REVIEW

Metabolomics for plant stress response

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Stress in plants could be defined as any change in growth condition(s) that disrupts metabolic homeostasis and requires an adjustment of metabolic pathways in a process that is usually referred to as acclimation. Metabolomics could contribute significantly to the study of stress biology in plants and other organisms by identifying different compounds, such as by-products of stress metabolism, stress signal transduction molecules or molecules that are part of the acclimation response of plants. These could be further tested by direct measurements, correlated with changes in transcriptome and proteome expression and confirmed by mutant analysis. In this review, we will discuss recent application of metabolomics and system biology to the area of plant stress response. We will describe approaches such as metabolic profiling and metabolic fingerprinting as well as combination of different 'omics' platforms to achieve a holistic view of the plant response stress and conduct detailed pathway analysis.

Introduction

Environmental stress could be defined in plants as any change in growth condition(s), within the plant's natural habitat, that alters or disrupts its metabolic homeostasis. Such change(s) in growth condition requires an adjustment of metabolic pathways, aimed at achieving a new state of homeostasis, in a process that is usually referred to as acclimation (Mittler 2006, Suzuki and Mittler 2006). Several different phases are thought to be involved in acclimation. In the initial stages, the change in environmental condition is sensed by the plant and activates a network of signaling pathways. In later phases, the signal transduction pathways activated in the first phase trigger the production of different proteins and compounds that restore or achieve a new state of homeostasis. From the standpoint of metabolomics, at least three different types of compounds are important for these processes: (1) compounds involved in the acclimation process such as antioxidants or osmoprotectants; (2) by-products of stress that appear in cells because of the disruption of normal homeostasis by the alteration(s) in growth conditions; and (3) signal transduction molecules involved in mediating the acclimation response. The signal transduction molecules could be newly synthesized compounds or compounds that are released from their conjugated form(s), such as the plant hormone salicylic acid, or they could be different by-products of stress metabolism (similar to point 2 mentioned above) that signal disruption of cellular homeostasis. The second type of signaling molecules could include by-products of membrane degradation, different reactive oxygen species (ROS) or various oxidized compounds such as phenolic compounds or even some antioxidants (Mittler 2002, Mittler et al. 2004).

Abbreviations – CE-MS, capillary electrophoresis–mass spectrometry; DFA, discriminant function analysis; EI, electron impact; FT-IR, Fourier transform infrared; GAs, genetic algorithms; GC, gas chromatography; LC-MS, liquid chromatography–mass spectrometry; PCA, principal component analysis; PLS, partial least squares; ROS, reactive oxygen species; SOMs, self-organizing maps; TOF, time-of-flight.

A few examples for plant metabolites involved in biotic/abiotic stress responses include compounds such as polyols mannitol and sorbitol; dimethylsulfonium compounds, such as dimethylsulfoniopropionate, glycine betaine; sugars such as sucrose, trehalose and fructan; or amino acids such as proline and ectoine that serve as osmolytes and osmoprotectant to protect plants under extreme salt, drought and desiccation stresses. A variety of epicuticular waxes protect plants from excess water loss during drought and serves as a mechanical barrier to confront pathogens. The saturation level of membrane fatty acids can significantly alter chilling tolerance. Many small molecules protect plants from oxidative damage associated with a variety of stresses. Ascorbic acid, glutathione, tocopherols, anthocyanins and carotenoids protect plant tissues by scavenging active oxygen intermediates generated during oxidative stress. The plant defense response is associated with the production of phytoalexins, activation of the general phenylpropanoid pathway and induction of lignin biosynthesis. Salicylic acid, methyl salicylate, jasmonic acid, methyl jasmonate and other small molecules produced as a result of stress can also serve as signaling molecules activating systemic defense and acclimation responses.

