

Recent advances in plant metabolomics and greener pastures

Lloyd W Sumner

Address: The Samuel Roberts Noble Foundation, Plant Biology Division, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA

Email: lwsunmer@noble.org

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Abstract

Metabolomics is an extension of the *omics* concept and experimental approaches. However, is metabolomics just another trendy *omics* fashion perturbation or is metabolomics actually delivering novel content and value? This article highlights some recent advances that definitely support the role of plant metabolomics in the movement toward greener pastures.

Introduction and context

Approximately 10 years ago, the seeds of plant metabolomics were sown and fertilized. These seeds have grown and plant metabolomics is in full bloom. Metabolomics, aka the large-scale profiling of metabolites, is the progressive extension of the *omics* technologies to the large-scale study of the small-molecule component of living organisms. The small molecules in cells represent the consequential end products of gene expression and offer a high-resolution biochemical phenotype of cells, tissues, and/or organisms. The financial cost of a metabolomics experiment is approximately an order of magnitude lower than that of a transcriptome or microarray experiment, adding to its practical implementation. Plant metabolomics has ripened into a very valuable tool for advancing our understanding of primary and secondary metabolism in plants and is revolutionizing the field of plant biology. This article highlights numerous recent advances describing how this revolution is being achieved.

Major recent advances

Biological advances

Plant metabolomics has come of age and is now a proven high-resolution biochemical phenotyping tool that is yielding advanced understanding of primary and secondary metabolism [1-5]. It is providing critical insight into the molecular and biochemical events that occur during mutualistic and pathogenic plant-microbe interactions [6-8], and it is a powerful functional

genomics tool for the discovery of novel metabolites and their correlated biosynthetic genes [9,10]. Some of the most eloquent examples are emerging from the Riken Plant Science Center (Kanagawa, Japan), where the group led by Kazuki Saito is using integrated metabolomics and transcriptomics for gene discoveries related to sulfur metabolism [11] and flavonoid biosynthesis [12,13].

Metabolomics can also be envisioned as metabolic marker analysis to the nth power with substantial predictive power. Accordingly, metabolomics is emerging as a promising tool in metabolomics-assisted breeding. Metabolomics-assisted breeding offers unique opportunities for enhancing traits of commercial value, and a recent review on the use of metabolomics in exploiting natural variance for the improvement of crop nutrition which is ultimately dependent upon metabolic composition has been published [14]. This logic is further evidenced by the recent work of Meyer and colleagues [15], who reported a highly significant canonical correlation (0.73) between biomass and a specific combination of metabolites, and by Lisec and colleagues [16], who also showed that metabolic quantitative trait loci could be correlated with biomass accumulation. Thus, the predictive power of numerous qualitative and quantitative metabolite markers promises to be a powerful resource for breeding and biomass production, and it appears that greener pastures are truly on the horizon for plant metabolomics.

Plant metabolomics is impacting the methods by which food taste, quality, and nutrition are assessed. Traditionally, taste has been assessed with the sophisticated palates of highly trained sensory panel personnel. However, metabolomics can provide large-scale metabolic assessment of taste, quality, and nutrition which may soon put sensory panels out of work. For example, the group of Oliver Fiehn [17] recently showed that gas chromatography-mass spectrometry (GC-MS)- and proton nuclear magnetic resonance ($^1\text{H-NMR}$)-based metabolomics offer a competitive and sophisticated assessment of white wine 'body' or mouthfeel. A similar approach has been used to assess taste in scrumptious and nutritious tomatoes [18]. Metabolomics has been essential in the fundamental biochemical understanding of plant metabolism and its direct relationship to nutrition. It has also been central to advancing the efficiency of metabolic engineering of plant nutrition and nutraceuticals in plants [4]. Examples include carotenoids/lycopene [19], vitamin A [20], folate [21,22], anthocyanins [23], and lignin [24]. The expansion of this approach appears to be truly unlimited.

