 2007), during which an in silico approach was applied in order to identify the optimal protein-nitrogen distribution among 38 enzymes involved in photosynthetic carbon metabolism. If the total protein-nitrogen available to the enzymes is fixed,

any increase in the amount of some enzymes will require compensatory decreases in the amount of other enzymes. An experimental approach to identify the optimal protein-nitrogen distribution would involve prohibitive testing of a huge number of permutations. In contrast, the mathematical model developed by Zhu et al. (2007) tackled the question efficiently using an in silico approach. The model mathematically captured all the enzyme-catalyzed reactions of the Calvin-Benson cycle and end product processes, namely, starch and sucrose syntheses, as well as its interplay with photorespiration. Using an evolutionary algorithm, partitioning of a fixed total amount of protein-nitrogen between enzymes was allowed to vary in the model. The model successfully simulated the dynamics of photosynthetic carbon fixation and the metabolite concentrations. The conclusion was that manipulation of enzyme partitioning could improve carbon fixation without any increase in total protein-nitrogen investment in photosynthetic carbon metabolism (Zhu et al., 2007).

MATHEMATICAL MODELING METHODS

Here, we summarize methods that are being used in the context of plant metabolic modeling. To make the topic more approachable, we will avoid mathematical or biophysical detail. Table 2 provides definitions of a number terms commonly used in computational modeling.

Network Models

The most basic systems approach toward understanding a cellular biological process is establishing a network model. A network can be described as a graph in which biological entities such as metabolites, genes, transcripts, and proteins correspond to nodes, and the interactions between nodes, such as biochemical transformation, coexpression, and protein-protein interaction, correspond to edges (Yonekura-Sakakibara et al., 2013). The core networks in plant systems biology are gene-to-metabolite, protein-protein interaction, transcriptional regulation, gene regulation, and metabolic networks (Yuan et al., 2008). Network analysis refers to the use of algorithms to identify structurally important elements or network parts and graph-theoretic models, which use statistical methods to identify and infer complex functional interactions among the components (Yonekura-Sakakibara et al., 2013). However, this topological approach does not incorporate the dynamic behavior of the system, which requires other methods (Wiechert, 2002), as further described below.

Stoichiometric Models

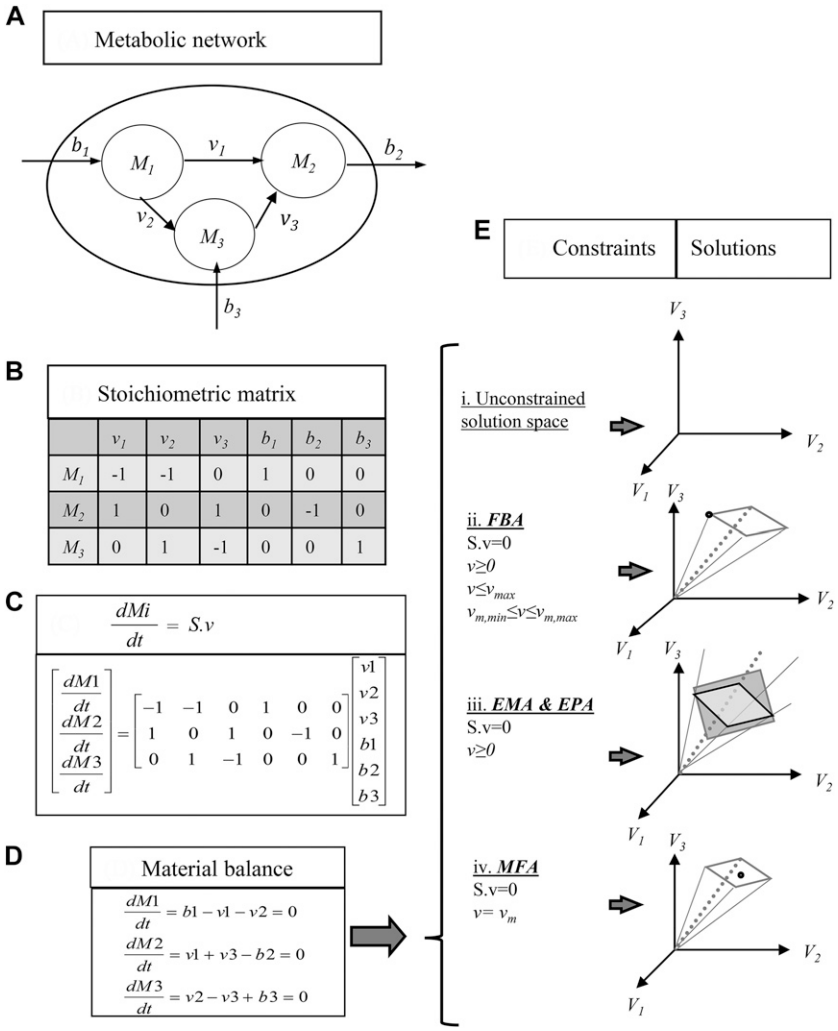
Stoichiometric models (Figure 1) are often applied to large metabolic networks (~1000 reactions) by imposing constraints to define the space of allowable metabolic flux states (Papp et al., 2011). Imposing such constraints allows the achievable flux distributions to be predicted. The basic feature of the stoichiometric approach is the assumption that a steady state prevails (Steuer, 2007). The three leading analytical approaches for characterizing a steady state flux distribution are flux balance analysis (FBA) (Figure 1E, ii), elementary mode analysis (EMA), and extreme pathway analysis (EPA) (Figure 1E, iii) (Schuster et al., 1999; Papin et al., 2004; Llaneras and Picó, 2008). Some further information regarding EMA and EPA is provided in Figure 2.

Genome-Scale Metabolic Models

The increasing volume of annotated genome sequence has allowed for the scaling-up of some metabolic models to form genome-scale metabolic models (Lee et al., 2011). In the ideal genome-scale metabolic model, every gene-to-protein-to-reaction

Table 2. Definitions of Terms Used in Metabolic Modeling

Term	Definition
Constraint	A restriction that must be satisfied for a solution to be permitted. It limits and defines a space where the feasible flux distributions occur.
Elementary modes	Unique sets of nondecomposable reactions within any network that are able to both sustain a steady state flux and operate independently.
Extreme pathways	Unique and minimal sets of reactions within any network that correspond to the extreme rays of a polyhedral cone and therefore completely characterize the steady state capabilities of metabolic networks.
Flux distribution	A specific set of reaction fluxes in a network.
Isotopic labeling experiment	An experiment in which isotopically labeled precursor (generally ^{13}C-labeled) is fed to the target, and the subsequent redistribution of the label is measured as a time course or after the system has reached an isotopic steady state.
Metabolic control analysis	A means to investigate the sensitivity of the steady state properties of a network by quantitative determination of enzymatic control coefficients.
Network robustness	The potential of a network to tolerate and respond to perturbation cause by a genetic and/or an environmental change.
Objective function	A function that must be maximized or minimized, reducing the solution space and defining fluxes that satisfy the objective of the model.
Steady state	The state wherein the quantity of a compound being produced is equal to the quantity being consumed.
Stoichiometric matrix	A matrix wherein, for each reaction, the stoichiometry of the metabolism is represented by a column and each metabolite is represented by a row.



	v_1	v_2	v_3	b_1	b_2	b_3
M_1	-1	-1	0	1	0	0
M_2	1	0	1	0	-1	0
M_3	0	1	-1	0	0	1

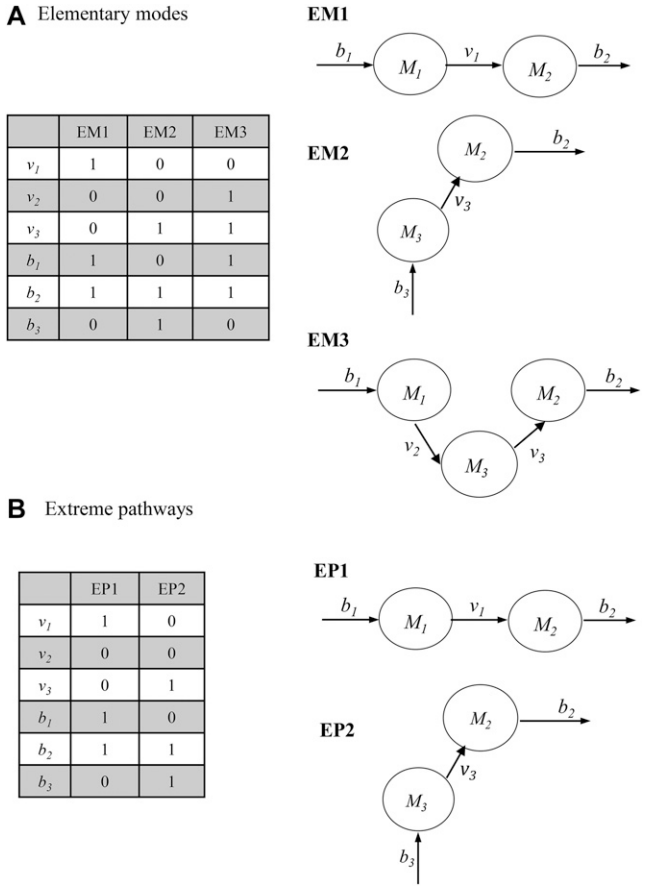
Figure 1. Steady State Modeling Approaches.

(A) A model reaction consisting of three metabolites (*M*), three exchanges (*b*), and three internal reactions (*v*).
(B) The reaction network represented in a stoichiometric matrix.
(C) The network rewritten in matrix form based on the equations.
(D) In a metabolic steady state, the product of the stoichiometric matrix (S) and the flux vector (v) returns a null vector (i.e., S.v = 0). Mass balance equations for each metabolite have been represented here.
(E) Constraints (shown in gray) and solution space. With no constraints, the flux distribution of a biological network reconstruction may lie at any point in a solution space (i). FBA (ii) solves the equation S.v = 0 by calculating intracellular fluxes from the measurement of a limited number of input and output fluxes. The solution (black dot) requires the definition of an objective function. By applying EMA and EPA (iii), the irreversible fluxes are constrained to be non-negative ($v \geq 0$), then the resulting space of flux distributions is a convex polyhedral cone, which represents the flux space of the metabolic system, containing all allowable flux distributions. MFA (iv) provides information concerning the contribution of a measured reaction (*m*) to the operational state of overall unmeasured fluxes and computes a metabolic flux vector specific for a particular growth condition (black dot).

association for each metabolic reaction in every pathway of a reconstructed network is represented (Collakova et al., 2012). The size and complexity of genome-scale metabolic models often imply that potential behavior can only be analyzed using constraint-based methods, in particular FBA. So although genome-scale metabolic model has become a popular modeling approach in recent years, it should not be considered as an independent mathematical model, but rather as an extended form of existing modeling platforms.

Steady State Metabolic Flux Analysis

Metabolic flux analysis (MFA) relies heavily on data acquired from experiments in which an isotopically labeled precursor (generally ^{13}C) is fed to the target, and the subsequent distribution of label into metabolic intermediates and end-products is analyzed when the system has reached a steady state (Figure 1E, iv). A model of the target metabolic network is used to predict the redistribution of the labels in a steady state situation. The experimentally observed



	EM1	EM2	EM3
v_1	1	0	0
v_2	0	0	1
v_3	0	1	1
b_1	1	0	1
b_2	1	1	1
b_3	0	1	0

	EP1	EP2
v_1	1	0
v_2	0	0
v_3	0	1
b_1	1	0
b_2	1	1
b_3	0	1

Figure 2. The Matrix and Figures Representing the Set of Three Elementary Modes and the Set of Two Extreme Pathways of the Network Depicted in Figure 1A.

With respect to the equation S.v = 0, S denotes the matrix formed by regarding each elementary mode (EM) **(A)** or each extreme pathway (EP) **(B)** as a column, and v is the vector containing their respective activity. Notice that the two EPs are also EMs, but the EM3 can be expressed as a combination of the others. This occurs because EPs are a subset of EMs, but no EP can be reconstructed as a linear combination of other EPs. Each EM represents a stoichiometrically and thermodynamically feasible route to the conversion of substrates into products, which cannot be decomposed into simpler routes. EMA reveals the adaptability and robustness of the metabolic network and EPA represents the margins of the derived steady state flux cone.

pattern is compared with the predicted labeling pattern and adjustments are made in the fluxes of the model until the agreement between the predicted and observed measurements becomes as close as possible. This process is repeated many times and finally leads to hundreds of flux maps from which it is then possible to deduce the model that provides the best prediction for the system (Kruger et al., 2012).

