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The Plant Subcellular Metabolome: Current State and Advances

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Abstract

Dissection of organismal metabolomes into smaller subunits of life holds the potential to unravel the details of operative metabolic pathways and metabolic compartmentation at the sub-cellular level. Although metabolomes have been characterized at tissue, cellular, and cell-population types, little efforts have been put towards separation of sub-cellular metabolomes. Obvious challenges in lack of pure preparations of organelles, shared metabolites among them, and complicated metabolic regulations in them has impeded our advances in this domain of metabolomics. However, in the post-genomic era, significant advances have been made in predicting plant protein and transcriptomic localization to subcellular organelles through computational approaches. We summarize the recent efforts and progresses made in directions of understanding the plant sub-cellular (organellar) metabolomes.

Keywords: metabolome, mitochondria, organelle, plastid, proteomics, vacuole

The metabolic compartmentation adds a complex dimension to subcellular metabolomes

Systems biology approaches, including bioinformatics, genomics, transcriptomics, proteomics, and metabolomics have begun to contribute to our growing knowledge of cellular signaling and metabolism. However, the extensive and unique metabolic compartmentation is characteristic of eukaryotic cells, such as plant cells, thus rendering the analysis of compartmentalized metabolic networks very challenging mostly due to the separation and parallelization of pathways and the involvement of intracellular transport (Wahrheit et al., 2011). Consequently, the study of plant cellular metabolomic networks is even more challenging (Toubian et al., 2013). Although the single cell and single-cell type metabolomics studies (Misra et al., 2014) bring in homogeneity in preparations to reflect on cellular (micro-metabolome) as the basic unit of life, the subcellular (nano-metabolome) pose a great deal of challenges for their investigation. Major plant subcellular structures include (but are not limited) to apoplast, cell plate, cell wall, endoplasmic reticulum, endosome, Golgi apparatus, microfilament, microtubule, mitochondrion, oil bodies, nucleus, peroxisome, plasma membrane, plastid and related structures, and vacuole. Plants are complex systems, where the photosynthetic energy in the forms of carbon is partitioned differentially into organellar compartments in a species dependent manner. Metabolic pathways are clearly segregated in different subcellular organelles (Browsher and Tobin, 2011). Undoubtedly, the compartmentalization of plant metabolites, add another complex dimension to principal regulatory aspects in plants, apart from the temporal dimensions. In addition, the diffusion of metabolites, the role of active transport by membrane-based transporters, and limitations in labeling and visualization of metabolites in cells render the localization even more difficult. As such, pathways of communication between various organelles of a plant cell are complex and interdependent, for example the rampant signaling between organelles such as chloroplasts and nuclei (Jung and Chory, 2009). Thus efforts to understand their individual metabolites would aid in understanding of these

complex regulatory exchanges, in addition to what is established at the levels of transcripts and proteins.

Omics-based tools and approaches in identifying subcellular functionalities are powerful resources

There have been considerable efforts to catalog the information content in organelles starting from imaging to omics-based systems biology approaches. For instance, the objective of the plant organelles database (http://podb.nibb.ac.jp/Organellome) is to provide insights on organellar dynamics such as their function, biogenesis, differentiation, movement and interactions (Mano et al., 2013). However, genomics-based efforts are more prevalent. For instance, a unique database of RNA-editing sites from plant organelle genes with the results mapped onto amino acid sequences and 3D structures (Yura et al., 2009) are highly useful. In addition, to catalog fluorescent protein expression, public repositories such as the Maize Cell Genomics (MCG) database (http://maize.jcvi.org/cellgenomics) was developed that represents major subcellular structures and developmentally important progenitor cell populations (Krishnakumar et al., 2014). Another noteworthy approach was the use of subcellular organelle expression microarray to study the organic acid changes in post-harvest *Citrus* fruit (Sun et al., 2013) and organelle membrane proteome studies during germination and tube growth of lily pollen (Pertl et al., 2009). In addition, proteomics efforts have revealed secretome, extracellular matrix, cell wall, vacuoles, plastids, and peroxisomes-specific changes in plants are catalogued (Liley and Dupree, 2007; Dai and Chen, 2012). Similarly, proteomics-based approaches for characterization of seed proteomes have been reviewed recently (Repetto and Gallardo, 2012). Rapid subcellular fractionation combined to targeted proteomics allowed estimation of subcellular protein concentrations in attomole per 1000 cells of *Chlamydomonas reinhardtii* (Weinkeeop et al., 2010). Although, the importance of spatial resolution of plant cellular metabolomes has been discussed elsewhere (Sumner et al., 2011) but robust efforts and databases are missing for plant subcellular metabolomes.