In view of the above, it is clear that the field of metabolic profiling could contribute significantly to the study of stress biology in plants and other organisms. A detailed time-course metabolic profiling analysis of plants subjected to stress could lead to the identification of many of the compounds mentioned above. These could be further tested by direct measurements, correlated with changes in transcriptome and proteome expression, and confirmed by mutant analysis. With respect to the studies described above, metabolic profiling could in fact be the most important tool in identifying the early compounds that signal the perception of stress because these would act even before any change(s) in the transcriptome or proteome could be detected. In this review, we will summarize some of the key tools and techniques used to study the metabolome of plants during stress.

Metabolomics technology

Metabolomics is a rapidly developing technology. Major approaches currently used in plant metabolomics research include metabolic fingerprinting, metabolite profiling and targeted analysis (Fiehn 2002, Halket et al. 2005, Shulaev 2006). Depending on the question asked in each particular study, specific metabolomics approaches or their combination are used. Some of these are described below.

Metabolic fingerprinting

Metabolic fingerprinting is largely used to identify metabolic signatures or patterns associated with a particular stress response without identification or precise quantification of all the different metabolites in the sample. Pattern recognition analysis is then performed on the data to identify features specific to a fingerprint. Fingerprinting can be performed with a variety of analytical techniques, including NMR (Krishnan et al. 2005), MS (Goodacre et al. 2003), Fourier transform ion cyclotron resonance mass spectrometry or Fourier transform infrared (FT-IR) spectroscopy (Johnson et al. 2003).

One of the limitations of NMR spectroscopy is its low sensitivity, which makes it difficult to detect low-abundance cellular metabolites. MS has an advantage over NMR in terms of resolving power, providing higher sensitivity and lower limit of detection. However, MS generates more complex spectrum because of the formation of product ions and adducts, and its results comes in a form of discriminant ions. This can provide a significant challenge for data validation. Using MS with different classification tools, a larger subset of metabolites associated with the phenotype can be identified.

Metabolic fingerprints can be analyzed with a variety of pattern recognition and multivariate statistic techniques (Sumner et al. 2003). Both unsupervised and supervised algorithms have been used in fingerprinting, although supervised techniques generally show greater discrimination power. Unsupervised techniques most often used with metabolomics data include principal component analysis (PCA), self-organizing maps (SOMs) and hierarchical clustering, while supervised algorithms include discriminant function analysis (DFA), partial least squares (PLS) and ANOVA. Most metabolomics data sets are underdetermined, meaning they contain many more variables than samples (Kohane et al. 2003), and for proper statistical analysis, it is important to reduce the number of variables to obtain uncorrelated features in the data. This can be achieved by using evolutionary algorithms such as genetic algorithms (GAs) or genetic programming (Pena-Reyes and Sipper 2000). For metabolomics applications, evolutionary algorithms are typically combined with the secondary algorithm (e.g. DFA or PLS) (Goodacre 2005).

In order to increase sample throughput mass spectra are usually obtained using direct infusion of the analytical sample into a mass spectrometer, i.e. without fractionation. However, direct infusion has problems, mostly because of a phenomenon known as cosuppression where the signal of many analytes can be lost at the mass spectrometer interface.

To minimize the cosuppression effect, samples can be separated using very rapid gradients with a short chromatographic column and the HPLC-MS data can then be analyzed using multivariate analysis to identify the discriminant ions. To confirm the fingerprinting results, samples are then re-analyzed with long HPLC gradient. This two-step fingerprinting/validating strategy was used to characterize the wound response in Arabidopsis (Grata et al. 2007).

