**Introduction**

Plants are our major renewable source of biomass due primarily to their capacity to capture the sun’s energy (in the form of light) and convert it into energy-rich molecules such as sucrose, polysaccharides (starch and/or fructans), proteins or fats. These molecules are subsequently catabolised to release energy for growth, development, and defence. Plants are vital to the survival of mankind as a source of photosynthetic oxygen, as our primary food source and as a major source of fuel, fibre, timber and pharmaceuticals (for example, aspirin is a natural plant secondary metabolite). Plants may be either microscopic in size and relatively simple in structure/organisation, such as unicellular algae, or magnificent multicellular, multi-organ (roots/leaves/stems), complex systems, such as trees typified by the majestic eucalypts, the world’s tallest organisms. Plants are generally distinguished from animals in that they possess a chlorophyll-containing plastid (plastid), are usually immobile, are devoid of a central nervous system and their cells are surrounded by a polysaccharide (and sometimes lignin)-rich cell wall that constitutes the skeleton of the plant kingdom.

The study of plant metabolism is central to the discipline of plant physiology/biochemistry. The early work of Melvin Calvin and Andrew Benson, who discovered the photosynthetic ‘dark’ reactions, today commonly called the ‘Calvin cycle’ (1), provided the basis for an in-depth investigation of other plant-specific metabolic pathways, including the starch biosynthetic pathway, cell wall biosynthesis, vitamin production, sucrose synthesis and recycling, and amino acid biosynthesis, as well as fatty acid synthesis and degradation. These early studies paved the way for more ‘holistic’ approaches towards understanding how these individual pathways are regulated in a co-ordinated fashion to effect plant growth and development and determine plant response(s) to environmental assaults. High-throughput approaches to determine the expression levels of all genes (transcriptomics) and the detection, identification and quantification of soluble and membrane proteins (proteomics) have more recently been combined with metabolomic approaches and powerful informatic tools to mine the data in an integrated ‘systems biology’ approach and to transform our understanding of plant growth and development. Metabolomics, which is the separation, detection and quantification of ‘all’ metabolites in a sample using either gas chromatography (GC) or liquid chromatography (LC) coupled to mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (NMR), has been applied to many areas of plant sciences (Fig. 1; for summary, see refs 2 and 3).

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**Fig. 1.** Schematic workflow of a metabolomics approach from tissue harvest through to data interpretation using complementary analytical instrumentation for greater comprehensiveness of metabolite detection and quantification. Figure modified with kind permission of Springer Science+Business Media (Figure 3.10. in Roessner and Beckles (2009), Chapter 3, pp 59, in Plant Metabolic Networks, ISBN 978-0-387-78744-2).
Dos and Don’ts of Plant Metabolomics

When working with plant metabolites, it is important to consider that plant metabolism is highly dependent on the time of the day and the quantity and quality of light the plant is exposed to during growth. Metabolite levels can alter quite dramatically between day and night, a process known as diurnal fluctuation (4). A number of metabolic pathways are dependent on carbon availability and therefore on the rate of photosynthesis and respiration, which change with light availability. Thus special care must be taken when plant tissue samples are harvested; ideally, all sampling should be conducted at the same time of the day or within a very small time frame in order to minimise this biological variation (Fig. 1).

When samples are harvested for a metabolomics experiment (irrespective of the biological source of tissue), it is important to quench metabolism as quickly as possible. A number of procedures have been developed for quenching plant metabolism, but the simplest and most effective appears to be snap freezing in liquid nitrogen or freeze clamping (Fig. 1) (2). Freezing in liquid nitrogen is the most common technique, but care has to be exercised not to freeze overly large pieces of tissue as this could create a gradient of arrest of metabolism across the sample at the time of harvest, i.e., a non-biological variation. Frozen plant tissue samples can be stored at -80°C until extraction or freeze-dried for longer storage as long as samples are kept in a dry environment to avoid any water absorption which would activate enzymatic reactions and/or cause degradation of chemically labile metabolites, such as phosphorylated compounds.

The next crucial step in metabolite analyses is the extraction of the low molecular weight metabolites prior to their separation and detection. Plant tissues and cells are inherently resistant to extraction because of the presence of the cell wall and surface structures, such as cuticles, and usually need to be ground to a powder prior to extraction with a mixture of solvents of varying hydrophilicity/hydrophobicity. These extraction procedures allow separation of the small molecules (metabolites) from the macromolecules, such as proteins, polysaccharides and nucleic acids (DNA and RNA). A range of homogenisation and extraction methods have been described (for review, see ref 2). The suitability of each of those methods has to be optimised and tailored for each plant species/tissue type and targeted to the particular class of metabolites under investigation.

Most plant metabolomics approaches have used either whole plants, organs such as leaves or roots, or parts of tissues which are homogenised and extracted from a powder. Since plant organs are heterogeneous, many different cell types will be present (plants have over 40 cell types) and hence any analysis of a whole organ is an ‘averaged’ composition rather than one that reflects the metabolome of a particular cell type. Recent developments are focussing on using either 1. laser micro-dissection technology to excise particular cell types, which are then analysed by conventional metabolomics approaches outlined above or 2. in situ mass spectrometry for metabolite imaging across a particular tissue (for example, see ref 5). The latter approach involves mounting tissue sections on a MALDI plate and exposure to laser pulses. Molecules are ablated by the laser and ionised by the matrix to be carried into the MS for analysis. This results in a mass spectrum representing molecules present in the area of the laser pulses, which represents an area as small as 50 μm. Sophisticated software then enables deconvolution of individual masses across the tissue and the generation of a tissue ‘image’. Although the technology is in its infancy, exciting reports are emerging, demonstrating its potential in plant metabolomics.