Recently, the need for understanding the challenges in cellular compartmentalization for successful plant metabolic engineering was identified (Heining et al., 2013). The enrichment of other omics-based subcellular localization tools would allow understanding of the metabolic pathways operative in them for tinkering them for commercial success. Some widely used computational approaches for proteome level assignment of localization include, prediction programs such as TargetP, http://www.cbs.dtu.dk/services/TargetP/, Predotar, http://www.inra.fr/predotar/, iPSORT, http://hc.ims.u-tokyo.ac.jp/iPSORT/, and SubLoc, http://www.bioinfo. tsinghua.edu.cn/SubLoc/, etc. For example, LocDB is a manually curated database with experimental annotations for the subcellular localizations of proteins in *A. thaliana* (Rastogi and Rost, 2011). Recently, the Peroxisome database (http://www.peroxisomeDB.org) was released which serves as a huge resource for cross-lineage comparison of functional genomic and metabolomic information on organisms such as fungi, yeasts, plants, human and lower eukaryotes, with an ensemble of 139 peroxisomal protein families and ~2706 putative peroxisomal protein homologs (Schlüter et al., 2010). Databases like SUBA (Arabidopsis SUBcellular Database, http://suba.plantenergy.uwa.edu.au/) (Heazlewood et al., 2007) are useful resources of subcellular compartmentation supported by experimental evidence collated from organellar proteome studies, which enable the integration of experimentation and prediction (Tanz et al., 2012). In addition, resources such as AraGEM (Arabidopsis genome-scale model), a genome-scale model of *Arabidopsis* metabolism are available where the vast majority of reactions are assigned to the cytosol (with 1265, 60, 159, and 98 reactions assigned to cytosol,

mitochondria, plastid, and peroxisome, respectively) (de Oliveira Dal'Molin et al., 2010). However, there are no available collage of information on subcellular metabolomes of plants to our knowledge.

Plant subcellular metabolome studies revisited: non-aqueous fractionation (NAF) methods