Kinetic Models

A kinetic model dynamically defines the metabolic network and, hence, is the most detailed and predictive mathematical description. Kinetic modeling requires the input of the dynamic behavior of enzymes and is generally applied to small portions of the metabolic network (10 to 50 reactions) (Morandini, 2009). Unlike the steady state stoichiometric approach, dynamic models calculate the time-dependent behavior of the system in terms of both fluxes and metabolite concentrations (Rohwer, 2012). In this approach, each reaction is defined in terms of an enzyme that catalyzes the conversion of its substrate into a product and reactions are modeled using differential equations (representing reactions rates) such as Michaelis-Menten kinetics. First, values are specified for the kinetic parameters v_{max}, K_m, the reaction stoichiometry, and the initial metabolite concentrations. Then, the velocity (v) of the reaction can be numerically simulated according to the Michaelis-Menten equation $(v_{max}[S])/(K_m + [S])$. Given the initial concentration of each metabolite, it is possible to simulate the system by considering the relevant rate equations and kinetic parameters (Schallau and Junker, 2010). However, since precise kinetic formulae are not available for many enzymes, assumptions must be made from heterologous systems or the literature (Sweetlove et al., 2008). A further challenge is to fit parameters to ensure a unique solution (Schallau and Junker, 2010). A reliable kinetic model can identify key control points via metabolic control analysis (Rohwer, 2012). In well-defined networks, where full information regarding individual enzyme-substrate kinetics is available, applying metabolic control analysis leads to the control coefficients, which predict the response of the system to perturbations (Toya and Shimizu, 2013). In instances where the kinetic parameters of a reaction (or reactions) are not known or, indeed, even the form of the kinetic relationship is not defined, control coefficients can be determined experimentally by altering the amount of a specific individual enzyme and measuring the impact on flux or other output variables of the network (Cascante et al., 2002).

MODELING SOFTWARE

The support of software toolsets is required for each step of the modeling workflow. This issue is particularly critical for model analysis and simulation (Ghosh et al., 2011). Advances in these toolsets enable us to assemble data into models in more efficient ways. MATLAB and the user-friendly tool Complex Pathway Simulator (COPASI) are perhaps the two most commonly used toolsets (Libourel and Shachar-Hill, 2008; Ghosh et al., 2011). MATLAB has been exploited for FBA in *Arabidopsis* (de Oliveira Dal'Molin et al., 2010a) for analyzing flux distributions in the leaves of C_4 species (de Oliveira Dal'Molin et al., 2010b) and for the analysis of storage metabolism in oilseed rape (*Brassica napus*) (Hay and Schwender, 2011a, 2011b). COBRA, a MATLAB-based toolbox designed for the constraint-based prediction of cellular behavior (Becker et al., 2007), has been applied to study both storage metabolism in the developing barley (*Hordeum vulgare*) endosperm (Grafahrend-Belau et al., 2009a) and the metabolic properties of *B. napus* seed (Pilalis et al., 2011). COPASI is an open-source software application for the simulation and analysis of biological processes such as biochemical reaction networks, cell-signaling pathways, and regulatory networks (Hoops et al., 2006). An example of its use is the detailed kinetic model of the aspartate-derived amino acid pathway in *Arabidopsis* (Curien

et al., 2009). Unlike COPASI, the use of MATLAB requires a background knowledge of mathematics and programming, but offers a substantial level of flexibility (Alves et al., 2006). A Python-based modeling tool known as ScrumPy has been developed to combine the advantages of COPASI and MATLAB (Poolman, 2006), and this has been successfully applied to construct and analyze genome-scale metabolic models of *Arabidopsis* (Poolman et al., 2009; Cheung et al., 2013) and rice (Poolman et al., 2013). Other tools for FBA include CellNetAnalyzer (Klamt et al., 2007) and FBASimViz (Grafahrend-Belau et al., 2009b), which provides a graphical front end to the COBRA toolbox. One can also find modeling studies, which have opted to apply other analytical approaches such as the General Algebraic Modeling System (Lakshmanan et al., 2013). To visually explore fluxes from experimental or simulated studies, tools such as FluxMap can be employed (Rohn et al., 2012).

In addition to simulation and analysis toolkits, software platforms are required to facilitate the exchange of data created by different researchers or different toolkits (Libourel and Shachar-Hill, 2008; Ghosh et al., 2011). To meet this requirement, three major standards have been developed, namely, SBML (Hucka et al., 2003), SBGN (Le Novère et al., 2009), and MIRIAM (Le Novère et al., 2005). Plant modelers have also started to apply these platforms for their models, which makes it convenient to exchange models and will increase the efficiency of future plant metabolic modeling. With rare exceptions such as the MIRIAM-compliant model of *Arabidopsis* (Mintz-Oron et al., 2012), many recent plant flux models have been made available in SBML, including a barley model (Grafahrend-Belau et al., 2009a; Grafahrend-Belau et al., 2013), AraGem (de Oliveira Dal'Molin et al., 2010a), C4GEM (de Oliveira Dal'Molin et al., 2010b), *B. napus* models (Hay and Schwender, 2011a, 2011b; Pilalis et al., 2011), the maize (*Zea mays*) model (Saha et al., 2011), the model of heterotrophic *Arabidopsis* cells in culture (Cheung et al., 2013), and a diel model that integrates temporally separated metabolic networks in C_3 and Crassulacean acid metabolism (CAM) leaves (Cheung et al., 2014). Many manually curated as well as computationally derived draft models represented in SBML and partially represented in SBGN can be found in BioModelsDB (Li et al., 2010).

DEVELOPMENTS IN PLANT METABOLIC MODELING

Further details concerning FBA, kinetic modeling, MFA, and genome-scale metabolic modeling have been provided in a number of recent reviews: Sweetlove and Ratcliffe (2011), Rohwer (2012), Kruger et al. (2012), and Collakova et al. (2012), respectively. Here, we discuss attempts to improve their effectiveness and the challenges that still lie ahead.

Network Models

The ever-increasing amount of high throughput data has opened new horizons in the application of purely structural network-based approaches for studying plant metabolism. One of the latest developments for network-based analysis of plant metabolites is correlation-based networks (CNs). This approach can be readily applied in plant science to gain novel insights into the complex regulation of biochemical reactions (Toubiana et al., 2013). Depending on the biological question to be answered and the type of data being studied, the CNs can help to identify regulatory mechanisms between distinct metabolic pathways in response to perturbation and to indicate the existence of uncharacterized metabolic pathways or to highlight phylogenetic relationships (Sulpice et al., 2013). For instance, CNs derived from metabolomics and transcriptomics data collected during time-resolved experiments of *Arabidopsis* rosette leaves and roots were used to study allosteric regulations in *Arabidopsis* and led to the identification of flavonoid biosynthetic genes (Sulpice et al., 2010). In a more recent study by Sulpice et al. (2013), the same approach was applied for studying the impact of carbon and nitrogen supply on metabolism/biomass interactions. A panel of 97 genetically different *Arabidopsis* accessions was grown in three different environmental conditions. CNs were created from the genotype-dependent variation in each condition to reveal sets of metabolites that indicated coordinated changes across accessions. The findings indicated that the networks were mostly specific for a single growth condition and the overall conclusion was that robust prediction of biomass across a range of conditions would require condition-specific measurement of metabolic traits to consider the effect of environment-dependent changes on the underlying networks (Sulpice et al., 2013).

Stoichiometric Models

EMA and EPA have been used to develop effective metabolic engineering strategies in microorganisms (Schuster et al., 2000; Tomar and De, 2013). However, their application for pathway analysis of large and highly entangled metabolic networks leads to the problem of the combinatorial explosion of possible routes across the network (Klamt and Stelling, 2002; Dandekar et al., 2003). Even though methods have been suggested to tackle larger systems by using these approaches (Dandekar et al., 2003), neither have been much employed for analyzing plants, most likely because their application to a larger metabolic network presents such a computational challenge (Klamt and Stelling, 2002). However, a comparison between EMA and MFA of *B. napus* cells indicated that the network structure described by the EMA captured a significant part of the metabolic activity in this biological system (Beurton-Aimar et al., 2011). The combination of EMA with kinetic modeling has proved successful for studying sucrose synthesis in sugarcane (Rohwer and Botha, 2001), and the combination of EMA with MFA was found informative for modeling oil synthesis in *B. napus* (Schwender et al., 2004). Moreover, using EMA, Steuer et al. (2007) outlined the structural and stoichiometric properties of the tricarboxylic acid (TCA) system. Aiming for transition from structure to dynamics of the system, they established a structural kinetic model of TCA.

Considering recent publications, it is apparent that among stoichiometric approaches, FBA has dominated the general trend in plant modeling studies. A successful FBA model relies on a complete and correct list of enzymatic reactions and more importantly the accuracy of the experimentally measured constraints (Sweetlove et al., 2013), which in many cases are not available for plants. The other major challenge of FBA in plants is that they are exposed to a wide range of environmental conditions.

Table 3. Summary of Objective Functions Used in the Literature to Optimize Plant FBA Models

Species	Model Name	Reference	Objective Function
Arabidopsis	–	Poolman et al. (2009)	Minimize total flux
Arabidopsis	AraGEM	de Oliveira Dal'Molin et al. (2010a)	Minimize uptake of biomass rate (photon for photosynthesis/photorespiration and sucrose for heterotrophic metabolism)
Arabidopsis	–	Mintz-Oron et al. (2012)	Minimize metabolic adjustment (MOMA)
Arabidopsis	–	Cheung et al. (2013)	25 combinations of five objective functions, including minimization of overall flux, maximization of biomass, minimization of glucose consumption, maximization of ATP production, and maximization of NADPH production
Arabidopsis	Diel model	Cheung et al. (2014)	Minimize total flux
Barley	–	Grafahrend-Belau et al. (2009a)	Maximize growth (linear optimization) Minimize overall flux (quadratic optimization)
Barley	–	Grafahrend-Belau et al. (2013)	Minimize carbon uptake (linear optimization) Minimize overall flux (quadratic optimization)
B. napus	bna572	Hay and Schwender (2011a), (2011b)	Flux balance: minimize substrate and light uptakes Flux variability: minimize and maximize each reaction while objective function is fixed
B. napus	–	Pilalis et al. (2011)	The actual biomass production based on measurements from the literature
Maize	iRS1563	Saha et al. (2011)	Maximize flux of biomass reaction
Maize, rice, sorghum, sugarcane	C4GEM	de Oliveira Dal'Molin et al. (2010b)	Same as in AraGEM
Rice	–	Poolman et al. (2013)	Minimize total flux
Rice	–	Lakshmanan et al. (2013)	Flux balance: maximize biomass production Flux variability: minimize and maximize each reaction while objective function is fixed

Therefore, a realistic description of the multiple behaviors of the cells likely will require multiple and more complex objective functions (Collakova et al., 2012). Another obstacle is the current insufficient knowledge regarding the constraints affecting all species and all environmental condition (Allen et al., 2009). A range of different objective functions can be used in FBA, including maximizing ATP yield per unit flux, minimizing energy usage, minimizing substrate uptake (at fixed biomass efflux), minimizing reaction steps or total flux, and maximizing biomass yield per total flux. However, none of these objective functions are consistently successful in predicting growth rates (Chen and Shachar-Hill, 2012).

In plant FBA modeling, the most popular constraint is the requirement to synthesize biomass of appropriate proportions and at a certain rate. The objective function is usually based on either minimization of the total reaction fluxes in the network or maximization of the carbon conversion efficiency (Table 3). A problem that arises here is that net biomass synthesis consumes a small proportion of the total energy budget. Therefore, when FBA is constrained solely by biomass synthesis, the fluxes through the energy-transforming pathways are greatly neglected (Sweetlove et al., 2013). Hence, because of the major energetic demand due to transport costs and cell maintenance, the biomass constraint alone is not sufficient to predict realistic fluxes in central heterotrophic metabolism of plant cells (Cheung et al., 2013). Accordingly, when Cheung et al. (2013) studied the effect of different constraints and objective functions on the accuracy of flux prediction by a FBA model of heterotrophic *Arabidopsis* cells in culture, they found accounting for energy costs (transport and maintenance costs) in the network system to be more important than the choice of objective function. In this regard, they developed a method to account for both the ATP and reductant costs of cell maintenance on the basis of the measured flux ratio between the oxidative steps of the oxidative pentose phosphate pathway (OPPP) and glycolysis.