In our laboratory, we have been employing a similar approach to metabolic fingerprinting where we actually carry out a chromatographic or electrophoretic separation prior to the MS. This is similar to what is performed for metabolite profiling, except that we do not attempt to identify all the molecules responsible for the peaks in the separation, rather, we focus on those that demonstrate to be discriminant between groups. This approach significantly reduces the cosuppression effect seen in direct infusion MS, the dominant method used for fingerprinting. Fig. 1 shows the comparison of the summary mass spectrum obtained following chromatographic separation using capillary column or direct infusion. Distribution of m/z within the acquisition mass range of 100–1500 atomic mass units using chromatography prior to MS shows ions at m/z 404, 579, 636, 740, 824, 1173, 1343 and 1392 corresponding to important plant metabolites including flavonoids and anthocyanins. These and many other ions are almost undetectable in the mass spectrum obtained by direct infusion because of the matrix suppression effect. Following the data acquisition, we have a data cube consisting of thousands of mass spectra at different elution times. This is then transformed into a single cumulative mass spectrum that is equivalent to what a direct infusion mass spectrum would be minus the cosuppression interference. This cumulative spectrum is then used for sample discrimination using statistical and machine-learning algorithms. Since all the original data cube where the separation data are actually kept for later analysis, we can inspect its details and identify specific molecules of interest against a library without a need for additional experiments.

Metabolite profiling

Metabolite profiling is aimed at a simultaneous measurement of all or a set of metabolites in a sample. Multiple analytical techniques can be used for metabolite profiling (Shulaev 2006, Sumner et al. 2003). These techniques include NMR, GC-MS, liquid chromatography–mass spectrometry (LC-MS), capillary electrophoresis–mass spectrometry (CE-MS) and FT-IR spectroscopy. The advantages and disadvantages of each technique for metabolite profiling were previously discussed (Shulaev 2006, Sumner et al. 2003).

To date, GC-MS is the most developed analytical platform for plant metabolite profiling. Historically, it was one of the first techniques used for high-throughput metabolite profiling in plants (Roessner et al. 2000). The GC-MS is generally performed using electron impact (EI) quadrupole or time-of-flight (TOF) mass spectrometry (Fiehn et al. 2000, Roessner et al. 2000). Using GC-MS, it is possible to profile several hundred compounds
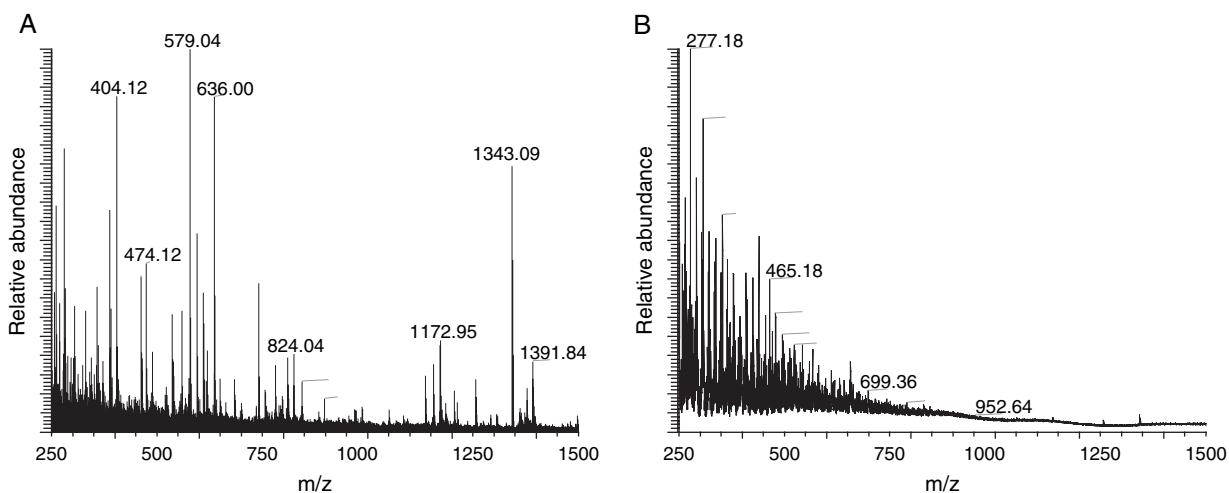

Fig. 1. Summary mass spectrum of Arabidopsis leaf extract following either chromatographic separation (A) or direct infusion (B). Ions were detected for positive ionization full-scan MS. Chromatography was performed on a 0.1 × 450 mm monolithic C18 column. Summary mass spectrum, which derives from adding up all mass scans over the chromatographic run, shows distribution of m/z within the acquisition mass range of 100–1500 atomic mass units, exceeding S/N > 6.

belonging to diverse chemical classes including sugars, organic acids, amino acids, sugar alcohols, aromatic amines and fatty acids.