There have been several successful attempts at obtaining the qualitative and quantitative snap shots of sub-cellular metabolomes in plants. These efforts relied on fractionation or isolation of pure organelles followed by characterization of the metabolomes by gas chromatography mass-spectrometry (GC-MS), liquid chromatography- mass spectrometry (LC-MS) among other approaches. Cell fractionation and immunohistochemical studies in the past few decades have revealed the extensive compartmentation of plant metabolism from protein-based information (Lunn, 2007). Majority of the classical studies in compartmentation of plant metabolism focused on plastids, mitochondria, and vacuole and reflected on their structural and functional heterogeneity (Lunn, 2007, Bowsher and Tobin, 2011). Plastids are involved in carbon and nitrogen metabolism, in particular nitrate and ammonium assimilation, the Calvin cycle, oxidative pentose-phosphate pathway, glycolysis, and terpenoid biosynthesis, and these have been reviewed from a metabolic perspective (Tobin and Bowsher, 2005). Thus plastidial proteomics have interested researchers for a long time (van Wijk and Baginsky, 2011). Analysis of the chloroplast proteome indicated the presence of biosynthesis of fatty acids, lipids, amino acids, nucleotides, hormones, alkaloids, and isoprenoids, Calvin cycle enzymes and proteins belonging to the light-harvesting apparatus and photosynthetic electron transport chain (van Wijk, 2004). Protoplast fractionation and enzymatic determination of metabolites are popular methods to quantify metabolites such as adenylates, phosphorylated sugars and Calvin cycle intermediates in different sub-cellular compartments (Kueger et al., 2012). The metabolomes of highly purified barley vacuoles isolated from mesophyll cell protoplasts by silicon oil centrifugation revealed the presence of 59 primary metabolites and ~200 secondary metabolites by GC-MS and FT-MS (Fourier transform-mass spectrometry) such as amino acids, organic acids, sugars, sugar alcohols, shikimate pathway intermediates, vitamins, phenylpropanoids, and flavonoids, of which 12 were found exclusively in the vacuole (Tohge et al., 2011). Similarly, a single vacuole of single cell of the alga *Chara australis* revealed the localization and dynamics of 125 known metabolites (Oikawa et al., 2011). In plants, vacuoles are known for detoxification of xenobiotics (Coleman et al., 1997). In addition, the analysis of subcellular metabolite levels of potato tubers (*Solanum tuberosum*) revealed that either the cytosol or apoplast leads to a decrease in sucrose and an increase in glucose whereas the hexoses accumulate in the vacuole independently of their site of biosynthesis (Farre et al., 2008). Furthermore, in the medicinal plant *Catharanthus roseus*, LC-MS analysis of the phenolics from isolated leaf vacuoles allowed identification of three caffeoylquinic acids and four flavonoids (Ferreres et al., 2010). Another example of the use of vibrational (Raman) spectroscopy in metabolomics was exemplified in the localization of β-carotene by its 1150 and 1515 cm^{-1} Raman bands with subcellular resolution (~550 nm/ pixel) in the cells of alga *Euglena gracilis*. Complementary single-cell MS data further revealed that colocalization of β-carotene to the internal antennae of photosystem II in plastids (Urban et al., 2011).

Non-aqueous fractionation (NAF) is the most popular tool available for investigating metabolite pool sizes at subcellular resolution in plants (Kueger et al., 2012), where NAF relies on the enrichment of organelles within a continuous non-aqueous density gradient instead of purifying individual intact organelles. This method associated with metabolomics studies allows for the analysis of metabolites at subcellular level (Farre et al., 2001, Krueger et al., 2011), i.e., mitochondria (Fly et al., 2015). The metabolome compartmentation of soybean leaves using non-aqueous fractionation by GC-

MS indicated that out of a total of 100 compounds a greater number of compounds were identified in vacuole when compared to cytosol or stroma (Benkeblia et al., 2007). Furthermore, the NAF method allowed the identification and quantification of the subcellular distributions of metabolites in developing potato (*Solanum tuberosum* L. cv Desiree) tubers which revealed that ~60% of most sugars, sugar alcohols, organic acids, and amino acids were found in the vacuole, whereas the substrates for starch biosynthesis, hexose phosphates, and ATP were found in plastid, while pyrophosphate was located in only cytosol (Farré et al., 2011). Similarly, in *A. thaliana* leaves, using NAF methods about 1,000 proteins and 70 metabolites, including 22 phosphorylated intermediates were separated into plastidial, cytosolic, and vacuolar metabolites and proteins which indicated that cytosolic, mitochondrial, and peroxisomal proteins clustered together. Both metabolites and proteins from the Calvin-Benson cycle, photorespiration, starch and sucrose synthesis, glycolysis, and the tricarboxylic acid cycle were localized to specific compartments, thus indicating NAF as a powerful tool for the study of the organellar and sub-organellar distribution of both, metabolites and proteins.