Technology advances

NMR and MS have been staple tools of chemists for decades, and recent Nobel Prizes for magnetic resonance imaging (2002) and MS (2002) further emphasize the impact of these technologies on the biological sciences. These technologies have been readily incorporated in the majority of metabolomics programs, but most groups tend to specialize in one or the other. However, this author believes that a substantial advantage is gained through the union of these technologies. The correlation of NMR and MS data through 'statistical heterospectroscopy' [25] is one example that exploits intrinsic covariance between chemical shifts in the NMR to m/z values in the MS data. Both datasets provide quantitative measures for the metabolites; however, the combined and correlated data provide significant advantages in metabolite identifications. Although this approach has not yet been demonstrated in plant metabolomics, several physical combinations of liquid chromatography (LC)-NMR, LC-solid-phase extraction (SPE)-NMR, and LC-MS-SPE-NMR have. (For a recent review, see [26]).

Another technical advance that offers great promise for metabolomics is dynamic nuclear polarization (DNP) [27]. DNP uses low temperatures, high magnetic fields, and microwaves to strongly polarize nuclear spins prior to NMR analysis. This approach yields enhanced high-resolution NMR signals resulting in sensitivity increases up to 10,000 fold or substantial reduction in measurement times or both. Such sensitivity enhancements can

dramatically expand a variety of *in vitro* and *in vivo* metabolomics applications, including endogenous metabolite imaging [28]. Unfortunately, the instrumental resources necessary for DNP NMR are still quite costly and highly specialized.

Recent technical advances have also been realized in the field of MS imaging. MS imaging using secondary ion MS has been possible for some time; however, the performance of this technique is not well suited for biological molecules. Recent coupling of direct laser desorption [29] and matrix-assisted laser desorption ionization [30,31] with time-of-flight MS imaging have made the molecular imaging of metabolites a practical reality. These technologies have the ability to spatially resolve metabolites in tissues and cells and offer powerful new tools to better understand the function and segregated biochemistry of cells and organs. Unfortunately, the resolution of the majority of commercial instruments is still somewhat limiting with current resolutions at an approximately 100- μm diameter for the incident circular laser pixel, because many biological questions require much higher spatial resolution. Advanced instruments with 10- μm resolution have been constructed and are expected to be commercially available in the near future. However, the 10- μm resolution is still at the multicellular level for many plants, and pixels of not more than 1-2 μm will be necessary for single-cell resolution. Subcellular metabolite imaging will require substantial improvements in laser optics and cycle times and these improvements are not likely in the immediate near future.

Advances in resources

Additional indicators that metabolomics has moved into mainstream include the increasing abundance of resources now available. There have been several journal special issues dedicated to plant metabolomics, including *Physiologia Plantarum* (2008, vol. 132, issue 2), *Metabolomics* (2007, vol. 3, issue 3), *Trends in Analytical Chemistry* (2008, vol. 27, issue 3), and *Phytochemistry* (2003, vol. 62, issue 6). Numerous other special issues and books have focused on metabolomics in general. These special issues reveal both a growing audience and an expanding field of practitioners. Numerous plant metabolomics databases and informatics resources are now available, and these have been recently reviewed [32,33]. Specific informatics resources for plant metabolomics include Platform for Riken Metabolomics (PRIME) [34], which contains multiple spectral and data processing tools; MassBank, a high-resolution mass spectral database [35]; The Golm Metabolome Database [36,37]; the Plant Metabolic Network [38,39]; and the Madison Metabolomics Consortium Database [40].

Metabolomics research requires tangible funding, and substantial investments have been made by most research universities and institutions, at least at the instrumental level. The federal government is also investing in plant metabolomics. For example, a national consortium has recently been funded by National Science Foundation's Arabidopsis 2010 Program, which is focused upon the use of metabolomics as a functional genomics tool for deciphering *Arabidopsis* genes of currently unknown function [41]. Other federal funding has been awarded for animals and microbes, and the National Institutes of Health (NIH) has had two specific metabolomics program solicitations and awards. However, it is interesting that many other nations (The Netherlands, Australia, and Canada) appear to have invested dramatically more heavily in metabolomics than the US. Hopefully, this trend will change.