The major advantage of GC-MS for metabolomics is the availability of both commercially and publicly available EI spectral libraries (Halket et al. 2005). The limitation of the GC-MS profiling is that it can only analyze volatile compounds or compounds that can be volatilized following chemical derivatization.

For non-volatile compounds, LC-MS and CE-MS provide a better alternative. LC-MS application in metabolomics is steadily increasing especially after the recent adoption of the ultra performance liquid chromatography technology that can dramatically increase separation efficiency and decrease analysis time (Giri et al. 2007, Granger et al. 2007). CE-MS provides a viable alternative for metabolite profiling due to its high resolving power, low sample volume requirements and the ability to separate cations, anions and uncharged molecules simultaneously (Soga et al. 2003).

Targeted analysis

Untargeted metabolite profiling is often paralleled with metabolomics because it is most often used in metabolomics studies. It is particularly useful to obtain a global view of the metabolism of cells or identify new metabolites/pathways. A substantial drawback of untargeted profiling is that it is semiquantitative, i.e. it provides relative concentration data based on the same 'surrogate' internal standard. These semiquantitative data have to be further validated using targeted quantitative assays. Targeted profiling is used when it is necessary to determine the precise concentration of a limited number of known metabolites and provides a very low limit of detection. Targeted analysis has been widely used to follow the dynamics of a limited number of metabolites known to be involved in a particular stress.

Targeted analysis can also be used for comparative metabolite profiling of a large number of known metabolites. For example, highly parallel targeted assays based on SRM can be used for very sensitive simultaneous analysis of over 100 metabolites in a single chromatographic run [see review by Bajad and Shulaev (2007)].

For truly quantitative measurement, targeted compounds should be available in a pure form and preferably labeled with stable isotope, which provides a significant challenge for plant stress research because many plant metabolites involved in stress response and their intermediates are not available in a pure form. A joint effort of the plant community and the chemical industry is required to synthesize these compounds and make them available to researchers.

An alternative approach for quantitative profiling may provide in vivo enrichment of metabolites with stable isotopes like ^{13}C and ^{15}N. This can be achieved by growing plants or plant cells in liquid media containing ^{15}N-labeled inorganic nitrogen sources ($K^{15}NO_3$, $^{15}NH_4^{15}NO_3$) or ^{13}C-labeled carbon dioxide or glucose (Hegeman et al. 2007, Huege et al. 2007). This approach allows for in vivo synthesis of stable isotope-labeled plant metabolites that can be used for quantitative metabolite analysis using stable isotope dilution method. Furthermore, extract of the fully labeled plant can be used as a complex internal standard for simultaneous quantitative profiling of the large number of known metabolites. Uniform metabolic labeling combined with MS has been successfully used for quantitative metabolic profiling in microorganisms (Lafaye et al. 2005, Mashego et al. 2004, Wu et al. 2005). In addition, in vivo stable isotope enrichment followed by metabolite analysis over the time-course experiment can provide information on metabolic fluxes and overall dynamics of metabolism (Hellerstein 2003, Huege et al. 2007, Kleijn et al. 2007, Matsuda et al. 2003). This information is essential for mathematical modeling of metabolic networks.

Metabolomics data analysis and the 'plant metabolome' challenge

Metabolomics, similar to transcriptomics and proteomics, generates huge volumes of data that require specialized bioinformatics and data mining tools to gain knowledge. Metabolomics requires automated raw data processing software that can handle data from various instruments, extensive mass spectral libraries and powerful database management systems that can store both raw and metadata.