Another issue is that while FBA is solely constrained by biomass synthesis, flux through the OPPP is absent in the vast majority of plant FBA models. Considering that these models account for the synthesis of biomass in sufficient quantities, this illustrates that the optimizing algorithm chooses other dehydrogenase enzymes to satisfy the NADPH demand of metabolism (Sweetlove et al., 2013). Cheung et al. (2013) show that the presence of thermodynamically implausible transhydrogenase cycles in the models can also lead to the absence of a predicted OPPP flux. Constraining these cycles to zero leads immediately to non-zero OPPP fluxes. Yet, there are studies showing that FBA, in its current standard form, has been very effective in predicting metabolic fluxes in plants. For instance, it was shown that FBA can predict net CO_2 evolution in a range of plant tissues and in response to environment (Sweetlove et al., 2013). More interestingly, by applying a set of appropriate constraints, the FBA framework has been used to establish a more representative model of leaf metabolism by solving the two phases of day and night photosynthetic cycles as a single optimization problem (a diel flux balance model). Applying only minimal changes to the constraints of this model enabled it to accurately capture CAM over a diel cycle (Cheung et al., 2014). Other studies have also shown that FBA has the capability to establish a condition-specific

metabolic model that is predictive under different environmental conditions (Williams et al., 2010; Cheung et al., 2013; Poolman et al., 2013). The extent to which FBA can successfully predict networks fluxes in plant metabolism is surprising as this method makes no reference to enzyme kinetic or regulation. This implies that enzyme regulation (i.e., allosteric regulation and posttranslational modifications) acts in such a way as to maintain metabolic steady state rather than as a key driver of the flux distribution across the network. Instead, it seems that the output demands are the main drivers of the flux distribution in central metabolism (Sweetlove et al., 2014).

Nevertheless, continuing efforts have been undertaken to improve the technical and practical aspects of plant FBA. So far, plant FBA models have been based primarily on data averaged across different cell types (Sweetlove and Ratcliffe, 2011). Given that many plant metabolic functions are based on interactions between different subcellular compartments, cells, tissues, and organs, the reconstruction of FBA models at the cell-type, tissue-specific, or even organ specific level is a prerequisite for their use in metabolic engineering (Grafahrend-Belau et al., 2013). A clear shift in this area has occurred with an increasing number of tissue-specific (de Oliveira Dal'Molin et al., 2010b; Hay and Schwender, 2011a, 2011b; Lakshmanan et al., 2013) and organ-specific (Mintz-Oron et al., 2012) FBA models or a whole-plant scale model (Grafahrend-Belau et al., 2013).

A key issue that may arise in the use of constraint-based models is the existence of alternate optimal solutions in which the same objective function can be achieved through different flux distributions. Flux variability analysis (FVA) is an efficient strategy for calculating flux variability that can exist to achieve optimal and suboptimal objectives (Tomar and De, 2013) and has been used to explore the metabolic capabilities of oil metabolism in a model of developing *B. napus* embryos (Hay and Schwender, 2011a). FVA was also applied to understand how oxygen influences the internal flux distributions in a model of rice, representing two tissue types: germinating seeds and photorespiring leaves (Lakshmanan et al., 2013). Cheung et al. (2014) also applied FVA to determine the feasible range of all fluxes in order to compare the predictions of a diel-modeling framework with the fluxes predicted in a constant light model.

Genome-Scale Metabolic Models

Over the past decade, genome-scale metabolic modeling has successfully provided unique insights into the metabolism of prokaryotic microorganisms (Toya and Shimizu, 2013; Xu et al., 2013a). Genome-scale models of prokaryotes can be analyzed with a wide range of optimization based tools and algorithms for rational design in metabolic engineering studies. Three of the most popular tools are OptKnock, OptORF, and OptFlux, which are used to simulate the simultaneous up- or downregulation (or knockout) of multiple genes (Lee et al., 2011; Tomar and De, 2013). Yet in plants, the application of genome-scale metabolic modeling is quite new, and it was not until 2009 that the first genome-scale model for *Arabidopsis* cell suspension culture (Poolman et al., 2009) became available. Since then, genome-scale metabolic modeling has been applied to studying the central metabolism of various C4 plants (de Oliveira Dal'Molin et al., 2010b; Saha et al., 2011), *Arabidopsis* (de Oliveira Dal'Molin et al., 2010a; Mintz-Oron et al., 2012), and rice (Poolman et al., 2013).

In general, these plant genome-scale metabolic models have proved to be functional, robust, and accurate in predicting qualitative changes in selected aspects of central carbon metabolism (Collakova et al., 2012; de Oliveira Dal'Molin and Nielsen, 2013). However, in the context of metabolic engineering, there are some concerns when it comes to comparing the application of genome-scale metabolic models to plants and microorganisms as unlike microorganisms, plants generally are not grown under a highly controlled environmental regime. Broadly speaking, extending the network flux analysis results of microorganisms to plant metabolic engineering studies will require some caution (Stitt et al., 2010). In this regard, Shachar-Hill (2013) provided an illustrative study using the case of lysine production, which is a metabolic engineering target common to plants and microbes. Mathematical modeling methods have been used successfully to improve lysine production in bacterial fermentation systems of *Corynebacterium glutamicum*. These tools have helped to identify possible metabolic bottlenecks and significant changes, leading to significant increase in lysine production. However, when the same approach was applied to maize endosperm, the general conclusion was that such limitations might not exist (Shachar-Hill, 2013). Another concern is that although genome-scale metabolic models may have been validated for selected aspects of central metabolism, they do not usually extend to secondary metabolism (Collakova et al., 2012). One exception to this can be found in a model of *Arabidopsis*, which includes some aspects of secondary metabolism (Mintz-Oron et al., 2012). Other challenges facing plant genome-scale metabolic models include uncertainty about the subcellular localization of reactions and the incomplete annotation of plant genomes (Sweetlove and Fernie, 2013). Approaches have been suggested for dealing with these challenges, such as applying subcellular localization prediction software for compartmentalizing metabolic reactions and comparative genomics for annotating undiscovered genomic content (Seaver et al., 2012; Lakshmanan et al., 2013).

Integration of genome-scale modeling and transcriptomics or proteomics data sets is another approach that can be used to extend understanding of the complex metabolic behavior of plants (Töpfer et al., 2012, 2013). An integrative approach was used to predict the metabolic response of *Arabidopsis* to changing conditions, and it was found that including the transcriptomic data improved the predictions even though transcript levels do not relate directly to fluxes (Töpfer et al., 2013). Further analysis has shown that this approach can successfully bridge the gap between flux- and metabolite-centric methods (Töpfer et al., 2014). In general, the fact that plant genome-scale models rely on constraint-based analysis makes them particularly suitable for defining the outer limits of a system's behavior rather than for making accurate predictions. An ideal progression would be to build a genome-scale kinetic model of a metabolic network, although the determination of kinetic parameters can be expected to be difficult, perhaps even to the point where it becomes too complex for calculation. A first attempt at building a parameterized genome-scale kinetic model of yeast metabolism

has been described by Smallbone et al. (2010). However, this approach still requires extensive development before it can be applied to higher eukaryotic systems. Given the acceleration in the sequencing of diverse plant genomes and the increasing interest in genome-scale metabolic models as a tool for examining plant metabolic networks, there is every reason to expect that with further improvement in available data and accordingly further refinement of the models their application will make an important contribution to plant metabolic engineering.

MFA

Despite the fact that steady state MFA techniques have addressed important questions, including the role of Rubisco in developing seeds and the regulation of oil seed metabolism (Kruger et al., 2012), their application to higher organisms (such as plants and mammalian systems) faces challenges, such as complex media formulations, subcellular compartmentation, and slow labeling dynamics (Allen et al., 2009). The major application of MFA to date has been on isolated cells or tissues, where typically 50 to 100 reactions are monitored (Allen et al., 2009). Technical difficulties in extending the analysis to plant networks have encouraged the development of alternative techniques (Sweetlove and Ratcliffe, 2011), such as the combinations MFA/EMA (Schwender et al., 2004) and MFA/FVA, which have been applied to study developing *B. napus* embryos (Hay and Schwender, 2011a, 2011b). Also, to avoid the long time period that MFA requires to achieve isotopic steady state, the isotopically nonstationary MFA (INST-MFA) technique has been developed. INST-MFA analyzes the metabolite labeling patterns obtained during the transient labeling period prior to isotopic steady state. This technique has been successfully applied to human cell studies (Murphy et al., 2013) and has also been used to study photosynthesis (Young et al., 2011; Szecowka et al., 2013). Steady state MFA is inapplicable to photoautotrophic tissues because labeling with $^{13}CO_2$ leads to uniform labeling of all metabolites in the steady state (Roscher et al., 2000). Therefore, while steady state MFA is a well-established technique for studying heterotrophic and mixotrophic plant tissues, it cannot be used to study photosynthesis. Moreover, achieving an isotopic steady state in leaves is in any case unlikely because of complications introduced by the light-dark cycle and the slow turnover of metabolite pools (Sweetlove et al., 2013). To address this problem, Young et al. (2011) applied the INST-MFA technique to the cyanobacterium *Synechocystis.* They obtained a comprehensive flux map for all the Calvin-Benson cycle reactions and some side reactions, including those catalyzed by Glc-6-phosphate dehydrogenase, malic enzyme, and the photorespiratory pathway. In this analysis, the metabolic pool sizes were fitted as free parameters, whereas in the application of a similar approach, kinetic flux balancing, to *Arabidopsis*, the model was constrained with measured pool sizes obtained by mass spectrometry, as well as nonaqueous fractionation to provide information on subcellular pool sizes. In this study, Szecowka et al. (2013) deduced a set of intracellular fluxes in intact illuminated *Arabidopsis* rosettes. They analyzed the dynamic redistribution of label from $^{13}CO_2$ supplied to leaves, from which a small set of fluxes were calculated. This approach allowed them to determine kinetic changes in isotope patterns of 40 metabolites of primary carbon metabolism and to benchmark them against four classically determined flux signatures of photosynthesis (Szecowka et al., 2013).

Kinetic Modeling

Where there is enough reliable data a kinetic model can be both comprehensive and predictive (Schallau and Junker, 2010; Wang et al., 2014). An example is the kinetic model of monolignol biosynthesis in *Populus trichocarpa* (Wang et al., 2014), which was constructed by performing a comprehensive study to obtain the reaction and inhibition kinetic parameters of all the relevant enzymes based on functional recombinant proteins. However, few such comprehensive models have been presented in plant metabolism because of the difficulty in obtaining the required information (Wang et al., 2014).

Structural-kinetic modeling could provide a potential way around this deficiency. This method represents a transitional bridge between the stoichiometric approach and the various dynamic kinetic models. Although it does not define actual dynamic behavior, it describes the stability and robustness of a specific metabolic state, and clarifies related interactions and parameters governing the system's dynamic properties. Detailed mathematical information, as well as the proposed workflow for modeling, have been provided by Steuer et al. (2006). A structural kinetic model consisting of 18 metabolites and 20 reactions was established to analyze the Calvin-Benson cycle. The model successfully extracted dynamic properties of the system without relying on any particular assumption about the functional form of the kinetic rate equations (Steuer et al., 2006). The same approach has been applied to the TCA cycle in plants (Steuer et al., 2007) to detect and quantify the dynamic behavior.

A second approach to address the problem has been to assemble the kinetic model in a "top-down" fashion, amounting to fitting the model to the observed metabolite concentrations and fluxes. This approach was used to model the benzenoid network in the petunia (*Petunia hybrida*) flower, leading to the successful identification of the key flux-controlling steps (Colón et al., 2010). A "bottom-up" kinetic modeling approach has been described in modeling phloem flow in sugarcane (*Saccharum officinarum*) in the form of an advection-diffusion reaction framework. This pioneering model can probably be adapted to other plant species and perhaps even be extended to study xylem flow. It has been suggested that the same framework could form the basis for creating an integrated kinetic model of whole plant physiological function (Rohwer, 2012).