Finally, as the field of plant metabolomics has matured, substantial efforts have been made toward standardizing metabolomics. In conjunction with the Metabolomics Society, the NIH convened a special meeting on standards in 2005 that facilitated the Metabolomics Standards Initiative (MSI) [42]. After much discussion, the MSI moved forward and the *Metabolomics* journal published a special issue on the MSI [43] which contained a large number of articles that proposed minimum metabolomics reporting standards related to multiple topics, including plant biology [44], chemical analyses [45], and data processing [46]. Several exemplary plant metabolomics studies that have adopted these standards have now been published [7,47]. It is envisioned that future articles and journals are highly likely to require a substantial level of compliance with these evolving standards.

Future directions

Although plant metabolomics has proven to be a valuable tool, there is still substantial room for growth and improvement. The major challenges that still face plant and all other metabolomics are temporal and spatial analyses, dynamic range limitations, lack of comprehensiveness, and limited metabolite annotations.

Many approaches to plant metabolomics still sample bulk tissues for analysis. However, plant biochemistry and physiology are highly spatially and temporally segregated at the subcellular and multicellular levels. Thus, complex sampling of anatomically and temporally resolved tissues will be necessary if we are ever to understand the sophisticated spatial and temporal organization of plant biochemistry and physiological functions. This logic also translates to the subcellular level.

The concentration range in cellular metabolites is estimated to be greater than 10^{12} . However, the best dynamic range of modern MS and NMR is approximately 10^6 . Thus, a million-fold increase in dynamic range is necessary to observe all of the cellular metabolites and an even larger increase is necessary if accurate quantification is to be achieved. Such an advance in instrumental dynamic range is not likely to be achieved in the near future given that half a century of instrumental research has led to modern MS and NMR. However, incremental advances are still reasonably expected.

The current practice of metabolomics relies upon a diversity of instrumental platforms, of which GC-MS, LC-MS, ultra-performance LC-MS, capillary electrophoresis-MS, and NMR [48,49] are the most common. These tools, individually or in combination, provide qualitative and quantitative characterization of large numbers of metabolites. Although the individual and combined tools provide substantial metabolome coverage, they are still far from being comprehensive [50]. Currently, one of the most sophisticated plant metabolomics consortiums [41] is composed of the expertise from six laboratories that use nine instrumental platforms. The combined consortium coverage is estimated at approximately 1800 metabolites, of which 900 are chemically defined. Although this is impressive, it still pales in comparison with the currently unknown, but estimated, metabolome within a given plant species as 10,000 or more. Thus, current metabolome depth of coverage is approaching 20% and there is substantial room for improvement. As the coverage increases, pastures that are more productive are to be expected.

The above comparison also emphasizes the need for greater metabolite identifications within current and future metabolomics profiles. Even though it is possible to differentiate a large number of metabolic components based upon the instrumental data (i.e., chromatographic retention, accurate mass, MS or MS/MS fragmentation, one-dimensional or two-dimensional NMR chemical shifts, and so on), biological context is directly linked to chemical identity. Expansion of the chemical context will provide a wider field of vision and a greater depth of biological understanding.

Although the above text itemizes multiple areas for future improvement, the great news is that metabolomics, in its current state, is an informative tool that is revolutionizing the biological sciences. Future improvements can and will lead to greater utility and greener pastures!

Abbreviations

DNP, dynamic nuclear polarization; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; MSI, Metabolomics Standards Initiative; MS/MS, tandem mass spectrometry (mass spectrometry/mass spectrometry); NIH, National Institutes of Health; NMR, nuclear magnetic resonance; SPE, solid-phase extraction.

Competing interests

The author declares that he has no competing interests.

