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Review

Green pathways: Metabolic network analysis of plant systems

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ABSTRACT

Metabolic engineering of plants with enhanced crop yield and value-added compositional traits is particularly challenging as they probably exhibit the highest metabolic network complexity of all living organisms. Therefore, approaches of plant metabolic network analysis, which can provide systems-level understanding of plant physiology, appear valuable as guidance for plant metabolic engineers. Strongly supported by the sequencing of plant genomes, a number of different experimental and computational methods have emerged in recent years to study plant systems at various levels: from heterotrophic cell cultures to autotrophic entire plants. The present review presents a state-of-the-art toolbox for plant metabolic network analysis. Among the described approaches are different *in silico* modeling techniques, including flux balance analysis, elementary flux mode analysis and kinetic flux profiling, as well as different variants of experiments with plant systems which use radioactive and stable isotopes to determine *in vivo* plant metabolic fluxes. The fundamental principles of these techniques, the required data input and the obtained flux information are enriched by technical advices, specific to plants. In addition, pioneering and high-impacting findings of plant metabolic network analysis highlight the potential of the field.

Contents

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1. Introduction

The increasing demand for food and feed, the shortage of arable land and extreme weather conditions call for improved plants with enhanced crop yield and better tolerance against drought, disease and predation (Long et al., 2015). In addition, plants are promising for the sustainable production of therapeutics, pigments, chemicals and biofuels (Yan and Kerr, 2002). Plant species often do not exhibit the desired phenotypes, which has initiated global efforts to metabolically engineer them towards improved performance. Meanwhile, zinc-finger nucleases (ZNFs) and transcription activator-like effector nucleases (TALENs), as well as clustered regularly interspaced short palindromic repeats (CRISPR) enable precise and site-specific genome editing of plants (Yuan and Grotewold, 2015). Targeted metabolic engineering of plants, however, is hampered by the fact that we still lack substantial knowledge about the metabolic functions and regulations that mediate desired plant performance. That such knowledge is ultimately important to guide metabolic engineers is illustrated by the achieved successes in breeding superior microorganisms on a global scale through systems metabolic engineering (Ajikumar et al., 2010; Becker et al., 2011; Hwang et al., 2014; Kim et al., 2014; Kind et al., 2014; Paddon et al., 2013; Poblete-Castro et al., 2013). These systems-level approaches are what plant metabolic engineers aim for (Shachar-Hill, 2013). Without doubt, the excellence in engineering microbial cell factories largely builds on solid understanding of the underlying metabolic and regulatory networks (Becker et al., 2005). Particularly, systems metabolic engineering has benefitted from understanding of the underlying metabolic networks in providing targets for genetic improvement (Kelleher, 2001; Stephanopoulos, 1999). For industrial microorganisms, a rich set of *in vivo* metabolic flux data (Becker et al., 2011; Kiefer et al., 2004; Kind et al., 2014; Wittmann et al., 2007) as well as *in silico* predictions on metabolic network properties (Becker et al., 2011; Hwang et al., 2014; Kim et al., 2014; Poblete-Castro et al., 2013; Trinh, 2012) have been derived and contributed to a great extent to microbial systems metabolic engineering. In this regard, metabolic network analysis of plant systems promises a huge next step towards understanding of their metabolic functions and superimposed regulation mechanisms towards superior plants.

Admittedly, plant metabolic networks are extremely complex, which makes network analysis a far greater challenge as compared to microorganisms. Higher plants are compartmentalized at the organ, tissue and subcellular level, leading to interconnected pathways, nutrient transport and cell signaling (O'Grady et al., 2012). In addition, they exhibit prominent characteristics: being sessile, photoautotrophic and possessing a vast amount of secondary metabolites (Allen et al., 2009a). The resulting high degree of complexity and connectivity has to be contemplated for successful genetic engineering of plant systems, especially considering single gene alterations (Allen et al., 2009a), as many alternative metabolic routes are available to the plant. In recent years, the field of plant metabolic network analysis has strongly evolved. Particularly, the rapid increase in the sequencing of

Table 1
Sequenced plant genomes that led to the construction of genome-scale metabolic networks.

Plant	Genome size	Model size (reactions × metabolites)	Organ
Chlamydomonas reinhardtii (green algae)	111 Mb, 17,737 genes (Merchant et al., 2007)	1725 × 1862 (De Oliveira Dal'Molin et al., 2011)	Cell culture
		2190 × 1068 (Chang et al., 2011)	Cell culture
Arabidopsis thaliana (thale cress)	135 Mb, 27,000 genes (TAIR, 2015)	1406 × 1253 (Poolman et al., 2009)	Heterotrophic tissue culture
		1567 × 1748 (De Oliveira Dal'Molin et al., 2010a)	Individual models for leaf, stem and root
		1363 × 1078 (Mintz-Oron et al., 2012)	Individual models for of leaf, stem, root, flower, etc.
		2769 × **** (Cheung et al., 2013)	Heterotrophic tissue culture, fully compartmented
		345 × 236 (Arnold and Nikoloski, 2014)	Core model
		9727 × **** (De Oliveira Dal'Molin et al., 2015)	Multi-tissue model of leaf, stem and root
Brassica napus (rapeseed)	1130 Mb (Chalhoub et al., 2014)	671 × 666 (Hay et al., 2014)	Seed
Oryza sativa (rice)	430 Mb, 50,000 genes (Kawahara et al., 2013)	3316 × 2986 (Liu et al., 2013)	Leaf
		1736 × 1484 (Poolman et al., 2014, 2013)	Leaf
Hordeum vulgare (barley)	5100 Mb, 26,159 genes (The international barley genome sequencing consortium, 2012)	257 × 234 (Grafahrend-Belau et al., 2009b)	Seed
		702 (269) × 890 (Grafahrend-Belau et al., 2013)	Multi-organ model (transport)
Sorghum bicolor (sorghum)	730 Mb, 34,496 genes (Paterson et al., 2009)	1755 × 1588 (De Oliveira Dal'Molin et al., 2010b)	Leaf
Zea mays (maize)	2400 Mb, 39,656 genes (Gramene, 2015)	1755 × 1588 (De Oliveira Dal'Molin et al., 2010b)	Leaf
		1985 × 1825 (Saha et al., 2011)	Leaf
		8525 × 9153 (Simons et al., 2014)	Leaf
		2322 × 2635 (Seaver et al., 2015)	Leaf
		2304 × 2636 (Seaver et al., 2015)	Embryo
		2280 × 2636 (Seaver et al., 2015)	Endosperm
		18000 × **** (Bogart and Myers, 2015)	Leaf (mesophyll and bundle sheath cells)

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