NEW INSIGHTS INTO METABOLISM AND ENGINEERING PLANT SYSTEMS

One of the most important goals of metabolic engineering is the optimization of metabolic pathways for the production of industrially important metabolites. A major challenge is to accurately select the target pathways and then to tune and optimize

the expression level of each enzyme for the selected pathways (Xu et al., 2013b). Models of plant metabolism have begun to address this challenge by providing a more rigorous basis for future genetic engineering. One such example is the identification of some key regulatory points within the pathway of monoterpene metabolism in peppermint, using a dynamic MFA approach. The model-derived results of this study have been experimentally verified and demonstrated the potential to guide the manipulation of metabolism to enhance monoterpene accumulation (Rios-Estepa et al., 2008). Another example is provided in a study on *Arabidopsis* seed, where the FBA model was used to computationally design metabolic engineering strategies for vitamin E overproduction (Mintz-Oron et al., 2012). A third example is a kinetic model of monolignol biosynthesis in *P. trichocarpa*, which revealed mechanisms involved in the regulation of lignin biosynthesis (Wang et al., 2014). This work provides a platform for future engineering of lignin production as well as improvements in other related areas such as the resistance to biotic and abiotic stresses and new biomaterials production (Wang et al., 2014).

The compounds synthesized within the plant cell can be classified as either primary metabolites or secondary metabolites (Bu'Lock, 1965; Luckner, 1972; Richter, 1978). Manipulation of secondary metabolic networks typically is less complex than that of primary metabolism, allowing them to be readily broken down into more manageable entities and therefore offering more favorable opportunities for pathway engineering (Sweetlove et al., 2010). Moreover, despite the remarkable diversity of secondary metabolism, they can still be organized into groups of structurally related compounds. This facilitates the categorizing of pathways and even their order to make their modeling more tractable. Grouping metabolites of similar biosynthetic origin forms the logical basis of organization of models of fluxes as well as kinetic models (Morgan and Shanks, 2002; Fernie and Morgan, 2013).

Nevertheless, secondary metabolite engineering in plants is less developed than in other organisms, perhaps because of the highly complicated network connections which link primary and secondary metabolisms. For instance, some transcription factors associated with the production of a particular group of secondary metabolites coactivate the expression of genes encoding metabolic enzymes linked with primary pathways that provide precursors to these secondary metabolites (Aharoni and Galili, 2011). Such links have been responsible for frustrating a number of attempts to engineer plant secondary metabolism, producing unanticipated outcomes or trivial alterations to the system (Colón et al., 2010; Stitt et al., 2010). Therefore, studying the central metabolism network may promote the engineering of both primary and secondary metabolism.

Photosynthesis

Numerous experiments have been conducted to enhance crop productivity by genetic manipulation of photosynthetic electron transport, RuBP regeneration, Rubisco activity, and the associated flow to photorespiration (Peterhansel et al., 2008; Raines, 2011). These results reaffirm the importance of mathematical models for a better understanding of photosynthetic reactions (Arnold and Nikoloski, 2014). A functional model of photosynthesis should include not only the individual metabolic steps, but also the major regulatory mechanisms affecting these steps. Such comprehensive models ideally would predict the photosynthetic metabolic network response to environmental or genetic perturbations and would have implications for the redirection of carbon to high value natural products and ultimately the improvement of crop yield (Szecowka et al., 2013; Zhu et al., 2013).

Although many aspects of photosynthetic networks have been subjected to modeling studies, the Calvin-Benson cycle has become a favored target, as it is the primary pathway in plants, producing starch and sucrose from CO_2 (Arnold and Nikoloski, 2014). However, a neglected aspect of the C_3 photosynthesis network is the control of flux from the Calvin-Benson cycle to the output pathways of starch, sucrose, isoprenoids, shikimate, and nucleotides. Because of this, it is difficult to predict in a comprehensive fashion the way the relative flux to these pathways changes during development or in response to environmental changes (Raines, 2011). Nevertheless, the existing models potentially provide a good starting point for extending and improving future photosynthesis models. In this regard, Arnold and Nikoloski (2011) compared 15 Calvin-Benson cycle models assembled over the past 30 years and provided a detailed classification on the basis of the model boundaries, the level of cellular organization, the complexity of the kinetics, and the regulatory processes that were included. They ranked the models on several criteria, including sensitivity, stability, robustness, and the residual sum of squares at the resulting steady states. Their target was to identify model candidates that provided quantitatively accurate predictions for use in metabolic engineering. Based on their analysis, they categorized the existing models into two groups: those suitable for carbon fixation and those suitable for metabolic engineering. The most suitable models appear to be those proposed by Farquhar et al. (1980) and Poolman et al. (2000), respectively.

The advantage of the model proposed by Farquhar et al. (1980) is that it links Rubisco with in vivo measurements of photosynthetic rate and therefore is capable of predicting net rates of photosynthetic CO_2 fixation in response to variable environmental conditions. Because of these advantages, the model has been studied and extensively validated over many years, and derivatives of the model have been established which are currently used in large-scale ecological modeling studies (Sweetlove et al., 2013). The Farquhar model and its derivatives consist exclusively of algebraic equations that can only capture the steady state behavior through restricting assumptions (Arnold and Nikoloski, 2013). However, photosynthesis is rarely at steady state in the natural environment due to fluctuating conditions of environment. Therefore, a highly mechanistic, well-validated model is required to study photosynthesis in a more practical approach. In this regard, a kinetic model (e-photosynthesis), which includes each discrete process from light capture to carbohydrate synthesis, has been recently described for C_3 photosynthesis. The e-photosynthesis model effectively mimics many typical kinetic of photosynthetic features and provides a workable platform for guiding engineering of improved photosynthetic efficiency (Zhu et al., 2013).

Because of its steady state nature, Farquhar model and its derivatives are incapable of capturing dynamic changes that occur in the relationship between photosynthesis and photorespiration at varying light intensities and concentrations of CO_2 and O_2. Recent experimental evidence indicates that photorespiration is also involved in nitrate assimilation, energy production of photosynthesis, exchange of redox equivalents between compartments, one-carbon (C1) metabolism, and redox signal transduction (Arnold and Nikoloski, 2013). Accurate quantitative modeling of photorespiration is thus of major importance to understand how the fine tuning of the levels of intermediates and fluxes maintains optimal CO_2 assimilation in response to perpetually changing conditions (Fernie et al., 2013). The Farquhar model's derivatives aside, even kinetic modeling approaches of photorespiration have neglected its complex role and have mostly coupled a far too simplified version with photosynthetic metabolism. However, the e-photosynthesis model (Zhu et al., 2013) has included photorespiration in more detail.

A promising approach toward modeling photorespiration could be assembling a complete metabolic network in such a way that the connection of nitrogen metabolism is also taken into account. For instance, through perturbing nitrogen-specific reactions in such a model, the interplay of photorespiration and photosynthesis as well as effects on the whole system could be tested. An extension to such an approach could be undertaken by adding the recent findings regarding regulatory and signaling events of photorespiration into genome-scale models (Arnold and Nikoloski, 2013).

While the balance between photosynthesis and respiration is a key determinant of the carbon economy, another flaw in the Farquhar model and its derivatives is that they predict respiration on the basis of its correlation with other processes not as an independent metabolic phenomenon (Sweetlove et al., 2013). To attain the goal of an applicable mechanistic respiration model, several challenges must be addressed. It seems that the term "respiration" should be defined in such a way as to capture the light-independent metabolic networks, which lead to the net CO_2 production. In this regard, Sweetlove et al. (2013) suggest substituting the poorly defined term "respiration" with "net CO_2 evolution," which is defined as the sum of all the CO_2-producing steps minus the sum of all the CO_2-consuming steps, excluding photosynthesis and photorespiration. Concentrating on net CO_2 evolution results in the identification of two precise challenges for the modeling process. First, one must identify all the metabolic processes contributing to net CO_2 production and this is generally regarded as extremely difficult. Second, any predictive model must allow for differences between tissue types and the impact of a change in conditions on the processes contributing to net CO_2 evolution. In addressing these challenges, quantification of biochemical processes leading to net CO_2 evolution by MFA shows that the contribution of different processes to the CO_2 balance is highly variable. It also shows that the variability in CO_2 evolution between species and tissues might be greater than between growth conditions. This finding would indirectly reflect the need for a robust central metabolic network in the face of suboptimal environmental conditions. In addition to MFA, the potential for FBA as a tool to predict net CO_2 evolution has been assessed. Although there are relatively few FBA studies for which experimentally constrained metabolic flux data are available as a point of comparison/validation, the conclusion is that FBA has the potential to predict the metabolic origin of evolved CO_2 in different tissues/species and under different conditions (Sweetlove et al., 2013).

However, most of these models assume that the organism grows in constant light, which is unlike the natural situation where the interaction between light and dark metabolism is a major feature of metabolism of photosynthetic organisms. To establish a more representative model of leaf metabolism, Cheung et al. (2014) constructed a diel flux balance model that accounted for metabolic fluxes in the light and dark phases of leaf metabolism by simulating them simultaneously in a single optimization problem. The diel model was obtained by applying a specific framework of constraints to an existing genome-scale model of *Arabidopsis* metabolism (Cheung et al., 2013). The model successfully captured many known features of C_3 leaf metabolism including the role of citrate synthesis and accumulation at night (through the mitochondrial tricarboxylic acid cycle) and its export from the vacuole during the day as a precursor for the provision of carbon skeletons for amino acid synthesis. Generally, this model discovered some important features of interactions between light and dark metabolism and successfully predicted the metabolic fluxes in the light in C_3 photosynthesis.

C_4 plants possess a characteristic leaf anatomy, which supercharges photosynthesis by concentrating CO_2 in the vicinity of Rubisco and significantly reducing the oxygenation reaction (Wang et al., 2012). A system understanding of the distinctive anatomy and unique physiology is a prerequisite to effective modeling of C_4 metabolism. In order to achieve a system-level understanding of spatial regulation of photosynthesis in C_4 plants, a genome-scale metabolic model (C4GEM) was developed and applied to investigate the flux distribution between two interacting tissues of bundle sheath and mesophyll during C_4 photosynthesis. The model is an extension of an *Arabidopsis* model (AraGEM) (de Oliveira Dal'Molin et al., 2010a), which represents three different C_4 subtypes NADP-ME (NADP-dependent malic enzyme), NAD-ME (NAD-dependent malic enzyme), and PEPCK (phosphoenolpyruvate carboxykinase) (de Oliveira Dal'Molin et al., 2010b). In an extension to this study, Wang et al. (2012) simulated the influence of each subtype on biomass synthesis and CO_2 fixation and concluded that the PEPCK subtype is superior to NADP-ME and NAD-ME subtypes under sufficient supply of water and nitrogen. Moreover, the C4GEM model highlighted differences in the relative fluxes through photosystem I and photosystem II (PSII) in the different cell types and in each of the three C_4 subtypes. The model also predicted that the NAD-ME and PEPCK subtypes have substantial PSII activity in the bundle sheath tissues, while NADP-ME species have little PSII and more cyclic electron transport (CET) in their bundle sheath cells. While C_4 plants require more ATP than C_3 plants to assimilate CO_2, it has not been elucidated how the extra ATP is produced. Interestingly, simulations have shown that CET occurring in the bundle sheath is an efficient means for supplying the extra ATP needed in the NADPH-ME subtype. The model compared the minimum photon requirement for CET in the mesophyll bundle sheath. The results showed that

CET in the bundle sheath is energetically more efficient as it requires fewer photons to produce the extra ATP than CET that is active in the mesophyll (de Oliveira Dal'Molin et al., 2010b).

A system-level understanding of how C_4 photosynthesis operates and differs from C_3 plants is also a prerequisite to understanding how carbon shuttling enzymes are tuned by controlling networks (Wang et al., 2012; Weissmann and Brutnell, 2012). The study by Wang et al. (2012) compared a C_3 metabolic network (AraGEM, for *Arabidopsis*) with a C_4 metabolic network (C4GEM, for maize). To this end, they first made some improvement to both models and compared them using graph theory analysis (which allows the comparison of important topological parameters). They found out that the C_3 network has a denser topology than C_4. This is probably a reflection of the anatomical difference between C_4 and C_3 leaf structure, as the former includes both mesophyll and bundle sheath cells, while the latter consists of single cell types. The simulation of enzyme knockouts (single reaction deletion) showed that more than 86% (where the objective function is biomass maximal) and more than 96% (where the objective function is CO_2 fixation) of reactions have no influence when deleted in C_4 and C_3 networks. This demonstrates the robustness of these networks. Further to this, a comparison of the redundancy of the primary metabolic network between C_4 and C_3 showed that, regardless of the type of objective function, the C_4 plant is more robust to gene mutation or environmental changes (Wang et al., 2012).