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References

- Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey R, Willmitzer L: **Metabolite profiling for plant functional genomics.** *Nat Biotechnol* 2000, **18**:157-61.
- Sumner LW, Mendes P, Dixon RA: **Plant metabolomics: large-scale phytochemistry in the functional genomics era.** *Phytochemistry* 2003, **62**:817-36.
- Guy C, Kopka J, Moritz T: **Plant metabolomics coming of age.** *Physiologia Plantarum* 2008, **132**:113-6.
- Hall RD, Brouwer ID, Fitzgerald MA: **Plant metabolomics and its potential application for human nutrition.** *Physiol Plant* 2008, **132**:162-75.
- Schauer N, Fernie AR: **Plant metabolomics: towards biological function and mechanism.** *Trends Plant Sci* 2006, **11**:508-16.
- Farag M, Deavours BE, Naoumkina M, De-Fatima A, Dixon RA, Sumner LW: **Integrated metabolite and transcript profiling identifies a mechanism for the biosynthesis of an auroone phytoalexin in *Medicago truncatula* cell cultures.** *Plant Physiol* 2009, **151**:1096-113.
- Farag MA, Huhman DV, Dixon RA, Sumner LW: **Metabolomics reveals novel pathways and differential mechanistic and elicitor-specific responses in phenylpropanoid and isoflavonoid biosynthesis in *Medicago truncatula* cell cultures.** *Plant Physiol* 2008, **146**:387-402.
- Naoumkina M, Farag MA, Sumner LW, Tang Y, Liu CJ, Dixon RA: **Inaugural article: different mechanisms for phytoalexin induction by pathogen and wound signals in *Medicago truncatula*.** *Proc Natl Acad Sci U S A* 2007, **104**:17909-15.
- Saito K, Matsuda F: **Metabolomics for functional genomics, systems biology, and biotechnology.** *Annu Rev Plant Biol* 2008, [Epub ahead of print].
- Saito K, Hirai MY, Yonekura-Sakakiba K: **Decoding genes with coexpression networks and metabolomics - 'majority report by precogs'.** *Trends Plant Sci* 2008, **13**:36-43.
- Hirai MY, Sugiyama K, Sawada Y, Tohge T, Obayashi T, Suzuki A, Araki R, Sakurai N, Suzuki H, Aoki K, Goda H, Nishizawa OI, Shibata D, Saito K: **Omics-based identification of *Arabidopsis* Myb transcription factors regulating aliphatic glucosinolate biosynthesis.** *Proc Natl Acad Sci U S A* 2007, **104**:6478-83.
- Tohge T, Nishiyama Y, Hirai MY, Yano M, Nakajima J, Awazuhara M, Inoue E, Takahashi H, Goodenowe DB, Kitayama M, Noji M, Yamazaki M, Saito K: **Functional genomics by integrated analysis of metabolome and transcriptome of *Arabidopsis* plants over-expressing an MYB transcription factor.** *Plant J* 2005, **42**:218-35.
- Yonekura-Sakakibara K, Tohge T, Matsuda F, Nakabayashi R, Takayama H, Niida R, Watanabe-Takahashi A, Inoue E, Saito K: **Comprehensive flavonol profiling and transcriptome coexpression analysis leading to decoding gene-metabolite correlations in *Arabidopsis*.** *Plant Cell* 2008, **20**:2160-76.
- Fernie AR, Schauer N: **Metabolomics-assisted breeding: a viable option for crop improvement?** *Trends Genet* 2009, **25**:39-48.
- Meyer RC, Steinfath M, Lisec J, Becher M, Witucka-Wall H, Törjék O, Fiehn O, Eckardt A, Willmitzer L, Selbig J, Altmann T: **The metabolic signature related to high plant growth rate in *Arabidopsis thaliana*.** *Proc Natl Acad Sci U S A* 2007, **104**:4759-64.

