Feature Review

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Plant single-cell and single-cell-type metabolomics

Biswapriya B. Misra[1], Sarah M. Assmann[2], and Sixue Chen[1,3]

[1] Department of Biology, Genetics Institute, Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32610, USA
[2] Department of Biology, Penn State University, 208 Mueller Laboratory, University Park, PA 16802, USA
[3] Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32610, USA

In conjunction with genomics, transcriptomics, and proteomics, plant metabolomics is providing large data sets that are paving the way towards a comprehensive and holistic understanding of plant growth, development, defense, and productivity. However, dilution effects from organ- and tissue-based sampling of metabolomes have limited our understanding of the intricate regulation of metabolic pathways and networks at the cellular level. Recent advances in metabolomics methodologies, along with the post-genomic expansion of bioinformatics knowledge and functional genomics tools, have allowed the gathering of enriched information on individual cells and single cell types. Here we review progress, current status, opportunities, and challenges presented by single cell-based metabolomics research in plants.

Metabolomics at cellular resolution

Metabolites add an important extra dimension to the information flow from DNA to RNA to protein. With the advent of high-end instrumentation and an accelerated understanding of the roles of metabolites in organismal functions [1], our appreciation of the importance of metabolites is increasing. By definition, the metabolome is the entire set of small molecules (molecular mass less than 2,000 Da) or metabolites in a given biological sample. Metabolomics is the unbiased analytical assessment of the metabolome, whereas metabolite/metabolic profiling deals with the detection and quantification of a group of metabolites using a particular analytical approach [2]. The total number of metabolites found in plants is currently estimated at 200,000, with around 7,000–15,000 being found in any individual species [3,4], 3,000–5,000 of which reside in leaves [5,6]. These large groups of structurally diverse, temporally and spatially dynamic compounds pose great challenges for modern analytical technologies [7] compared with the comparatively straightforward polymeric assemblies of four nucleotides as DNA molecules. In addition, species specificity in the distribution of specialized metabolites, rapid endogenous turnover rates, an inability to amplify metabolites, the limited ability to capture more than a snapshot of the metabolites in an organism at any one time, the suboptimal quality of extraction methods, and the dynamic ranges of abundance, detection capabilities, ionization and polarizability render metabolomics far more difficult than other 'omics'. It is no doubt that metabolomics studies are of essential importance for a comprehensive understanding of cellular functions [8,9], and metabolomics has become important in studying the genetic regulation of metabolite synthesis and function [10]. Large-scale discovery and quantitation of metabolites is imperative for comprehensive understanding of plant signaling and biochemical processes. In addition, many phytochemicals have immense significance in food, medicine, and agriculture.

Alongside tissue-, organ-, and organism-level metabolome studies, tools and techniques have been developed to overcome the averaging effect that is inevitable in studies on populations of mixed cell types. Indeed, advances in the isolation of both single cells and populations of an individual cell type have made metabolomics at the cellular level a reality, thus enhancing unbiased detection and quantification of metabolites at this basic level of biological organization [11]. Cell types have been defined on the basis of their appearance, location, expression of specific marker genes, or performance of specific functions [12]. To date, the choice of cell systems to study has been dictated largely by the ease of isolation, handling, purity and homogeneity of preparations, state of differentiation, and importance to the plant's physiological status. Here, we define single-cell metabolomes as those derived from one individual cell (e.g., a single epidermal cell), whereas single-cell-type metabolomes are defined as those derived from a population of cells representing a common origin and physiological role (e.g., trichomes or guard cells).

Higher plants are multicellular organisms: for example, in rice 40 different cell types are documented [13]. To date, enriched or pure preparations of many cell types from different plant species have been used for single-cell or single-cell-type metabolomics studies (Table 1, Figure 1). Excellent topical reviews have appeared that summarize substantial progress in the areas of single-cell genomics [14], transcriptomics [15], and proteomics [16] of plants. In this review, we present recent advances in plant single-cell and single-cell-type metabolomics. We highlight the sampling tools, techniques, and various analytical platforms,

Corresponding author: Chen, S. (schen@ufl.edu).
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Table 1. Recent studies highlighting the analytical tools used and results obtained in single cell and single cell-type metabolomics