CAM represents a temporal separation of metabolic events in which CO_2 is initially fixed at night in the form of carboxylic acids (mainly malic acid) and then decarboxylated during the day to provide CO_2 for conventional photosynthesis (Cheung et al., 2014). CAM maximizes water use efficiency and maintains high biomass productivity by concentrating CO_2 around Rubisco, favoring carboxylase activity. CAM also represents a simpler anatomical structure, as its photosynthetic metabolism occurs in a single mesophyll cell instead of in the two separate cells as in C_4 photosynthesis. Modeling could provide a key approach for comprehensive systemic understanding of the enzymatic and temporal regulatory events that control the carboxylation-decarboxylation of carboxylic acids and the concurrent metabolic fluxes through glycolysis-gluconeogenesis (Borland et al., 2014). Relatively little effort has been made toward studying CAM in a systems level. To address this, Cheung et al. (2014) used an innovative technical approach by making some changes to the constraints of the original diel C_3 model in order to capture the classical CAM cycle of a mature leaf and to predict the metabolic flux over a diel cycle. While the model successfully predicted metabolic fluxes consistent with the well-known CAM cycle, it also showed that despite the potential for suppression of photorespiration through CO_2 concentration, there are unlikely to be significant energetic benefits in CAM photosynthesis over C_3. The model predicted that the energetic savings of enzymatic machinery, which have been achieved by suppression of photorespiration, are probably offset by the higher flux demand of the CAM cycle.

In addition to Rubisco, which is the carboxylating enzyme operating in the Calvin-Benson cycle, nature employs several other carbon fixation pathways. This diversity of natural solutions offers the chance of utilizing a combination of modeling together with synthetic biology, to assemble fully innovative CO_2 fixation pathways that may be more efficient than the C_3 cycle. With the aim of designing synthetic metabolic pathways for improved carbon fixation, growth, and yield, Bar-Even et al. (2010) considered the entire range of ~5000 metabolic enzymes known to occur in nature as components and used an FBA to systemically discover all possibilities that can be devised with these enzymes as building blocks. This led to several promising synthetic carbon fixation pathways, which then they compared with the natural pathways using physiochemical criteria. The comparison suggested that some of proposed synthetic pathways could have a significant quantitative advantage over the natural ones.

Besides the Calvin-Benson cycle, which supports most of the global carbon fixation, there are currently five known naturally occurring carbon fixation pathways: the reductive TCA cycle, the 3-hydroxypropionate/malyl-CoA cycle, the reductive acetyl-CoA pathway, the 3-hydroxypropionate/4-hydroxybutyrate cycle, and the dicarboxylate/4-hydroxybutyrate cycle. Boyle and Morgan (2011) compared the thermodynamics and efficiency of these six pathways using FBA. Based on comparisons of either the energy demand or photon requirement for conversion of photoassimilate into biomass, it was shown that the reductive TCA cycle is the most efficient way of generating biomass from solar energy. However, the reductive TCA cycle is only trivially more efficient than Calvin-Benson cycle. Overall, this study emphasizes the role of the Calvin-Benson cycle, which has evolved to operate in the current oxidative environment of the earth (Boyle and Morgan, 2011).

TCA Cycle

In microorganisms, in silico pathway analysis has suggested a significant potential for TCA cycle optimization (Kjeldsen and Nielsen, 2009); hence, efforts have been initiated to engineer it (Becker et al., 2009). Engineering the TCA cycle in plants could also be useful because of the high value metabolites derived from carbon skeletons provided by this pathway, including amino acids, fatty acids, flavonoids, pigments, alkaloids, and isoprenoids. In plants, there are significant hurdles in advancing engineering of the TCA cycle, perhaps because of the overall complexity of the system. However, almost all the genes encoding the enzymes involved in TCA cycle have been cloned from different plant species, and many of the encoded proteins have been biochemically characterized. Efforts have also been intensified to understand the modular organization of TCA cycle (Carrari et al., 2003). These achievements have provided the basis for efforts to genetically modify TCA cycle and to enhance the organic acid content in plants (Morgan et al., 2013).

Genetic and metabolic experiments have shown that the conventional TCA cycle is not the only pathway through which TCA flux passes (Sweetlove et al., 2010), raising numerous as yet unanswered questions concerning the balance between the cyclic and the noncyclic flux modes. Answers to these questions could help to efficiently engineer the plant TCA cycle. To this end, modeling experiments have been of great benefit in showing that when the demand for ATP is low, the cyclic flux

mode is not necessarily maintained (Sweetlove et al., 2010). Similarly, a large-scale model of cellular metabolism in developing embryos of the *B. napus* developing seed demonstrated that cyclic TCA activity is reduced as the photosynthetic output of NADPH and ATP rises (Hay and Schwender, 2011a, 2011b), while an FBA-based model of heterotrophic *Arabidopsis* metabolism demonstrated that cyclic TCA flux is only required when there is a high demand for ATP (Poolman et al., 2009). In the barley endosperm, FBA has been used to show that the gradual switch from cyclic TCA (in aerobic tissue) to noncyclic TCA (in hypoxic tissue) occurs during the process of grain maturation, probably because succinate dehydrogenase (which connects the TCA cycle with the mitochondrial electron transport chain) is associated with only a minor flux (Grafahrend-Belau et al., 2009a). In agreement with this report, in silico flux maps of seed-derived suspension culture rice cells grown under anoxic conditions showed a truncated TCA cycle operation between fumarate and oxaloacetate while a fully operational TCA cycle was characterized under aerobic conditions. This difference was mainly due to the limited regeneration of redox cofactors since mitochondrial respiration was impaired under anoxia. Interestingly, FBA revealed the possible role of γ-aminobutyric acid shunt in the conversion of α-ketoglutarate to succinate instead of α-ketoglutarate dehydrogenase and succinate-CoA ligase under anaerobic conditions. Furthermore, in contrast to anaerobic conditions, a significant amount of pyruvate was converted to acetyl-CoA under aerobic conditions, thus enabling its entry into the TCA cycle for energy production (Lakshmanan et al., 2013). The genome-scale metabolic model of a developing leaf cell of rice predicted that the responses of the three neighboring enzymes succinate dehydrogenase, fumarate, and malate dehydrogenase are different under different light intensities. This study pointed out that the TCA cycle has the ability to reconfigure its reactions to fulfill different requirements under different conditions or developmental stages (Poolman et al., 2013). This view of the TCA cycle is supported by experimental evidence from other studies (Studart-Guimarães et al., 2007; Rocha et al., 2010).

Sucrose Metabolism

Sucrose accumulation in storage tissues is accompanied by recurring cleavage and synthesis, during which ATP is wasted (Schäfer et al., 2004). The genetic inhibition of this futile cycle might be expected to increase crop productivity. Identifying the candidate genes for transgenic regulation would require a laborious gene-by-gene approach (Rohwer, 2012), whereas modeling could radically short-cut this process. Applying a combination of EMA and kinetic modeling, 14 elementary modes were detected during sucrose accumulation in sugarcane, five of which were associated with a futile cycle. The model also predicted that the attenuation of neutral invertase and the overexpression of a vacuolar sucrose importer and plasma membrane glucose and fructose transporters would provide an efficient means of reducing futile cycling (Rohwer and Botha, 2001). These predictions were partially validated in an analysis of suspension cell cultures in which neutral invertase activity had been downregulated by RNA interference, since these cells were more capable of accumulating sucrose compared with the wild type (Rohwer, 2012). MFA of the maize kernel similarly showed that depending on the subcellular location of glyceradehyde 3-phosphate and the identity of the enzymes involved, futile cycling could waste between 18 and 47% of the ATP pool (Alonso et al., 2011). However, Kruger et al. (2007) argue that this value is unlikely to be as high as reported and that reliable ^{13}C MFA measurements of the flux from hexose phosphate to glucose (sucrose cycling), will only be possible if the labeling pattern is known for both the cytosolic and vacuolar glucose pools.

Seed Oil Synthesis

During seed storage deposition, the biosyntheses of different storage compounds need different proportions of energy cofactors (ATP and NADPH), as well as different proportions of metabolic precursors (Hay and Schwender, 2011b). As a predictive model of oil metabolism is helpful in manipulation of seed composition, many efforts have been conducted in this regard. An important feature influencing the seed oil yield is the average carbon conversion efficiency (CCE), a measure of the efficiency of conversion of substrates into storage product (Alonso et al., 2011). CCE is a straightforward definition of metabolic efficiency and highlights the proportion of resources devoted to accumulation of structural, storage, and reproductive biomass (Chen and Shachar-Hill, 2012). CCE estimates have been obtained for the sunflower (*Helianthus annuus*) embryo (50%) (Alonso et al., 2007), the maize endosperm (76 to 92%) (Alonso et al., 2011) and embryo (56 to 71%) (Alonso et al., 2010), and the *B. napus* seed (>80%) (Alonso et al., 2007). Studying the metabolic basis underlying these differences may promote insights into how genetic engineering can be used to increase oil content and to improve its composition. Several models have been established for description of oil production, including in *B. napus* (Schwender et al., 2004; Schwender, 2008; Hay and Schwender, 2011a, 2011b), maize (Alonso et al., 2010, 2011), and sunflower (Alonso et al., 2007).

Modeling storage metabolism in the developing *B. napus* embryo has highlighted the potential participation of various pathways, including the formation of the lipid precursor pyruvate and the potential role of PEP carboxylation in either nitrogen assimilation or in lipid synthesis. No increased uptake or changed use of amino acids, as possible lipid precursor, was predicted using MFA or FVA (Hay and Schwender, 2011a, 2011b). The same studies also characterized the bypass of glycolytic reactions by Rubisco to lipid synthesis. This "Rubisco bypass" pathway can explain the observed increase in CCE. However, due to the energy requirement of the bypass, this contribution is only predicted to be beneficial if the light intensity is above a certain threshold (Hay and Schwender, 2011a).

In an elegant study, an FBA model for cultured *B. napus* (Hay and Schwender, 2011a, 2011b) was combined with high-resolution measurements of in planta developing embryos in order to get an in-depth insight into the spatial variation in metabolic fluxes across different tissues of oilseed. Unlike the FBA model prediction, this study predicted that the Rubisco bypass occurs only in the outer cotyledon, hypocotyl, and radicle, but not in the

inner cotyledon. This probably happens due to the shape of the seed because as the seed gets bigger, light penetration into the inner tissues gets smaller (Hay and Schwender, 2011a; Borisjuk et al., 2013).

MFA of the developing maize embryo and endosperm has also revealed that flux through the OPPP is greater in the embryo than in endosperm. Nevertheless, even the carbon amount entering the embryo cannot fully meet the NADPH demand for fatty acid synthesis and may limit oil production, while NADPH is not a limiting factor for lipid synthesis in the endosperm. MFA studies also revealed the key role for plastidic NADP-depended malic enzyme activity in providing reductant and carbon for fatty acid synthesis in developing maize embryo (Alonso et al., 2010, 2011).

Metabolism and the Environment

The level of metabolites is dramatically influenced by environmental adversity. However, the connection between environmental conditions and metabolism is hidden by the complex networks linking them. Understanding this connection becomes more important when we try to recognize the role of metabolism in acclimation to abiotic stress. Metabolic modeling has been applied to this issue, seeking answers to questions such as to what extent the functioning of metabolic pathway(s) may be associated with environmental change and whether the network connectivity is conserved or changes between different growth conditions.

One of the first attempts at stoichiometric modeling of plant metabolism was performed to analyze the storage pattern of developing barley seed endosperm in response to oxygen depletion (Grafahrend-Belau et al., 2009a). Since then, several modeling investigations of plant-environment interactions have studied the impact of stress (increased temperature and hyperosmotic stress) (Williams et al., 2010; Cheung et al., 2013), carbon and nitrogen availability (Sulpice et al., 2013), light and temperature condition (Töpfer et al., 2013), and nitrogen supply (nitrate or ammonium) (Masakapalli et al., 2013) on heterotrophic metabolism in *Arabidopsis*.