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- Lisec J, Meyer RC, Steinfath M, Redestig H, Becher M, Witucka-Wall H, Fiehn O, Törjék O, Selbig J, Altmann T, Willmitzer L: **Identification of metabolic and biomass QTL in *Arabidopsis thaliana* in a parallel analysis of RIL and IL populations.** *Plant J* 2008, **53**:960-72.
- Skogerson K, Runnebaum R, Wohlgenuth G, de Ropp J, Heymann H, Fiehn O: **Comparison of gas chromatography-coupled time-of-flight mass spectrometry and 1H nuclear magnetic resonance spectroscopy metabolite identification in white wines from a sensory study investigating wine body.** *J Agric Food Chem* 2009, **57**:6899-907.
- Zanor MI, Rambla JL, Chaib J, Steppa A, Medina A, Granell A, Fernie AR, Causse M: **Metabolic characterization of loci affecting sensory attributes in tomato allows an assessment of the influence of the levels of primary metabolites and volatile organic contents.** *J Exp Bot* 2009, **60**:2139-54.
- DellaPenna D, Pogson BJ: **Vitamin synthesis in plants: tocopherols and carotenoids.** *Annu Rev Plant Biol* 2006, **57**:711-38.
- Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, Potrykus I: **Golden Rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency.** *J Nutr* 2002, **132**:506S-510.
- DellaPenna D: **Biofortification of plant-based food: enhancing folate levels by metabolic engineering.** *Proc Natl Acad Sci U S A* 2007, **104**:3675-6.
- Bekaert S, Storozhenko S, Mehrshahi P, Bennett MJ, Lambert W, Gregory JF 3rd, Schubert K, Hugenholtz J, Van Der Straeten D, Hanson AD: **Folate biofortification in food plants.** *Trends Plant Sci* 2008, **13**:28-35.
- Xie D-Y, Sharma SB, Wright E, Wang Z-Y, Dixon RA: **Engineering plants for introduction of health-beneficial proanthocyanidins.** *Plant J* 2006, **45**:895-907.
- Vanholme R, Morreel K, Ralph J, Boerjan W: **Lignin engineering.** *Curr Opin Plant Biol* 2008, **11**:278-85.
- Crockford DJ, Holmes E, Lindon JC, Plumb RS, Zirah S, Bruce SJ, Rainville P, Stumpf CL, Nicholson JK: **Statistical heterospectroscopy, an approach to the integrated analysis of NMR and UPLC-MS data sets: application in metabolomic toxicology studies.** *Anal Chem* 2006, **78**:363-71.
- Ward JL, Baker JM, Beale MH: **Recent applications of NMR spectroscopy in plant metabolomics.** *FEBS J* 2007, **274**:1126-31.
- Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K: **Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR.** *Proc Natl Acad Sci U S A* 2003, **100**:10158-63.
- Golman K, Ardenkjaer-Larsen JH, Petersson JS, Mansson S, Leunbach I: **Molecular imaging with endogenous substances.** *Proc Natl Acad Sci U S A* 2003, **100**:10435-9.
- Nemes P, Barton AA, Vertes A: **Three-dimensional imaging of metabolites in tissues under ambient conditions by laser ablation electrospray ionization mass spectrometry.** *Anal Chem* 2009, [Epub ahead of print].
- Burrell MM, Earnshaw CJ, Clench MR: **Imaging matrix assisted laser desorption ionization mass spectrometry: a technique**