Unfortunately, organelles extracted from whole tissue homogenates are generally derived from diverse cell types (Bowsher and Tobin, 2001), but from specific organs such as leaves. The single largest study depicting the compartmentalized *A. thaliana* metabolome (Krueger et al., 2011) used GC-MS and LC-MS based approaches to capture the subcellular distribution of 1,117 polar and 2,804 lipophilic metabolite features associated to known and unknown compounds, where 81.5% of the metabolic data could be associated to one of three subcellular compartments: the cytosol (including mitochondria), vacuole, or plastids. Advances in MS-based lipidomics have enabled the simultaneous identification and quantification of lipid species from complex structures at the tissue, cellular and organelle resolution levels (Horn and Chapman, 2012). The authors showed that at the nano scale, 'direct organelle MS' (DOMS) holds immense potential organellar lipid profiling by extracting lipids from isolated organelles, or from intact cells, within a capillary tip, followed by their qualitative and quantitative evaluation using direct-infusion nanospray MS. Furthermore, fluorescent protein technology can be used to image subcellular dynamics at spatio-temporal resolution, and to manipulate the distribution of fluorescent markers for identification of genes involved in organellar functioning by means of light microscopy and genomics (Sparkes and Brandizzi, 2012). Recently, to address and resolve the spatio-temporal metabolic complexity of plant sub-cellular metabolome, isotopic labelling (i.e., $^{13}CO_2$) and high resolution mass spectrometry (GC-MS and LC-MS) has been used to construct metabolic flux models (Allen et al., 2009; Ma et al., 2014).

Conclusion and future prospects

Although popularly used, NAF is static, invasive, has little cellular resolution, and is sensitive to artifacts (Looger et al., 2005). Thus, NAF needs further validation before further successful implementation and applications (Klie et al., 2011). Spectroscopic methods such as nuclear magnetic resonance (NMR) imaging and positron emission tomography (PET) provide dynamic data, but poor spatial resolution. Thus, genetically encoded fluorescence resonance energy transfer (FRET) sensors (i.e., green florescence protein (GFP)-based, enzyme based etc.) have been proposed for imaging metabolites at subcellular resolution (Looger et al., 2005). Recently, several flux-balance models of plant metabolism have been published including genome-scale models of *A. thaliana* metabolism (Sweetlove and Ratcliffe, 2011). Approaches for flux balance analysis have been reviewed elsewhere (Lee et al., 2011; Lakshmanan et al., 2012). To achieve greater insights into metabolic fluxes across subcellular metabolomes several flux analyses tools are available, such as FiatFlux (Zamboni et al., 2005), OpenFLUX (Quek et al., 2009) that are based on ^{13}C-based analysis, OptFlux (Rocha et al., 2010),

FluxAnalyzer (Klamt et al., 2013), YANA (Schwarz et al., 2005). Model SEED, FAME, and MetaFlux include workflows to facilitate the reconstruction of genome-scale metabolic models (Lakshmanan et al., 2012). NAF methods for obtaining subcellular fractions allows direct quenching of metabolism by snap-freezing in liquid nitrogen, thus, the combination of NAF with metabolic flux analysis (MFA) using ^{13}C labeled CO_2 is a very attractive approach for the future (Keuger et al., 2012). On the other hand, MALDI associated secondary ion mass spectrometry (SIMS) imaging, on research-grade MALDI-MS instruments, MSI is possible with a spatial resolution of <1 µm at ambient pressures, and with very high mass accuracy and mass resolution which corresponds to subcellular resolution for many cell-types (Zenobi, 2013). These approaches certainly hold immense promises in pushing the field forward to decipher compartmentalized plant metabolomes.

Competing interests

The authors declare that there are no competing interests.

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References

Allen, D. K., Libourel, I. G., & Shachar-Hill, Y. A (2009). Metabolic flux analysis in plants: coping complexity. Plant, cell & environment, 32(9), 1257. http://dx.doi.org/10.1111/j.1365-3040.2009.019 PMid:19422611

Benkeblia, N., Shinano, T., & Osaki, M. (2 Metabolite profiling and assessment of metabo compartmentation of soybean leaves using aqueous fractionation and GC-MS ana Metabolomics, 3(3), 29 http://dx.doi.org/10.1007/s11306-007-0078-y

Dai, S., & Chen, S. (2012). Single-cell-type proteomics: toward a holistic understanding of plant function. Molecular & Cellular Proteomics, 11(12), 1622-1630. http://dx.doi.org/10.1074/mcp.R112.021550 PMid:22982375 PMCid:PMC3518137

de Oliveira Dal'Molin, C. G., Quek, L. E., Palfreyman, R. W., Brumbley, S. M., & Nielsen, L. K. (2010). AraGEM, a genome-scale reconstruction of the primary metabolic network in Arabidopsis. Plant Physiology, 152(2), 579-589. http://dx.doi.org/10.1104/pp.109.148817 PMid:20044452 PMCid:PMC2815881