The increasing availability of high throughput data for crop plants is leading to new modeling applications in studies of the interaction between crop plants and their environment. For instance, to elucidate metabolic flux profiles during abiotic stresses (flooding and drought stresses) a metabolic/regulatory network of rice cells was reconstructed for two different rice tissues, germinating seeds and photorespiring leaves (Lakshmanan et al., 2013). In another study, a genome-scale metabolic model of a developing leaf cell of rice was used over a range of photon flux values (Poolman et al., 2013).

The breeding of new crop varieties with improved performance under abiotic stress is becoming increasingly important. Therefore, it is expected that metabolic modeling will play a key role in this field in the near future.

CONCLUDING REMARKS

Plant computational modeling is evolving rapidly and will soon reach the point where it can begin to make an impact on plant metabolic engineering practice. However, there is still a need to overcome serious difficulties before plant metabolic models can be routinely incorporated as part of crop systems biology and there is a particular need for multiscale models (Baldazzi et al., 2012). By definition, a multiscale model explicitly integrates mechanisms that occur across multiple spatial or temporal scales and/or functions (Baldazzi et al., 2012; Walpole et al., 2013). Establishing such a model sometimes requires a range of diverse inputs from biochemical or mechanistic mechanisms to biomechanical phenomena, which leads to a hybrid multiscale model (Baldazzi et al., 2012). An excellent example of such a hybrid multiscale model was developed for the heart (Noble, 2011) in which the reaction-diffusion equations (as a description for the electro-mechanical contraction of heart) are coupled to a set of ordinary differential equations (as a description for ions transport at the cellular membrane). Examples in plant biology include models that correlate molecular level processes with plant development/morphogenesis in *Arabidopsis* (Vernoux et al., 2011; Grieneisen et al., 2012). However, the comprehensive physiological role of the metabolic network can only be fully understood from a whole-body perspective where individual cells, the surrounding tissue, and the whole organism interact continuously at a metabolic level (Krauss et al., 2012; Grafahrend-Belau et al., 2013).

Several approaches for combining metabolic models, covering different levels of biological organization in humans have been described (Krauss et al., 2012), while at the time of this review, the only multiscale metabolic model in plants was presented by Grafahrend-Belau et al. (2013). During this study, the multiorgan FBA model was combined with a dynamic whole-plant multiscale functional plant model. Dynamic FBA was performed by partitioning a selected plant growth phase into several time intervals and by computing a static FBA at the beginning of each time interval. To include dynamic processes, exchange fluxes that had been predicted by the functional plant model and are also time dependent were used to constrain the static FBA within each time interval.

Establishing a multiscale plant model requires simultaneous modeling of many different cell types in several connected tissues/organs. Considering the large scale of a multiorgan or whole-organism model, stoichiometric modeling, in particular FBA, is the most suitable approach. With the aim of achieving a multiscale metabolic model, first the validated subsystem specific models should be constructed separately, and then the separate parts can be coupled together to construct the multiscale model. This presents several technical and mathematical challenges. The first is that we need to formulate new constraints and/or objective functions, both at the level of subsystems and at the level of the integrated model to describe the behavior of the plant as realistically as possible. The second is the lack of tissue-specific information on metabolite uptake and secretion, which is required for FBA (Shlomi et al., 2008). It is obvious that coupling submodels together redraws the system boundaries. The question that arises during coupling subsystem models is to what extent the interdependencies of fluxes in subsystems will vary with those in the coupled metabolic network. Special mathematical analysis, such as flux-coupling analysis, has been developed to deal with this question (Marashi and Bockmayr, 2011; Marashi et al., 2012).

Plant metabolic engineering will be able to address human needs only when it begins to make meaningful changes on an industrial scale. To achieve this, multiscale modeling is a prerequisite for obtaining an improved understanding of metabolism at a systems level. However, before that, plant metabolic modeling needs to be supported by more advanced bioinformatics platforms and computational toolboxes. There is also a need to gain an improved understanding of the regulatory circuits governing cellular metabolism. Moreover, improved cellular resolution and enhanced sensitivity of metabolomics are also required.

AUTHOR CONTRIBUTIONS

All authors contributed to writing the article.

ACKNOWLEDGMENTS

We thank R. George Ratcliffe for his invaluable intellectual input and feedback throughout the development of this article.

Received July 25, 2014; revised September 22, 2014; accepted October 2, 2014; published October 24, 2014.

REFERENCES

Aharoni, A., and Galili, G. (2011). Metabolic engineering of the plant primary-secondary metabolism interface. Curr. Opin. Biotechnol. **22:** 239–244.

Allen, D.K., Libourel, I.G.L., and Shachar-Hill, Y. (2009). Metabolic flux analysis in plants: coping with complexity. Plant Cell Environ. **32:** 1241–1257.

Alonso, A.P., Dale, V.L., and Shachar-Hill, Y. (2010). Understanding fatty acid synthesis in developing maize embryos using metabolic flux analysis. Metab. Eng. **12:** 488–497.

Alonso, A.P., Val, D.L., and Shachar-Hill, Y. (2011). Central metabolic fluxes in the endosperm of developing maize seeds and their implications for metabolic engineering. Metab. Eng. **13:** 96–107.

Alonso, A.P., Goffman, F.D., Ohlrogge, J.B., and Shachar-Hill, Y. (2007). Carbon conversion efficiency and central metabolic fluxes in developing sunflower (*Helianthus annuus* L.) embryos. Plant J. **52:** 296–308.

Alves, R., Antunes, F., and Salvador, A. (2006). Tools for kinetic modeling of biochemical networks. Nat. Biotechnol. **24:** 667–672.

Arnold, A., and Nikoloski, Z. (2011). A quantitative comparison of Calvin-Benson cycle models. Trends Plant Sci. **16:** 676–683.

Arnold, A., and Nikoloski, Z. (2013). Comprehensive classification and perspective for modelling photorespiratory metabolism. Plant Biol (Stuttg) **15:** 667–675.

Arnold, A., and Nikoloski, Z. (2014). In search for an accurate model of the photosynthetic carbon metabolism. Math. Comput. Simul. **96:** 171–194.

Baldazzi, V., Bertin, N., de Jong, H., and Génard, M. (2012). Towards multiscale plant models: integrating cellular networks. Trends Plant Sci. **17:** 728–736.

Bar-Even, A., Noor, E., Lewis, N.E., and Milo, R. (2010). Design and analysis of synthetic carbon fixation pathways. Proc. Natl. Acad. Sci. USA **107:** 8889–8894.

Becker, J., Klopprogge, C., Schroder, H., and Wittmann, C. (2009). Metabolic engineering of the tricarboxylic acid cycle for improved lysine production by *Corynebacterium glutamicum*. Appl. Environ. Microbiol. **75:** 7866–7869.

Becker, S.A., Feist, A.M., Mo, M.L., Hannum, G., Palsson, B.O., and Herrgard, M.J. (2007). Quantitative prediction of cellular metabolism with constraint-based models: the COBRA toolbox. Nat. Protoc. **2:** 727–738.

Beurton-Aimar, M., Beauvoit, B., Monier, A., Vallée, F., Dieuaide-Noubhani, M., and Colombié, S. (2011). Comparison between elementary flux modes analysis and 13C-metabolic fluxes measured in bacterial and plant cells. BMC Syst. Biol. **5:** 95.

Borisjuk, L., et al. (2013). Seed architecture shapes embryo metabolism in oilseed rape. Plant Cell **25:** 1625–1640.

Borland, A.M., Hartwell, J., Weston, D.J., Schlauch, K.A., Tschaplinski, T.J., Tuskan, G.A., Yang, X., and Cushman, J.C. (2014). Engineering crassulacean acid metabolism to improve water-use efficiency. Trends Plant Sci. **19:** 327–338.

Boyle, N.R., and Morgan, J.A. (2011). Computation of metabolic fluxes and efficiencies for biological carbon dioxide fixation. Metab. Eng. **13:** 150–158.

Bu'Lock, J.D. (1965). The Biosynthesis of Natural Products: An Introduction to Secondary Metabolism. (London: McGraw-Hill).

Carrari, F., Urbanczyk-Wochniak, E., Willmitzer, L., and Fernie, A. R. (2003). Engineering central metabolism in crop species: learning the system. Metab. Eng. **5:** 191–200.

Cascante, M., Boros, L.G., Comin-Anduix, B., de Atauri, P., Centelles, J.J., and Lee, P.W.N. (2002). Metabolic control analysis in drug discovery and disease. Nat. Biotechnol. **20:** 243–249.

Chandran, A.K.N., and Jung, K.H. (2014). Resources for systems biology in rice. J. Plant Biol. **57:** 80–92.

Chen, X., and Shachar-Hill, Y. (2012). Insights into metabolic efficiency from flux analysis. J. Exp. Bot. **63:** 2343–2351.

Cheung, C.Y., Poolman, M.G., Fell, D.A., Ratcliffe, R.G., and Sweetlove, L.J. (2014). A diel flux balance model captures interactions between light and dark metabolism during day-night cycles in C3 and Crassulacean acid metabolism leaves. Plant Physiol. **165:** 917–929.

Cheung, C.Y.M., Williams, T.C.R., Poolman, M.G., Fell, D.A., Ratcliffe, R.G., and Sweetlove, L.J. (2013). A method for accounting for maintenance costs in flux balance analysis improves the prediction of plant cell metabolic phenotypes under stress conditions. Plant J. **75:** 1050–1061.

Collakova, E., Yen, J.Y., and Senger, R.S. (2012). Are we ready for genome-scale modeling in plants? Plant Sci. **191-192:** 53–70.

Colón, A.M., Sengupta, N., Rhodes, D., Dudareva, N., and Morgan, J. (2010). A kinetic model describes metabolic response to perturbations and distribution of flux control in the benzenoid network of *Petunia hybrida*. Plant J. **62:** 64–76.

Copeland, W.B., Bartley, B.A., Chandran, D., Galdzicki, M., Kim, K.H., Sleight, S.C., Maranas, C.D., and Sauro, H.M. (2012). Computational tools for metabolic engineering. Metab. Eng. **14:** 270–280.

Cramer, G.R., Urano, K., Delrot, S., Pezzotti, M., and Shinozaki, K. (2011). Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol. **11:** 163–176.

Curien, G., Bastien, O., Robert-Genthon, M., Cornish-Bowden, A., Cárdenas, M.L., and Dumas, R. (2009). Understanding the regulation of aspartate metabolism using a model based on measured kinetic parameters. Mol. Syst. Biol. **5:** 271–284.

Dandekar, T., Moldenhauer, F., Bulik, S., Bertram, H., and Schuster, S. (2003). A method for classifying metabolites in topological pathway analyses based on minimization of pathway number. Biosystems **70:** 255–270.

de Oliveira Dal'Molin, C.G., and Nielsen, L.K. (2013). Plant genome-scale metabolic reconstruction and modelling. Curr. Opin. Biotechnol. **24:** 271–277.

de Oliveira Dal'Molin, C.G., Quek, L.E., Palfreyman, R.W., Brumbley, S.M., and Nielsen, L.K. (2010a). AraGEM, a genome-scale reconstruction of the primary metabolic network in Arabidopsis. Plant Physiol. **152:** 579–589.

de Oliveira Dal'Molin, C.G., Quek, L.E., Palfreyman, R.W., Brumbley, S.M., and Nielsen, L.K. (2010b). C4GEM, a genome-scale metabolic model to study C4 plant metabolism. Plant Physiol. **154:** 1871–1885.

Elena, S.F., Carrera, J., and Rodrigo, G. (2011). A systems biology approach to the evolution of plant-virus interactions. Curr. Opin. Plant Biol. **14:** 372–377.

Farquhar, G.D., von Caemmerer, S., and Berry, J.A. (1980). A biochemical model of photosynthetic CO2 assimilation in leaves of C 3 species. Planta **149:** 78–90.

Fell, D. (1997). Understanding the Control of Metabolism. (London: Portland Press).

Fernie, A.R., and Morgan, J.A. (2013). Analysis of metabolic flux using dynamic labelling and metabolic modelling. Plant Cell Environ. **36:** 1738–1750.

Fernie, A.R., Aharoni, A., Willmitzer, L., Stitt, M., Tohge, T., Kopka, J., Carroll, A.J., Saito, K., Fraser, P.D., and DeLuca, V. (2011). Recommendations for reporting metabolite data. Plant Cell **23:** 2477–2482.