Metabolomics data handling, analysis and mining and its integration with other omics platforms have been dramatically improved in recent years because of the development of an array of publicly available bioinformatics tools. These include pathway databases and pathway viewers KEGG (http://www.genome.ad.jp/kegg/), Atomic Reconstruction of Metabolism database (http://www.metabolome.jp/), BioCyc (http://biocyc.org) (Paley and Karp 2006), MetaCyc (http://metacyc.org/) (Caspi et al. 2006), AraCyc (http://www.Arabidopsis.org/tools/aracyc/) (Zhang et al. 2005), MapMan (http://gabi.rzpd.de/projects/MapMan/) (Thimm et al. 2004), KaPPA-View (http://kpv.kazusa.or.jp/kappa-view/) (Tokimatsu et al. 2005) and BioPathAT (http://www.ibc.wsu.edu/research/lange/public%5Ffolder/) (Lange and Ghassemian 2005), the data model for plant metabolomics experiments ArMet (http://www.armet.org/), and the functional genomics databases MetNetDB (http://www.metnetdb.org/

MetNet_db.htm) and DOME (http://medicago.vbi.vt.edu).

One significant challenge for plant metabolomics is the lack of a fully described and annotated metabolome for any plant species. Estimates are that the plant kingdom produce 90 000–200 000 different metabolites (Fiehn et al. 2001), but the actual number of metabolites present in any individual plant species is still unknown. Even in microorganisms with a simpler and better understood metabolism, the exact number of metabolites in each cell remains unknown. Understanding the metabolome of each plant species is critical to understanding gene function and coupling each of these functions to plant traits.

Combination of the omics platforms and systems biology approach

The real power of the omics approach is the ability to look at the studied response on a number of different levels, including transcripts, proteins or metabolites. Integrated analysis of metabolite and transcript or metabolite and protein levels in several plant systems already identified several important features of plant metabolic regulation. Currently, most plant stress response studies use largely either one or a combination of two approaches, whereas integrated studies of the plant stress response using a combination of all three approaches are just appearing.

Integration of the transcriptomics and metabolomics data to elucidate gene-to-gene and metabolite-to-gene networks in Arabidopsis under sulfur deficiency was described by Hirai et al. (2005), while combined metabolomics and proteomics approach to study the Arabidopsis response to a cesium stress was described by Le Lay et al. (2006). It is important to mention that combined analysis of the metabolomics data with other omics data is quite challenging because of the data integration problem (Mehrotra and Mendes 2006). This hampers the wide use of combined data sets and requires further development of data integration and data fusion approaches. Another issue with combined omics studies is related to sample collection and processing. Most of the combined studies use different samples for transcripts, proteins and metabolite measurements. This can introduce a significant error in the subsequent analysis of the combined data and cause lack of correlation in RNA, protein and metabolite levels because of the time difference in quenching metabolism between different samples. Ideally, all three types of molecules should be analyzed from the same biological sample, and the proper quenching and sampling technique that allows for preservation of RNA, proteins and metabolites should be used (Martins et al. 2007, Weckwerth et al. 2004).

Furthermore, omics data should be combined with mathematical modeling of the biological systems in the so-called systems biology approach (van der Greef et al. 2004, Kell 2006, Kitano 2002, Sims et al. 2007). Systems biology approach allows not only to analyze the topology of the biochemical and signaling networks involved in stress response but also to capture the dynamics of the response.

Systems biology research requires close interaction of biologists and mathematicians in all steps of experimental design, data collection and data analysis and mining (Goel et al. 2006). One of the most critical aspects for successful systems biology study is the type of high-throughput data available for mathematical modeling. Time-course experiments can provide information on system's dynamics, but the exact time points for sample collection following initial perturbation should be properly selected, based on the systems behavior, to capture both fast and slow responses. Data providing absolute quantities of metabolites are more suited for mathematical modeling than semiquantitative data currently provided by many metabolomics studies. Additionally, data on enzyme activities rather than protein levels are required by most dynamic metabolic modeling approaches.