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- to map plant metabolites within tissues at high spatial resolution. *J Exp Bot* 2007, **58**:757-63.
31. Li Y, Shrestha B, Vertes A: **Atmospheric pressure infrared MALDI imaging mass spectrometry for plant metabolomics.** *Anal Chem* 2008, **80**:407-20.
 32. Tohge T, Fernie AR: **Web-based resources for mass-spectrometry-based metabolomics: a user's guide.** *Phytochemistry* 2009, **70**:450-6.
 33. Go EP: **Database resources in metabolomics: an overview.** *J Neuroimmune Pharmacol* 2009, [Epub ahead of print]
 34. Akiyama K, Chikayama E, Yuasa H, Shimada Y, Tohge T, Shinozaki K, Hirai MY, Sakurai T, Kikuchi J, Saito K: **PRIME: a Web site that assembles tools for metabolomics and transcriptomics.** *In Silico Biol* 2008, **8**:339-45.
 35. **About MassBank.** [<http://www.massbank.jp/en/about.html>]
 36. **GMD@CSB.DB: the Golm Metabolome Database.** [<http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html>]
 37. Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmüller E, Dörmann P, Weckwerth W, Gibon Y, Stitt M, Willmitzer L, Fernie AR, Steinhauser D: **GMD@CSB.DB: the Golm Metabolome Database.** *Bioinformatics* 2005, **21**:1635-8.
 38. **Plant Metabolic Network - Plant Metabolic Pathway Databases.** [<http://www.plantcyc.org>]
 39. Wurtele ES, Li L, Berleant D, Cook D, Dickerson JA, Ding J, Hofmann H, Lawrence M, Lee EK, Li J, Mentzen W, Miller L, Nikolau BJ, Ranson N, Wang Y: **MetNet: Systems biology software for Arabidopsis** In *Concepts in Plant Metabolomics*. Edited by Nikolau BJ, Wurtele ES: Dordrecht, The Netherlands: Springer; 2007:145-58.
 40. **Madison Metabolomics Consortium Database.** [<http://mmcd.nmrfam.wisc.edu>]
 41. **Arabidopsis Metabolomics Consortium - About Plantmetabolomics.** [http://lab.bcb.iastate.edu/sandbox/pbais05/alpha/plantmetabolomics_trimmed/index.php]
 42. Castle AL, Fiehn O, Kaddurah-Daouk R, Lindon JC: **Metabolomics Standards Workshop and the development of international standards for reporting metabolomics experimental results.** *Brief Bioinform* 2006, **7**:159-65.
 43. Fiehn O, Robertson D, Griffin J, van der Werf M, Nikolau B, Morrison N, Sumner L, Goodacre R, Hardy N, Taylor C, Fostel J, Kristal B, Kaddurah-Daouk R, Mendes P, van Ommen B, Lindon J, Sansone S-A: **The metabolomics standards initiative (MSI).** *Metabolomics* 2007, **3**:175-8.
 44. Fiehn O, Sumner L, Rhee S, Ward J, Dickerson J, Lange B, Lane G, Roessner U, Last R, Nikolau B: **Minimum reporting standards for plant biology context information in metabolomic studies.** *Metabolomics* 2007, **3**:195-201.
 45. Sumner L, Amberg A, Barrett D, Beale M, Beger R, Daykin C, Fan T, Fiehn O, Goodacre R, Griffin J, Hankemeier T, Hardy N, Harnly J, Higashi R, Kopka J, Lane A, Lindon J, Marriott P, Nicholls A, Reily M, Thaden J, Viant M: **Proposed minimum reporting standards for chemical analysis.** *Metabolomics* 2007, **3**:211-21.
 46. Goodacre RD, Broadhurst A, Smilde B, Kristal J, Baker J, Beger R, Bessant C, Connor S, Capuani G, Craig A, Ebbels T, Kell D, Manetti C, Newton J, Paternostro G, Somorjai R, Sjöström M, Trygg J, Wulfert F: **Proposed minimum reporting standards for data analysis in metabolomics.** *Metabolomics* 2007, **3**:231-41.
 47. Fiehn O, Wohlgemuth G, Scholz M, Kind T, Lee do Y, Lu Y, Moon S, Nikolau B: **Quality control for plant metabolomics: reporting MSI-compliant studies.** *Plant J* 2008, **53**:691-704.
 48. Dunn Warwick B, Ellis DE: **Metabolomics: current analytical platforms and methodologies.** *Trends Analyt Chem* 2005, **24**:285-94.
 49. Dettmer K, Aronov PA, Hammock BD: **Mass spectrometry-based metabolomics.** *Mass Spectrom Rev* 2007, **26**:51-78.
 50. Bino RJ, Hall RD, Fiehn O, Kopka J, Saito K, Draper J, Nikolau BJ, Mendes P, Roessner-Tunalı U, Beale MH, Trethewey RN, Lange BM, Wurtele ES, Sumner LW: **Potential of metabolomics as a functional genomics tool.** *Trends Plant Sci* 2004, **9**:418-25.