Farre, E. M., Fernie, A. R., & Willmitzer, L. (2008). Analysis of subcellular metabolite levels of potato tubers (Solanum tuberosum) displaying alterations in cellular or extracellular sucrose metabolism. Metabolomics, 4(2), 161-170. http://dx.doi.org/10.1007/s11306-008-0107-5 PMid:19816536 PMCid:PMC2758360

Farré, E. M., Tiessen, A., Roessner, U., Geigenberger, P., Trethewey, R. N., & Willmitzer, L. (2001). Analysis of the compartmentation of glycolytic intermediates, nucleotides, sugars, organic acids, amino acids, and sugar alcohols in potato tubers using a nonaqueous fractionation method. Plant Physiology, 127(2), 685-700. http://dx.doi.org/10.1104/pp.010280 PMCid:PMC125103

Ferreres, F., Figueiredo, R., Bettencourt, S., Carqueijeiro, I., Oliveira, J., Gil-Izquierdo, A., ... & Sottomayor, M. (2011). Identification of phenolic compounds in isolated vacuoles of the medicinal plant Catharanthus roseus and their interaction with vacuolar class III peroxidase: an H2O2 affair?. Journal of Experimental Botany, 62(8), 2841-2854. http://dx.doi.org/10.1093/jxb/erq458 PMid:21357771

Fly, R., Lloyd, J., Krueger, S., Fernie, A., & van der Merwe, M. J. (2015). improvements to define

mitochondrial metabolomics using nonaqueous fractionation. Plant mitochondria: Methods and Protocols, 197-210. http://dx.doi.org/10.1007/978-1-4939-2639-8_14

Heazlewood, J. L., Verboom, R. E., Tonti-Filippini, J., Small, I., & Millar, A. H. (2007). SUBA: the Arabidopsis subcellular database. Nucleic Acids Research, 35(suppl 1), D213-D218. http://dx.doi.org/10.1093/nar/gkl863 PMid:17071959 PMCid:PMC1635339

Horn, P. J., & Chapman, K. D. (2012). Lipidomics in tissues, cells and subcellular compartments. The Plant Journal, 70(1), 69-80. http://dx.doi.org/10.1111/j.1365-313X.2011.04868.x PMid:22117762

Klamt, S., Stelling, J., Ginkel, M., & Gilles, E. D. (2003). FluxAnalyzer: exploring structure, pathways, and flux distributions in metabolic networks on interactive flux maps. Bioinformatics, 19(2), 261-269. http://dx.doi.org/10.1093/bioinformatics/19.2.261 PMid:12538248

Krishnakumar, V., Choi, Y., Beck, E., Wu, Q., Luo, A., Sylvester, A., ... & Chan, A. P. (2014). A maize database resource that captures tissue-specific and subcellular-localized gene expression, via fluorescent tags and confocal imaging.(Maize Cell Genomics Database). Plant and Cell Physiology, pcu178. http://dx.doi.org/10.1093/pcp/pcu178

Kueger, S., Steinhauser, D., Willmitzer, L., & Giavalisco, P. (2012). High-resolution plant metabolomics: from mass spectral features to metabolites and from whole-cell analysis to subcellular metabolite distributions. The Plant Journal, 70(1), 39-50. http://dx.doi.org/10.1111/j.1365-313X.2012.04902.x PMid:22449042

Lakshmanan, M., Koh, G., Chung, B. K., & Lee, D. Y. (2012). Software applications for flux balance analysis. Briefings in Bioinformatics, bbs069. http://dx.doi.org/10.1093/bib/bbs069

Lee, J. M., Gianchandani, E. P., & Papin, J. A. (2006). Flux balance analysis in the era of metabolomics. Briefings in Bioinformatics, 7(2), 140-150. http://dx.doi.org/10.1186/1471-2105-7-140 http://dx.doi.org/10.1093/bib/bbl007 PMid:16772264