Species	Material	Analytical approach[a]	Metabolites identified	Class of metabolites	Refs
Subcellular metabolomics					
Hordeum vulgare	Vacuoles from mesophyll cell protoplasts	GC-MS, UPLC-FT-MS	259	Amino acids, organic acids, sugars, specialized metabolites	[45]
Single-cell metabolomics					
Torenia hybrida	Petal cell	Nano-HPLC-MS	5	Anthocyanins	[33]
Closterium acerosum	Single cell	MALDI-MS	4	Central metabolites	[79]
Pelargonium zonale	Single leaf, stem, petal cell	Nano-ESI-MS	22	Monoterpenoids	[36]
Allium cepa, Narcissus pseudonarcissus	Epidermal single cell of bulbs	AP-ESI-MS	32, 22	Specialized metabolites, oligosaccharides	[34]
Arabidopsis thaliana, Hypericum perforatum, Hypericum reflexum	Individual dark glands from petals, leaves; glandular trichomes	LDI-ToF-MS	15	Naphthodianthrones, flavonoids	[41]
Single-cell-type metabolomics					
Dilatris pillansii	Leaf and flower secretory cavities	Cryogenic NMR, HPLC	7	Methoxyphenylphenalenones	[50]
Eucalyptus spp.	Leaf subdermal secretory cavities	GC, GC-MS	24	Mono- and sesqui-terpenoids	[51]
Catharanthus roseus	Leaf epidermome	LC-ESI-MS	2	Oleanolic and ursolic acid	[52]
Picea abies	Phloem parenchyma cells	Cryogenic NMR	2	Stilbene glucosides, flavonoids	[43]
P. abies	Stone cells/sclereids	Cryogenic NMR, MS	2	Phenolic glycosides	[54]
Cucurbita maxima	Phloem latex (sieve tubes)	GC-ToF-MS, HPLC, FIE-MS	80	Amino acids, sugars	[55]
Glycine max	Root hairs	GC-MS, UPLC-QToF-MS	634	Amino acids, sugars, sugar alcohols, fatty acids, flavonoids, organic acids, nucleosides, phenolics, glucosinolates, saponins, alkaloids	[56]
Lilium longiflorum	Pollen grains	GC-ToF-MS	252	Sugars, organic acids, amino acids	[57]
Gossypium hirsutum	Fiber cells	GC-MS	86	Non-polar (sterols, alkanes) and polar (sugars, sugar alcohols, amino acids)	[59]
G. hirsutum	Fiber cells	GC-MS	27	Organic acids, amino acids, sugars	[60]
Citrus paradisi	Epithelial and parenchyma cells	GC, GC-MS, UPLC-QToF-MS	28	Terpenoid, sterols, fatty acids, carotenoids, oxygen heterocyclics	[62]
A. thaliana	Guard cell and mesophyll cell protoplasts	LC-(MRM)-MS/MS	85	Phytohormones, signaling molecules, phenolics, flavonoids, amino acids	[23]
Lycopersicon hirsutum	Glandular trichomes	GC-MS	7	Terpenoids	[66]
Solanum spp.	Trichomes	LC-MS	119	Terpenoid, flavonoids, fatty acids, alkaloids, acyl sugars	[65]
Solanum lycopersicum	Glandular trichomes	LC-ToF-MS	32	Acylated molecules, flavonoids, glycosides,	[67]
Artemisia annua	Glandular trichomes	GC-MS	12	Mono- and sesqui-terpenoids	[68]
Cannabis sativa	Trichome types	LC-MS, NMR	9	Cannabinoids	[69]
A. thaliana	Epidermal trichomes, basal/pavement cells	GC-ToF-MS	117	Amino acids, fatty acids and alcohols, alkanes, lipids, N-compounds, organic acids, polyhydroxy acids, polyols, sugars, sugar conjugates, phenylpropanoids	[63]
A. thaliana	Glandular trichomes	UPLC-ESI-QToF-MS	13	Glucosinolates	[64]
Colquhounia coccinea	Peltate glandular trichomes	UPLC-MS/MS, X-ray diffraction	3	Sesquiterpenoids (colquhounoids)	[42]
Nicotiana attenuate	Glandular trichomes	^{1}H-NMR, LC-QToF-MS	>2	Nicotine, phaseoloidin, acyl sugars, fatty acids	[70]
Ocimum basilicum	Peltate and capitate glandular trichomes	GC-MS, HPLC	15	Terpenoids, phenylpropenes	[71]
A. thaliana	Epidermal cell layer, palisade mesophyll cells, vascular bundle	MALDI-ToF-MS	18	Cell wall polysaccharides	[80]
A. thaliana	Endodermis, epidermis, columella, cortex, stele	GFP-FACS, UPLC-QToF-MS	50	Glucosinolates, phenylpropanoids, dipeptides	[81]

[a]Abbreviations: AP, atmospheric pressure; ESI, electrospray ionization; FACS, fluorescence activated cell sorting; FIE, flow injection electrospray; FT, Fourier transform; GC, gas chromatography; HPLC, high performance liquid chromatography; LC, liquid chromatography; LDI, matrix-free laser desorption/ionization; MALDI, matrix-assisted laser desorption/ionization; MRM, multiple reaction monitoring; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NMR, nuclear magnetic resonance; QToF, quadrupole time of flight; ToF, time of flight; UPLC, ultra performance liquid chromatography.