Fernie, A.R., et al. (2013). Perspectives on plant photorespiratory metabolism. Plant Biol (Stuttg) **15:** 748–753.

Fiehn, O., Barupal, D.K., and Kind, T. (2011). Extending biochemical databases by metabolomic surveys. J. Biol. Chem. **286:** 23637–23643.

Flint, H.J., Tateson, R.W., Barthelmess, I.B., Porteous, D.J., Donachie, W.D., and Kacser, H. (1981). Control of the flux in the arginine pathway of *Neurospora crassa*. Modulations of enzyme activity and concentration. Biochem. J. **200:** 231–246.

Ghosh, S., Matsuoka, Y., Asai, Y., Hsin, K.Y., and Kitano, H. (2011). Software for systems biology: from tools to integrated platforms. Nat. Rev. Genet. **12:** 821–832.

Go, E.P. (2010). Database resources in metabolomics: an overview. J. Neuroimmune Pharmacol. **5:** 18–30.

Gómez-Galera, S., Pelacho, A.M., Gené, A., Capell, T., and Christou, P. (2007). The genetic manipulation of medicinal and aromatic plants. Plant Cell Rep. **26:** 1689–1715.

Grafahrend-Belau, E., Schreiber, F., Koschützki, D., and Junker, B.H. (2009a). Flux balance analysis of barley seeds: a computational approach to study systemic properties of central metabolism. Plant Physiol. **149:** 585–598.

Grafahrend-Belau, E., Klukas, C., Junker, B.H., and Schreiber, F. (2009b). FBA-SimVis: interactive visualization of constraint-based metabolic models. Bioinformatics **25:** 2755–2757.

Grafahrend-Belau, E., Junker, A., Eschenröder, A., Müller, J., Schreiber, F., and Junker, B.H. (2013). Multiscale metabolic modeling: dynamic flux balance analysis on a whole-plant scale. Plant Physiol. **163:** 637–647.

Grennan, A.K. (2009). MoTo DB: a metabolic database for tomato. Plant Physiol. **151:** 1701–1702.

Grieneisen, V.A., Scheres, B., Hogeweg, P., and M Marée, A.F. (2012). Morphogengineering roots: comparing mechanisms of morphogen gradient formation. BMC Syst. Biol. **6:** 37.

Gutiérrez, R.A. (2012). Systems biology for enhanced plant nitrogen nutrition. Science **336:** 1673–1675.

Gutiérrez, R.A., Shasha, D.E., and Coruzzi, G.M. (2005). Systems biology for the virtual plant. Plant Physiol. **138:** 550–554.

Hay, J., and Schwender, J. (2011a). Computational analysis of storage synthesis in developing *Brassica napus* L. (oilseed rape) embryos: flux variability analysis in relation to ^{13}C metabolic flux analysis. Plant J. **67:** 513–525.

Hay, J., and Schwender, J. (2011b). Metabolic network reconstruction and flux variability analysis of storage synthesis in developing oilseed rape (*Brassica napus* L.) embryos. Plant J. **67:** 526–541.

Heinig, U., Gutensohn, M., Dudareva, N., and Aharoni, A. (2013). The challenges of cellular compartmentalization in plant metabolic engineering. Curr. Opin. Biotechnol. **24:** 239–246.

Heinrich, R., and Rapoport, T.A. (1974). A linear steady-state treatment of enzymatic chains. General properties, control and effector strength. Eur. J. Biochem. **42:** 89–95.

Heinrich, R., Rapoport, S.M., and Rapoport, T.A. (1977). Metabolic regulation and mathematical models. Prog. Biophys. Mol. Biol. **32:** 1–82.

Hoops, S., Sahle, S., Gauges, R., Lee, C., Pahle, J., Simus, N., Singhal, M., Xu, L., Mendes, P., and Kummer, U. (2006). COPASI—a COmplex PAthway SImulator. Bioinformatics **22:** 3067–3074.

Hucka, M., et al, SBML Forum (2003). The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics **19:** 524–531.

Jarboe, L.R., Zhang, X., Wang, X., Moore, J.C., Shanmugam, K.T., and Ingram, L.O. (2010). Metabolic engineering for production of biorenewable fuels and chemicals: contributions of synthetic biology. J. Biomed. Biotechnol. **2010:** 761042.

Kacser, H., and Burns, J.A. (1973). The control of flux. Symp. Soc. Exp. Biol. **27:** 65–104.

Keurentjes, J.J.B., Angenent, G.C., Dicke, M., Dos Santos, V.A., Molenaar, J., van der Putten, W.H., de Ruiter, P.C., Struik, P.C., and Thomma, B.P. (2011). Redefining plant systems biology: from cell to ecosystem. Trends Plant Sci. **16:** 183–190.

Kjeldsen, K.R., and Nielsen, J. (2009). In silico genome-scale reconstruction and validation of the *Corynebacterium glutamicum* metabolic network. Biotechnol. Bioeng. **102:** 583–597.

Klamt, S., and Stelling, J. (2002). Combinatorial complexity of pathway analysis in metabolic networks. Mol. Biol. Rep. **29:** 233–236.

Klamt, S., Saez-Rodriguez, J., and Gilles, E.D. (2007). Structural and functional analysis of cellular networks with CellNetAnalyzer. BMC Syst. Biol. **1:** 2.

Klipp, E., and Schaber, J. (2006). Modelling of signal transduction in yeast. In Understanding and Exploiting Systems Biology in Biomedicine and Bioprocesses, M. Canovas, J. Iborra, and A. Manjon, eds (Murica, Spain: Fundacion CajaMurica), pp. 15–30.

Kopka, J., et al. (2005). GMD@CSB.DB: the Golm Metabolome Database. Bioinformatics **21:** 1635–1638.

Krauss, M., Schaller, S., Borchers, S., Findeisen, R., Lippert, J., and Kuepfer, L. (2012). Integrating cellular metabolism into a multiscale whole-body model. PLOS Comput. Biol. **8:** e1002750.

Kruger, N.J., and Ratcliffe, R.G. (2012). Pathways and fluxes: exploring the plant metabolic network. J. Exp. Bot. **63:** 2243–2246.

Kruger, N.J., Le Lay, P., and Ratcliffe, R.G. (2007). Vacuolar compartmentation complicates the steady-state analysis of glucose metabolism and forces reappraisal of sucrose cycling in plants. Phytochemistry **68:** 2189–2196.

Kruger, N.J., Masakapalli, S.K., and Ratcliffe, R.G. (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.

Kurata, H., Zhao, Q., Okuda, R., and Shimizu, K. (2007). Integration of enzyme activities into metabolic flux distributions by elementary mode analysis. BMC Syst. Biol. **1:** 31–44.

Lakshmanan, M., Zhang, Z., Mohanty, B., Kwon, J.Y., Choi, H.Y., Nam, H.J., Kim, D.I., and Lee, D.Y. (2013). Elucidating rice cell metabolism under flooding and drought stresses using flux-based modeling and analysis. Plant Physiol. **162:** 2140–2150.

Le Novère, N., et al. (2005). Minimum information requested in the annotation of biochemical models (MIRIAM). Nat. Biotechnol. **23:** 1509–1515.

Le Novère, N., et al. (2009). The systems biology graphical notation. Nat. Biotechnol. **27:** 735–741.

Lee, S.Y., Park, J.M., and Kim, T.Y. (2011). Application of metabolic flux anaysis in metabolic engineering. In Synthetic Biology, Pt B: Computer Aided Design and DNA Assembly, C. Voigt, ed (San Diego, CA: Elsevier; Academic Press), pp. 67–93.

Li, C., et al. (2010). BioModels Database: An enhanced, curated and annotated resource for published quantitative kinetic models. BMC Syst. Biol. **4:** 92.

Libourel, I.G.L., and Shachar-Hill, Y. (2008). Metabolic flux analysis in plants: from intelligent design to rational engineering. Annu. Rev. Plant Biol. **59:** 625–650.

Llaneras, F., and Picó, J. (2008). Stoichiometric modelling of cell metabolism. J. Biosci. Bioeng. **105:** 1–11.

Luckner, M. (1972). Secondary Metabolism in Plants and Animals. (London: Chapman and Hall).

Marashi, S.-A., and Bockmayr, A. (2011). Flux coupling analysis of metabolic networks is sensitive to missing reactions. Biosystems **103:** 57–66.

Marashi, S.-A., David, L., and Bockmayr, A. (2012). On flux coupling analysis of metabolic subsystems. J. Theor. Biol. **302:** 62–69.

Masakapalli, S.K., Kruger, N.J., and Ratcliffe, R.G. (2013). The metabolic flux phenotype of heterotrophic Arabidopsis cells reveals a complex response to changes in nitrogen supply. Plant J. **74:** 569–582.

Mendes, P. (2002). Emerging bioinformatics for the metabolome. Brief. Bioinform. **3:** 134–145.

Mintz-Oron, S., Meir, S., Malitsky, S., Ruppin, E., Aharoni, A., and Shlomi, T. (2012). Reconstruction of Arabidopsis metabolic network models accounting for subcellular compartmentalization and tissue-specificity. Proc. Natl. Acad. Sci. USA **109:** 339–344.

Morandini, P. (2009). Rethinking metabolic control. Plant Sci. **176:** 441–451.

Morandini, P. (2013). Control limits for accumulation of plant metabolites: brute force is no substitute for understanding. Plant Biotechnol. J. **11:** 253–267.

Morgan, J.A., and Rhodes, D. (2002). Mathematical modeling of plant metabolic pathways. Metab. Eng. **4:** 80–89.

Morgan, J.A., and Shanks, J.V. (2002). Quantification of metabolic flux in plant secondary metabolism by a biogenetic organizational approach. Metab. Eng. **4:** 257–262.

Morgan, M.J., Osorio, S., Gehl, B., Baxter, C.J., Kruger, N.J., Ratcliffe, R.G., Fernie, A.R., and Sweetlove, L.J. (2013). Metabolic engineering of tomato fruit organic acid content guided by biochemical analysis of an introgression line. Plant Physiol. **161:** 397–407.

Mueller, L.A., Zhang, P., and Rhee, S.Y. (2003). AraCyc: a biochemical pathway database for Arabidopsis. Plant Physiol. **132:** 453–460.

Murphy, T.A., Dang, C.V., and Young, J.D. (2013). Isotopically nonstationary 13C flux analysis of Myc-induced metabolic reprogramming in B-cells. Metab. Eng. **15:** 206–217.

Noble, D. (2011). Successes and failures in modeling heart cell electrophysiology. Heart Rhythm **8:** 1798–1803.

Papin, J.A., Stelling, J., Price, N.D., Klamt, S., Schuster, S., and Palsson, B.O. (2004). Comparison of network-based pathway analysis methods. Trends Biotechnol. **22:** 400–405.

Papp, B., Notebaart, R.A., and Pál, C. (2011). Systems-biology approaches for predicting genomic evolution. Nat. Rev. Genet. **12:** 591–602.

Peterhansel, C., Niessen, M., and Kebeish, R.M. (2008). Metabolic engineering towards the enhancement of photosynthesis. Photochem. Photobiol. **84:** 1317–1323.

Pilalis, E., Chatziioannou, A., Thomasset, B., and Kolisis, F. (2011). An in silico compartmentalized metabolic model of Brassica napus enables the systemic study of regulatory aspects of plant central metabolism. Biotechnol. Bioeng. **108:** 1673–1682.

Pitkänen, E., Rousu, J., and Ukkonen, E. (2010). Computational methods for metabolic reconstruction. Curr. Opin. Biotechnol. **21:** 70–77.

Poolman, M.G. (2006). ScrumPy: metabolic modelling with Python. Syst. Biol. (Stevenage) **153:** 375–378.

Poolman, M.G., Fell, D.A., and Thomas, S. (2000). Modelling photosynthesis and its control. J. Exp. Bot. **51:** 319–328.

Poolman, M.G., Miguet, L., Sweetlove, L.J., and Fell, D.A. (2009). A genome-scale metabolic model of Arabidopsis and some of its properties. Plant Physiol. **151:** 1570–1581.

Poolman, M.G., Kundu, S., Shaw, R., and Fell, D.A. (2013). Responses to light intensity in a genome-scale model of rice metabolism. Plant Physiol. **162:** 1060–1072.

Pritchard, L., and Birch, P. (2011). A systems biology perspective on plant-microbe interactions: biochemical and structural targets of pathogen effectors. Plant Sci. **180:** 584–603.