Lilley, K. S., & Dupree, P. (2007). Plant organelle proteomics. Current Opinion in Plant Biology, 10(6), 594-599. http://dx.doi.org/10.1016/j.pbi.2007.08.006 PMid:17913569

Looger, L. L., Lalonde, S., & Frommer, W. B. (2005). Genetically encoded FRET sensors for visualizing metabolites with subcellular resolution in living cells. Plant Physiology, 138(2), 555-557. http://dx.doi.org/10.1104/pp.104.900151 PMid:15955913 PMCid:PMC1150369

Ma, F., Jazmin, L. J., Young, J. D., & Allen, D. K. (2014). Isotopically nonstationary 13C flux analysis of changes in *Arabidopsis thaliana* leaf metabolism due to high light acclimation. Proceedings of the National Academy of Sciences, 111(47), 16967-16972. http://dx.doi.org/10.1073/pnas.1319485111 PMid:25368168 PMCid:PMC4250135

Mano, S., Nakamura, T., Kondo, M., Miwa, T., Nishikawa, S. I., Mimura, T., ... & Nishimura, M. (2013). The Plant Organelles Database 3 (PODB3) update 2014: integrating electron micrographs and new options for plant organelle research. Plant and Cell Physiology, pct140. http://dx.doi.org/10.1093/pcp/pct140

Mintz-Oron, S., Meir, S., Malitsky, S., Ruppin, E., Aharoni, A., & Shlomi, T. (2012). Reconstruction of Arabidopsis metabolic network models accounting for subcellular compartmentalization and tissue-specificity. Proceedings of the National Academy of Sciences, 109(1), 339-344. http://dx.doi.org/10.1073/pnas.1100358109 PMid:22184215 PMCid:PMC3252957

Misra, B. B., Assmann, S. M., & Chen, S. (2014). Plant single-cell and single-cell-type metabolomics. Trends in Plant Science, 19(10), 637-646. http://dx.doi.org/10.1016/j.tplants.2014.05.005 PMid:24946988

Oikawa, A., Matsuda, F., Kikuyama, M., Mimura, T., & Saito, K. (2011). Metabolomics of a single vacuole reveals metabolic dynamism in an alga Chara australis. Plant Physiology, 157(2), 544-551. http://dx.doi.org/10.1104/pp.111.183772 PMid:21846815 PMCid:PMC3192564

Pertl, H., Schulze, W. X., & Obermeyer, G. (2009). The pollen organelle membrane proteome reveals highly spatial− temporal dynamics during germination and tube growth of lily pollen. Journal of Proteome Research, 8(11), 5142-5152. http://dx.doi.org/10.1021/pr900503f PMid:19799449

Quek, L. E., Wittmann, C., Nielsen, L. K., & Krömer, J. O. (2009). OpenFLUX: efficient modelling software for 13C-based metabolic flux analysis. Microbial Cell Factories, 8(1), 25. http://dx.doi.org/10.1186/1475-2859-8-25 PMid:19409084 PMCid:PMC2689189

Rastogi, S., & Rost, B. (2011). LocDB: experimental annotations of localization for Homo sapiens and Arabidopsis thaliana. Nucleic Acids Research, 39(suppl 1), D230-D234. http://dx.doi.org/10.1093/nar/gkq927 PMid:21071420 PMCid:PMC3013784

Repetto, O., & Gallardo, K. (2012). Organelle Proteomics of Developing Seeds: Comparison with Other Plant Tissue Organelles. In Seed Development: OMICS Technologies toward Improvement of Seed Quality and Crop Yield (pp. 213-246). Springer Netherlands.