Metabolomics Applications

There are a large number of potential applications of metabolomics technologies in plant sciences, including studying development, phenotyping of genetically altered plants, qualitative trait analysis, and systems biology (for review, see ref 3). In addition, metabolomics technologies can be utilised for the discovery and identification of markers of diseased and stressed plants, as well as following genetic modifications. In the past twenty years, major advances have been made in the ability to introduce additional endogenous or foreign genes into plants (genetic engineering) in order to increase, decrease or eliminate the activity of a gene. This has resulted in a need to determine the effects of these genetic modifications on the performance of the plants, as well as the influence of altered gene activity on the cellular composition of the plant (6,7). Metabolomics, as well as other ‘omics’ type multi-parallel technologies such as proteomics and transcriptomics, can be utilised to monitor the effects of transgenesis, not only under normal growth conditions, but also under stress, allowing one to estimate and assess the potential risks associated with transgenesis (8). Given the scope of this article, we will now describe one area of application of metabolomics that is being conducted within the Australian Centre for Plant Functional Genomics (ACPFG).

Metabolomics is an important tool in the repertoire of functional genomics techniques, enabling Australian researchers to investigate the adaptation mechanisms employed by plants in responding to abiotic (and biotic) stresses. This results in a better understanding of what occurs at the biochemical, cellular and whole plant levels, with the eventual aim of producing novel crop varieties that are better able to cope with these stresses. These stresses may include drought, salinity, mineral toxicity and deficiencies, and extreme temperatures, and cause major reductions in crop yield and grain quality, leading to major economic losses and food shortages. A well-known response to osmotic shock, a common consequence of many abiotic stresses, is to alter the types and concentrations of intracellular solutes. This is usually achieved by increased synthesis and accumulation of compatible solutes, commonly referred to as osmoprotectants, such as polyols (including glycerol and sorbitol), amino acids (especially proline), quaternary nitrogen compounds (glycine betaine), or tertiary sulfonium compounds (dimethylsulfonopropionate) (9,10). Metabolomics allows the determination of changes in the levels of these important osmolytes and osmoprotectants, as well as a range of other metabolites, providing a more accurate overview of cellular stress responses, which can
then be integrated with the physiology of the stress response. Exciting outcomes of the application of this technology are emerging. For example, Kaplan et al. (11) and Cook et al. (12) have determined the metabolic changes of Arabidopsis thaliana, the ‘lab rat’ of plants, in response to and following adaptation to different temperatures. Low temperatures had greater repercussions for metabolite levels than did high temperatures, and several previously unknown adaptive responses to cold stress were revealed, including changes in cellular amino acids, TCA cycle intermediates and carbohydrate metabolism.

Another exciting approach is to compare the metabolic responses of commercially important crop species with those of closely related wild species or landraces, which are characterised by higher tolerance levels to a particular stress condition (Fig. 2). A recent example has been reported where the metabolite responses to salinity were compared for two barley cultivars that differ in their tolerance to high salt conditions (13). Clipper, a commercial cultivar, showed extensive stress symptoms typified by growth reduction and necrotic leaf regions, whereas Sahara, an Algerian landrace, maintained its growth, similar to that of control untreated plants, and only showed some evidence of leaf necrosis after five weeks of salt treatment. The leaves of both Clipper and Sahara plants had similar, highly elevated sodium concentrations, suggesting a higher tissue tolerance level of Sahara to equivalent sodium levels. In order to gain a better understanding of the underlying mechanisms resulting in the greater tolerance level of Sahara, both plant varieties were grown in high salinity conditions and metabolite responses monitored and compared to the respective control plants. In the leaves of Clipper, the more sensitive cultivar with greater stress symptoms, the levels of most amino acids were elevated, which was correlated to the greater degree of cell damage/necrosis. In contrast, Sahara responded differently, with increased levels of organic acids and sugars following salt treatment. These compounds were proposed to be involved in cellular protection of the leaf tissues to high salt concentrations, offering an explanation for the greater tolerance of Sahara to salt. This example demonstrates that if individual metabolites or metabolic pathways are identified that underlie the adaptation and/or tolerance mechanism(s) of a plant variety/species to an abiotic stress, this could result in novel breeding strategies, leading to the production of crop varieties capable of better withstanding those stress conditions and maintaining yield under stress (Fig. 2).

Conclusions

The demands of a world where the human population continues to grow exponentially, combined with the impacts of global climate change and a finite fossil fuel resource, will place enormous demands on agricultural and forestry production systems. Better environmentally adapted crop (and forest) species will need to be generated to maintain yield. Delivering health outcomes through enhanced food quality (functional foods) will also lead to a better quality of life, as well as impacting positively on the health budgets of the developing world economies as the diseases of atherosclerosis, obesity and diabetes are directly related to the quantity and quality of the food we eat. Furthermore, the need for alternative transport fuels has led to a major push for ligno-cellulosic biomass, which will require the development of new crops that are optimised for biomass rather than for seed production, a fundamental paradigm shift in plant breeding strategies. All these developments will involve the need to fundamentally alter plant metabolism and tailor it for specific outcomes. Metabolites are at the heart of this process, yet our understanding of how metabolic pathways are regulated is at best rudimentary. The past few years have seen dramatic developments in high-throughput metabolite analysis (metabolomics), which, together with further advances that allow for cellular and subcellular resolution of metabolite analyses and the integration of these datasets with the other ‘-omics’ through bioinformatics, make us ideally placed to make significant inroads into understanding these processes and their regulation in planta, thereby enabling rational design of novel crops.

Fig. 2.
Example of effects of dryland salinity onto wheat cultivars differing in their ability to withstand salt.

References


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