Mathematical modeling of plant stress response using 'omics' data is quite limited. It is partially because of the lack of proper time-course data sets and insufficient 'top-down' modeling approaches that can utilize large transcriptomics, proteomics and metabolomics datasets. Morioka et al. (2007) used, for example, the linear dynamical system to model gene expression and metabolite time series data from Arabidopsis grown under sulfur starvation conditions. Using this variation of Markov model, the authors not only were able to detect known changes in gene expression and metabolite accumulation but also identified novel changes involved in the stress response (Morioka et al. 2007). This study shows the power of the systems biology approach in understanding and predicting the behavior of the plant system under stress.

Metabolomics studies of stress in plants

Metabolomics was used to study temperature (Cook et al. 2004, Kaplan et al. 2004; Kaplan et al., 2007), water and salinity (Brosche et al. 2005, Cramer et al. 2007, Gong et al. 2005, Johnson et al. 2003, Kim et al., 2007), sulfur (Nikiforova et al. 2004, Nikiforova et al., 2005a, Nikiforova et al., 2005b), phosphorus (Hernandez et al. 2007), oxidative (Baxter et al. 2007) and heavy metal (Le Lay et al. 2006) stress as well as a combination of multiple stresses (Rizhsky et al. 2004) in plants. Below, we briefly describe some of these studies.

Temperature stress

Metabolite profiling was used to understand the dynamics of the Arabidopsis response to temperature stress (Kaplan et al. 2004). The authors performed GC-MS profiling of Arabidopsis plants subjected to heat and cold stress and identified a set of known metabolites as well as unknown mass spectral tags that specifically respond to heat or cold stress or to both. Cold stress appears to cause a more dramatic metabolic response, but what was more surprising was that the majority of the metabolites affected by the heat were also affected by the cold and many of the metabolites induced by both stresses were previously unlinked to temperature stress. In a subsequent study, these data were coanalyzed with transcript profiling data to uncover mechanism underlying cold acclimation in Arabidopsis (Kaplan et al. 2007). Based on the fact that for some metabolic processes, transcript abundance correlated with metabolite abundance and for other metabolic processes, they did not correlate, the authors made a conclusion that regulatory processes independent of transcript abundance could play a key role in the metabolic adjustments of plants during cold acclimation (Kaplan et al. 2007).

Global GC-TOF-MS metabolite profiling of cold-stressed Arabidopsis plants that differ in freezing tolerance in comparison with plants overexpressing the C-repeat/dehydration responsive element-binding factor (CBF) 3 revealed that Arabidopsis metabolome is extensively reconfigured in response to low temperature and suggested a prominent role for the CBF cold response pathway in this process (Cook et al. 2004).

In an example of targeted profiling, Morsy et al. (2007) studied the carbohydrate metabolism of rice under chilling, salt and osmotic stress in different genotypes differing in chilling tolerance. Using a quantitative HPLC assay, the authors measured the levels of soluble carbohydrates in the chilling-tolerant and chilling-sensitive genotypes under chilling stress and identified differences in carbohydrate accumulation. The chilling-tolerant genotype accumulated galactose and raffinose under stress, while these sugars declined in the chilling-sensitive genotype. These genotypes also responded differently to salt and osmotic stress. Based on the carbohydrate profiling results combined with the measurements of oxidative products and antioxidative enzymes, the authors concluded that the chilling-tolerant genotype possess a more effective ROS-scavenging system (Morsy et al. 2007).