Rocha, I., Maia, P., Evangelista, P., Vilaça, P., Soares, S., Pinto, J. P., ... & Rocha, M. (2010). OptFlux: an open-source software platform for in silico metabolic engineering. BMC Systems Biology, 4(1), 45. http://dx.doi.org/10.1186/1752-0509-4-45 PMid:20403172 PMCid:PMC2864236

Schwarz, R., Musch, P., von Kamp, A., Engels, B., Schirmer, H., Schuster, S., & Dandekar, T. (2005). YANA–a software tool for analyzing flux modes, gene-expression and enzyme activities. BMC Bioinformatics, 6(1), 135. http://dx.doi.org/10.1186/1471-2105-6-135 PMid:15929789 PMCid:PMC1175843

Sparkes, I., & Brandizzi, F. (2012). Fluorescent protein-based technologies: shedding new light on the plant endomembrane system. The Plant Journal, 70(1), 96-107. http://dx.doi.org/10.1111/j.1365-313X.2011.04884.x PMid:22449045

Sumner, L. W., Yang, D. S., Bench, B. J., Watson, B. S., Li, C., & Jones, A. D. (2011). Spatially resolved plant metabolomics. Annual Plant Reviews, Biology of Plant Metabolomics, 43, 343. http://dx.doi.org/10.1002/9781444339956.ch11

Sun, X., Zhu, A., Liu, S., Sheng, L., Ma, Q., Zhang, L., ... & Deng, X. (2013). Integration of Metabolomics and Subcellular Organelle Expression Microarray to Increase Understanding the Organic Acid Changes in Post-harvest Citrus Fruit. Journal of Integrative Plant Biology, 55(11), 1038-1053. http://dx.doi.org/10.1111/jipb.12083 PMid:23758915

Sweetlove, L. J., & Ratcliffe, R. G. (2011). Flux-balance modeling of plant metabolism. Frontiers in Plant Science, 2. http://dx.doi.org/10.3389/fpls.2011.00038 PMid:22645533 PMCid:PMC3355794

Tanz, S. K., Castleden, I., Hooper, C. M., Vacher, M., Small, I., & Millar, H. A. (2012). SUBA3: a database for integrating experimentation and prediction to define the SUBcellular location of proteins in Arabidopsis. Nucleic Acids Research, gks1151. http://dx.doi.org/10.1093/nar/gks1151

PMid:20358043

Tobin, A. K., & Bowsher, C. G. (2005). Nitrogen and carbon metabolism in plastids: Evolution, integration, and coordination with reactions in the cytosol. Advances in Botanical Research, 42, 113-165. http://dx.doi.org/10.1016/S0065-2296(05)42004-2

Toubiana, D., Fernie, A. R., Nikoloski, Z., & Fait, A. (2013). Network analysis: tackling complex data to study plant metabolism. Trends in Biotechnology, 31(1), 29-36. http://dx.doi.org/10.1016/j.tibtech.2012.10.011 PMid:23245943

van Wijk, K. J., & Baginsky, S. (2011). Plastid proteomics in higher plants: current state and future goals. Plant Physiology, 155(4), 1578-1588. http://dx.doi.org/10.1104/pp.111.172932 PMid:21350036 PMCid:PMC3091083

Wienkoop, S., Weiß, J., May, P., Kempa, S., Irgang, S., Recuenco-Munoz, L., ... & Weckwerth, W. (2010). Targeted proteomics for Chlamydomonas reinhardtii combined with rapid subcellular protein fractionation, metabolomics and metabolic flux analyses. Molecular BioSystems, 6(6), 1018-1031. http://dx.doi.org/10.1039/b920913a

Yura, K., Sulaiman, S., Hatta, Y., Shionyu, M., & Go, M. (2009). RESOPS: a database for analyzing the correspondence of RNA editing sites to protein three-dimensional structures. Plant and Cell Physiology, 50(11), 1865-1873. http://dx.doi.org/10.1093/pcp/pcp132 PMid:19808808 PMCid:PMC2775959

Zamboni, N., Fischer, E., & Sauer, U. (2005). FiatFlux–a software for metabolic flux analysis from 13C-glucose experiments. BMC Bioinformatics, 6(1), 209. http://dx.doi.org/10.1186/1471-2105-6-209 PMid:16122385 PMCid:PMC1199586

Zenobi, R. (2013). Single-cell metabolomics: analytical and biological perspectives. Science, 342(6163), 1243259. http://dx.doi.org/10.1126/science.1243259 PMid:24311695