Raikhel, N.V., and Coruzzi, G.M. (2003). Achieving the in silico plant. Systems biology and the future of plant biological research. Plant Physiol. **132:** 404–409.

Raines, C.A. (2011). Increasing photosynthetic carbon assimilation in C3 plants to improve crop yield: current and future strategies. Plant Physiol. **155:** 36–42.

Richter, G. (1978). Plant Metabolism: Physiology and Biochemistry of Primary Metabolism. (Stuttgart, Germany: Georg Thieme Publishers).

Rios-Estepa, R., Turner, G.W., Lee, J.M., Croteau, R.B., and Lange, B.M. (2008). A systems biology approach identifies the biochemical mechanisms regulating monoterpenoid essential oil composition in peppermint. Proc. Natl. Acad. Sci. USA **105:** 2818–2823.

Rocha, M., Licausi, F., Araújo, W.L., Nunes-Nesi, A., Sodek, L., Fernie, A.R., and van Dongen, J.T. (2010). Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of *Lotus japonicus*. Plant Physiol. **152:** 1501–1513.

Rohn, H., Hartmann, A., Junker, A., Junker, B.H., and Schreiber, F. (2012). FluxMap: a VANTED add-on for the visual exploration of flux distributions in biological networks. BMC Syst. Biol. **6:** 33.

Rohwer, J.M. (2012). Kinetic modelling of plant metabolic pathways. J. Exp. Bot. **63:** 2275–2292.

Rohwer, J.M., and Botha, F.C. (2001). Analysis of sucrose accumulation in the sugar cane culm on the basis of in vitro kinetic data. Biochem. J. **358:** 437–445.

Roscher, A., Kruger, N.J., and Ratcliffe, R.G. (2000). Strategies for metabolic flux analysis in plants using isotope labelling. J. Biotechnol. **77:** 81–102.

Saha, R., Suthers, P.F., and Maranas, C.D. (2011). Zea mays iRS1563: a comprehensive genome-scale metabolic reconstruction of maize metabolism. PLoS ONE **6:** e21784.

Schäfer, W.E., Rohwer, J.M., and Botha, F.C. (2004). A kinetic study of sugarcane sucrose synthase. Eur. J. Biochem. **271:** 3971–3977.

Schallau, K., and Junker, B.H. (2010). Simulating plant metabolic pathways with enzyme-kinetic models. Plant Physiol. **152:** 1763–1771.

Schreiber, F., Colmsee, C., Czauderna, T., Grafahrend-Belau, E., Hartmann, A., Junker, A., Junker, B.H., Klapperstück, M., Scholz,

U., and Weise, S. (2012). MetaCrop 2.0: managing and exploring information about crop plant metabolism. Nucleic Acids Res. **40:** D1173–D1177.

Schuster, S., Dandekar, T., and Fell, D.A. (1999). Detection of elementary flux modes in biochemical networks: a promising tool for pathway analysis and metabolic engineering. Trends Biotechnol. **17:** 53–60.

Schuster, S., Fell, D.A., and Dandekar, T. (2000). A general definition of metabolic pathways useful for systematic organization and analysis of complex metabolic networks. Nat. Biotechnol. **18:** 326–332.

Schwender, J. (2008). Metabolic flux analysis as a tool in metabolic engineering of plants. Curr. Opin. Biotechnol. **19:** 131–137.

Schwender, J., Goffman, F., Ohlrogge, J.B., and Shachar-Hill, Y. (2004). Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. Nature **432:** 779–782.

Seaver, S.M., Henry, C.S., and Hanson, A.D. (2012). Frontiers in metabolic reconstruction and modeling of plant genomes. J. Exp. Bot. **63:** 2247–2258.

Shachar-Hill, Y. (2013). Metabolic network flux analysis for engineering plant systems. Curr. Opin. Biotechnol. **24:** 247–255.

Shlomi, T., Cabili, M.N., Herrgård, M.J., Palsson, B.Ø., and Ruppin, E. (2008). Network-based prediction of human tissue-specific metabolism. Nat. Biotechnol. **26:** 1003–1010.

Small, J.R., and Kacser, H. (1993). Responses of metabolic systems to large changes in enzyme activities and effectors. 1. The linear treatment of unbranched chains. Eur. J. Biochem. **213:** 613–624.

Smallbone, K., Simeonidis, E., Swainston, N., and Mendes, P. (2010). Towards a genome-scale kinetic model of cellular metabolism. BMC Syst. Biol. **4:** 6.

Steuer, R. (2007). Computational approaches to the topology, stability and dynamics of metabolic networks. Phytochemistry **68:** 2139–2151.

Steuer, R., Gross, T., Selbig, J., and Blasius, B. (2006). Structural kinetic modeling of metabolic networks. Proc. Natl. Acad. Sci. USA **103:** 11868–11873.

Steuer, R., Nesi, A.N., Fernie, A.R., Gross, T., Blasius, B., and Selbig, J. (2007). From structure to dynamics of metabolic pathways: application to the plant mitochondrial TCA cycle. Bioinformatics **23:** 1378–1385.

Stitt, M., Sulpice, R., and Keurentjes, J. (2010). Metabolic networks: how to identify key components in the regulation of metabolism and growth. Plant Physiol. **152:** 428–444.

Studart-Guimarães, C., Fait, A., Nunes-Nesi, A., Carrari, F., Usadel, B., and Fernie, A.R. (2007). Reduced expression of succinyl-coenzyme A ligase can be compensated for by up-regulation of the γ-aminobutyrate shunt in illuminated tomato leaves. Plant Physiol. **145:** 626–639.

Sulpice, R., Sienkiewicz-Porzucek, A., Osorio, S., Krahnert, I., Stitt, M., Fernie, A.R., and Nunes-Nesi, A. (2010). Mild reductions in cytosolic NADP-dependent isocitrate dehydrogenase activity result in lower amino acid contents and pigmentation without impacting growth. Amino Acids **39:** 1055–1066.

Sulpice, R., Nikoloski, Z., Tschoep, H., Antonio, C., Kleessen, S., Larhlimi, A., Selbig, J., Ishihara, H., Gibon, Y., Fernie, A.R., and Stitt, M. (2013). Impact of the carbon and nitrogen supply on relationships and connectivity between metabolism and biomass in a broad panel of Arabidopsis accessions. Plant Physiol. **162:** 347–363.

Sweetlove, L.J., and Ratcliffe, R.G. (2011). Flux-balance modeling of plant metabolism. Front. Plant Sci. **2:** 38.

Sweetlove, L.J., and Fernie, A.R. (2013). The spatial organization of metabolism within the plant cell. Annu. Rev. Plant Biol. **64:** 723–746.

Sweetlove, L.J., Last, R.L., and Fernie, A.R. (2003). Predictive metabolic engineering: a goal for systems biology. Plant Physiol. **132:** 420–425.

Sweetlove, L.J., Fell, D., and Fernie, A.R. (2008). Getting to grips with the plant metabolic network. Biochem. J. **409:** 27–41.

Sweetlove, L.J., Obata, T., and Fernie, A.R. (2014). Systems analysis of metabolic phenotypes: what have we learnt? Trends Plant Sci. **19:** 222–230.

Sweetlove, L.J., Williams, T.C., Cheung, C.Y., and Ratcliffe, R.G. (2013). Modelling metabolic CO_2 evolution—a fresh perspective on respiration. Plant Cell Environ. **36:** 1631–1640.

Sweetlove, L.J., Beard, K.F.M., Nunes-Nesi, A., Fernie, A.R., and Ratcliffe, R.G. (2010). Not just a circle: flux modes in the plant TCA cycle. Trends Plant Sci. **15:** 462–470.

Szecowka, M., Heise, R., Tohge, T., Nunes-Nesi, A., Vosloh, D., Huege, J., Feil, R., Lunn, J., Nikoloski, Z., Stitt, M., Fernie, A.R., and Arrivault, S. (2013). Metabolic fluxes in an illuminated Arabidopsis rosette. Plant Cell **25:** 694–714.

Tomar, N., and De, R.K. (2013). Comparing methods for metabolic network analysis and an application to metabolic engineering. Gene **521:** 1–14.

Töpfer, N., Jozefczuk, S., and Nikoloski, Z. (2012). Integration of time-resolved transcriptomics data with flux-based methods reveals stress-induced metabolic adaptation in *Escherichia coli*. BMC Syst. Biol. **6:** 148.

Töpfer, N., Scossa, F., Fernie, A., and Nikoloski, Z. (2014). Variability of metabolite levels is linked to differential metabolic pathways in Arabidopsis's responses to abiotic stresses. PLoS Comp. Biol. **10:** e1003656.

Töpfer, N., Caldana, C., Grimbs, S., Willmitzer, L., Fernie, A.R., and Nikoloski, Z. (2013). Integration of genome-scale modeling and transcript profiling reveals metabolic pathways underlying light and temperature acclimation in Arabidopsis. Plant Cell **25:** 1197–1211.

Toubiana, D., Fernie, A.R., Nikoloski, Z., and Fait, A. (2013). Network analysis: tackling complex data to study plant metabolism. Trends Biotechnol. **31:** 29–36.

Toya, Y., and Shimizu, H. (2013). Flux analysis and metabolomics for systematic metabolic engineering of microorganisms. Biotechnol. Adv. **31:** 818–826.

Vernoux, T., et al. (2011). The auxin signalling network translates dynamic input into robust patterning at the shoot apex. Mol. Syst. Biol. **7:** 508.

Walpole, J., Papin, J.A., and Peirce, S.M. (2013). Multiscale computational models of complex biological systems. Ann. Rev. Biomed. Eng. **15:** 137–154.

Wang, C., Guo, L., Li, Y., and Wang, Z. (2012). Systematic comparison of C3 and C4 plants based on metabolic network analysis. BMC Syst. Biol. **6** (Suppl 2)**:** S9.

Wang, J.P., et al. (2014). Complete proteomic-based enzyme reaction and inhibition kinetics reveal how monolignol biosynthetic enzyme families affect metabolic flux and lignin in *Populus trichocarpa*. Plant Cell **26:** 894–914.

Weissmann, S., and Brutnell, T.P. (2012). Engineering C4 photosynthetic regulatory networks. Curr. Opin. Biotechnol. **23:** 298–304.

Wiechert, W. (2002). Modeling and simulation: tools for metabolic engineering. J. Biotechnol. **94:** 37–63.

Williams, T.C., Poolman, M.G., Howden, A.J., Schwarzlander, M., Fell, D.A., Ratcliffe, R.G., and Sweetlove, L.J. (2010). A genome-scale metabolic model accurately predicts fluxes in central carbon metabolism under stress conditions. Plant Physiol. **154:** 311–323.

Xu, C., Liu, L., Zhang, Z., Jin, D., Qiu, J., and Chen, M. (2013a). Genome-scale metabolic model in guiding metabolic engineering of microbial improvement. Appl. Microbiol. Biotechnol. **97:** 519–539.

Xu, P., Bhan, N., and Koffas, M.A.G. (2013b). Engineering plant metabolism into microbes: from systems biology to synthetic biology. Curr. Opin. Biotechnol. **24:** 291–299.

Yonekura-Sakakibara, K., Fukushima, A., and Saito, K. (2013). Transcriptome data modeling for targeted plant metabolic engineering. Curr. Opin. Biotechnol. **24:** 285–290.

Young, J.D., Shastri, A.A., Stephanopoulos, G., and Morgan, J.A. (2011). Mapping photoautotrophic metabolism with isotopically nonstationary (13)C flux analysis. Metab. Eng. **13:** 656–665.

Yuan, J.S., Galbraith, D.W., Dai, S.Y., Griffin, P., and Stewart, C.N., Jr. (2008). Plant systems biology comes of age. Trends Plant Sci. **13:** 165–171.

Zhang, P., et al. (2010). Creation of a genome-wide metabolic pathway database for Populus trichocarpa using a new approach for reconstruction and curation of metabolic pathways for plants. Plant Physiol. **153:** 1479–1491.

Zhu, X.G., de Sturler, E., and Long, S.P. (2007). Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. Plant Physiol. **145:** 513–526.

Zhu, X.G., Wang, Y., Ort, D.R., and Long, S.P. (2013). e-Photosynthesis: a comprehensive dynamic mechanistic model of C3 photosynthesis: from light capture to sucrose synthesis. Plant Cell Environ. **36:** 1711–1727.