Water and salt stress

Several metabolomics studies on salt-stressed plants have been reported (J. Kopka this issue). Metabolic fingerprint-ing of salt stress in tomato was used to identify metabolic changes in fruits under salinity stress (Johnson et al. 2003). The authors studied two tomato varieties subjected to salinity stress. Whole fruit flesh extracts were finger-printed using FT-IR spectroscopy. Metabolic fingerprints were analyzed using unsupervised (PCA) and supervised (DFA) algorithms. PCA was not able to discriminate between control and salt-treated groups in any variety, while DFA was able to classify control and salt-treated groups in both varieties (Johnson et al. 2003). The authors also employed a GA to identify the regions within the FT-IR spectrum that are important for classification. These regions corresponded to saturated and unsaturated nitrile compounds, cyanide-containing compounds and a strong broad peak of NH_2 (an amino radical) and other nitrogen-containing compounds.

More detailed metabolic analysis of salt stress response was performed in a time-course experiment on salt-stressed Arabidopsis cell cultures (Kim et al. 2007). GC-MS and LC-MS profiling was performed at 0.5, 1, 2, 4, 12, 24, 48 and 72 h after a salt treatment at 100 mM NaCl. Bioinformatics analysis of the data using PCA and batch-learning self-organizing mapping analysis revealed that short-term responses to salt stress included the induction of the methylation cycle for the supply of methyl groups, the phenylpropanoid pathway for lignin production and glycine betaine production (Kim et al. 2007). The long-term effects were the coinduction of glycolysis and sucrose metabolism and coreduction of the methylation cycle.

GC-MS profiling, in combination with microarray analysis, was also used to compare salinity stress competence in the extremophile *Thellungiella halophila* with Arabidopsis (Gong et al. 2005). The authors found drastic differences in metabolic profiles of the two species. Generally, *Thellungiella* had a higher metabolite levels both without and with salt stress when compared with Arabidopsis. In Arabidopsis, 150 mM salt stress caused increase in sucrose, proline and an unknown metabolite (putative complex sugar). In *Thellungiella*, the response was more complex. In addition to having higher levels of many metabolites before stress, changes in other sugars, sugar alcohols, organic acids and phosphate were detected (Gong et al. 2005).

An integrated study of the early and late changes in transcript and metabolite profiles revealed difference in the dynamics of grapevine response to water and salinity stress (Cramer et al. 2007) and showed the differences in molecular response to water deficit and salinity. GC-MS profiling and anion-exchange chromatography with UV detection revealed that concentration of glucose, malate and proline is higher in water-deficit-treated plants, than in salt-stressed plants. These differences in metabolite

levels were correlated with differences in transcript levels of many genes involved in energy metabolism and nitrogen assimilation, suggesting a higher demand in water-deficit-treated plants to adjust osmotically, detoxify ROS and cope with photoinhibition than in salt-stressed plants (Cramer et al. 2007).

In another interesting study, transcript expression and metabolite profiling were used to study the salt-tolerant *Populus euphratica* plant grown within its native habitat, the Negev desert, in order to understand the mechanism underlying stress acclimation (Brosche et al. 2005).

Sulfur and phosphorus stress

Sulfur stress was studied using metabolomics by several groups and is discussed in a paper by Höefgen and Nikiforova in this issue. Therefore, we will just mention several published reports without discussing them in detail.

In a metabolomics study of sulfur deficiency in Arabidopsis, Nikiforova et al. (2005b) used untargeted GC-MS and LC-MS profiling to monitor the response of 134 known metabolites and 6023 unknown non-redundant ion traces to sulfur starvation. Based on the profiling data, the coordinated network of metabolic regulation induced by sulfur stress was successfully reconstructed (Nikiforova et al. 2005b). These data were subsequently analyzed together with transcriptomics data to reconstruct gene–metabolite correlation networks involved in Arabidopsis response to sulfur deprivation (Nikiforova et al. 2005a).

Combination of transcriptomics and metabolomics approaches was used to investigate transcriptional and metabolic responses of bean plants growing under P-deficient and P-sufficient conditions (Hernandez et al. 2007). GS-TOF-MS profiling of bean roots under phosphorus stress conditions identified a set of metabolites significantly changed in P-deficient roots. Most metabolites, including amino acids, polyols and sugars, were increased in P-stressed plants (Hernandez et al. 2007).

Oxidative stress

Despite the well-established role of several metabolic systems, including the ascorbate–glutathione system, in oxidative stress response, only few reports exist on using metabolomics to study oxidative stress response in plants. Baxter et al. (2007) studied the dynamics of metabolic change in response to oxidative stress caused by menadione in heterotrophic Arabidopsis cells. The authors used GC-MS profiling to measure the levels of 50 polar metabolites following stress treatment and correlated metabolic changes to changes in mRNA levels

measured in the same sample. In this study, oxidative stress initially caused dramatic inhibition of the TCA cycle and large sectors of amino acid metabolism followed by backing up of glycolysis and diversion of carbon into the oxidative pentose phosphate pathway (Baxter et al. 2007). Transcriptomics analysis of the same samples also revealed a coordinated transcriptional response to cope with the stress with a major switch from anabolic to catabolic metabolism.

Heavy metal stress

Metabolic consequences of stress induced by heavy metals in plants were studied using NMR-based metabolic fingerprinting (Bailey et al. 2003) and metabolite profiling (Le Lay et al. 2006).

Metabolic fingerprinting using NMR spectroscopy combined with multivariate statistics analysis was used to discriminate between control and cadmium-treated *Silene cucubalus* cell cultures (Bailey et al. 2003). Compounds that showed an increase in cadmium-treated cells were identified as malic acid and acetate, while glutamate and branched chain amino acids decreased.

Metabolite profiling of Arabidopsis cells exposed to cesium stress using NMR showed that metabolite changes because of a Cs stress, include mainly sugar metabolism and glycolytic fluxes, and depended on potassium levels in the cell (Le Lay et al. 2006).

Stress combination

Traditionally, abiotic stress conditions are studied in plants by applying a single stress condition such as drought, salinity or heat, and analyzing the different molecular aspects of plant acclimation. This type of analysis is, however, in sharp contrast to the conditions that occur in nature, in which plants are routinely subjected to a combination of different abiotic stresses. Drought and heat stress represent an excellent example of two different abiotic stress conditions that occur in the field simultaneously. A sum of all major US weather disasters between 1980 and 2004 reveals that a combination of drought and heat caused an excess of $120 billion in damages. In contrast, over the same period, drought that was not accompanied by heat stress caused some $20 billion in damages (Mittler 2006, Rizhsky et al. 2004). Metabolite profiling of plants subjected to drought, heat stress or a combination of drought and heat stress revealed that plants subject to a combination of drought and heat stress accumulated sucrose and other sugars such as maltose and gulose (Rizhsky et al. 2004). In contrast, Pro that accumulated in plants subjected to drought did not accumulate in plants during

a combination of drought and heat stress. Heat stress was found to exaggerate the toxicity of Pro to cells, suggesting that during a combination of drought and heat stress, sucrose replaces Pro in plants as the major osmoprotectant. These findings of different metabolic responses to stress combination in comparison with each individual stress highlight the need for further studies of different stress combinations at the metabolic level (Mittler 2006).

Perspective

Despite being a relatively new approach in plant biology, metabolomics is becoming one of the major tools in studying plant stress responses. Significant new discoveries have already been made in the field by using metabolomics alone or in combination with other omics disciplines. In the future, we envision more studies that include metabolomics as an integral part of the systems biology approach to study plant response to a variety of stress conditions. Combination of metabolomics, proteomics, transcriptomics and mathematical modeling will provide us with a holistic view of how plants respond to abiotic and biotic stress and enable us to develop advanced strategies to enhance the tolerance of different plants and crops to biotic and abiotic stress conditions.

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