Metabolomic Profiling of Plants to Understand Reasons for Plant Stress Resilience to Abiotic Stress

Prashat G. Rama and T. Vinutha

Large numbers of metabolites are produced by plants. They are of diversified structures and abundance and are important for plant growth, development and environmental response. These diverse metabolites are the chemical base of crop yield and quality, valuable nutrition and energy sources for human beings and live stocks (Hall et al. 2008). These metabolites are generally classified into primary and secondary metabolites. The primary metabolites are indispensable for the growth and development of a plant and secondary metabolites are not essential for growth and development but are necessary for a plant to survive under stress conditions by maintaining a delicate balance with the environment. Primary metabolites are highly conserved in their structures and abundances but secondary metabolites differ widely across plant kingdoms (Scossa et al. 2016). The diversity of plant metabolites and their role in complicated regulatory mechanism necessitates exploring the underlying biochemical nature (Hall et al. 2008). It will be very challenging to study the metabolome of plants because of the complexity of the diverse metabolic characteristics and abundances of molecules. Plant metabolism is disturbed by various abiotic stresses. The reconfiguration of metabolic networks of plant must happen under stress conditions to allow both the maintenance of metabolic homeostasis and production of compounds that ameliorate the stress.

When plants are subjected to unfavourable growth conditions, such as abiotic stress, the plant growth and productivity is retarded. Under most abiotic stress conditions, plant metabolism is disturbed either because of inhibition of metabolic enzymes, shortage of substrate, excess demand for specific compounds or a combination of these factors and many other reasons. So the metabolic network must be reconfigured in a way that essential metabolism is maintained and a new steady state is adopted for acclimatization of the prevailing stress conditions. The metabolic

P. G. Rama (✉)
Division of Genetics, ICAR-IARI, New Delhi, India

T. Vinutha
Division of Biochemistry, ICAR-IARI, New Delhi, India

S. H. Wani (ed.), *Recent Approaches in Omics for Plant Resilience to Climate Change*, https://doi.org/10.1007/978-3-030-21687-0_3

reprogramming is necessary to meet the demand for anti-stress agents including compatible solutes, antioxidants and stress-responsive proteins. The reactive oxygen species (ROS) accumulation is another problem which causes oxidation and dysfunction of cellular components and in the worst case cell death. The metabolic flux optimization via the organellar electron transport chains is crucial in order to lessen ROS production. The redox state maintenance in the cell is thus an important task to provide the reducing power required for ROS scavenging. Despite these important roles of metabolic regulation under stress conditions, our understanding of this process currently is fragmented and far from complete.

Despite the fact that metabolomics is downstream of the other functional genomics (transcriptomics and proteomics), the practical size of the metabolome of a species, unlike transcriptome or proteome, cannot be speculated directly by known genomic information via central dogma. Therefore, metabolomics is used to obtain a large amount of valuable information for the discovery of genes and pathways through accurate and high throughput corollary peak annotation via snapshotting the plant metabolome (Tohge et al. 2014). It seems that there is a complicated regulatory network among these small molecules in plants, and by detecting the interactions among these metabolites, metabolomic analysis contributes significantly to the understanding of the relation between genotype and metabolic outputs by tackling key network components (Toubiana et al. 2013). Plant metabolomics has become a powerful tool to explore various aspects of plant physiology and biology, which significantly broadens our knowledge of the metabolic and molecular regulatory mechanisms regulating plant growth, development and stress responses, and the improvement of crop productivity and quality. Plants have evolved a series of adaptive changes at both transcriptional and post-transcriptional levels to encounter various environmental stresses during their developmental processes, resulting in the reconfiguration of regulatory networks to maintain homeostasis (Verslues et al. 2006). Expression of stress responsive genes are activated once the plant receptors are stimulated by stress signals, resulting in subsequent biosynthesis of specialized metabolites to adapt to environmental stresses (Nakabayashi and Saito 2015).

The era of ‘Omics’ has witnessed huge developments in the fields of genomics, transcriptomics, epigenomics, proteomics, metabolomics and phenomics. The information generated by these ‘Omics’ approaches has enhanced the breeding programme precision and speed in developing climate smart and nutrition-rich germplasm for ensuring food security (Parry and Hawkesford 2012). The role of phenomics-based breeding has become more evident in recent years in improving the crop production and productivity (Khush, 2001; Langridge and Fleury 2011; Wang et al. 2017; Xavier et al., 2017). Compared with genomics, transcriptomics and proteomics, metabolomics provide a direct and global snapshot of all the metabolites. Metabolomics is as one of the major breakthroughs in science, paving the way for accurate metabolite profiling in microbes, plants and animals (Heyman and Dubery 2016; van Dam and Bouwmeester 2016; Wuolikainen et al. 2016). Metabolomics is the most complex of all the Omics approaches but received

inadequate attention in the field of crop science, particularly for trait mapping and plant selections. It has the ability to detect a vast array of metabolites from a single extract, thus allowing speedy and precise analysis of metabolites, offering a comprehensive view of cellular metabolites which participate in different cellular events, thus representing the absolute physiological state of a cell.

Metabolomics can be classified as untargeted metabolomics and targeted metabolomics based on the researchers' approach. Untargeted metabolomics, also called as discovery metabolomics, usually involves the comparison of the metabolome between the control and test groups, to identify the differences between their metabolite profiles which may be relevant to specific biological conditions. Targeted metabolomics is a quantitative method for the identification and quantitative analysis of targeted metabolic compounds in organisms. It provides information on the content and composition of metabolites, which are closely associated with the biological activities and can vary dramatically under different physiological conditions. Therefore, metabolomics methods are important for studying the biological function and comparing the metabolic systems of different organisms.

Rapid qualitative and quantitative analyses of metabolic responses of plants to environmental disturbances will not only help us to identify the phenotypic response of plants to abiotic stresses but also they reveal the genetic and biochemical mechanisms underlying the plant's responses to stresses. Metabolomics is a powerful tool by which a comprehensive perspective is gained of how metabolic networks are regulated and has indeed been applied by many researches in recent years. The plant plasticity for the future genetic engineering of stress resistant/tolerant plants can also be better understood. The output largely depends on the methodologies and instrumentations to comprehensively identify, quantify and localize every metabolite. Despite the fact that currently it is not possible to do accurate and exhaustive whole metabolome analysis of a biological sample, the methodologies and instrumentations of plant metabolomics are developing rapidly (Hegeman 2010). Large scale analysis of highly complex mixtures are made possible at present by a series of integrated technologies and methodologies, like non-destructive NMR (nuclear magnetic resonance spectroscopy), mass spectrometry (MS) based methods including GC–MS (gas chromatography–MS), LC–MS (liquid chromatography–MS) and CE–MS (capillary electrophoresis–MS), and FI-ICR–MS (Fourier transform ion cyclotron resonance–MS) (Okazaki and Saito 2012; Khakimov et al. 2014). Metabolomics could be performed in the subcellular level and even in a single cell with assistance from other technologies of sampling (Kueger et al. 2012; Moussaieff et al. 2013; Misra et al. 2014; Sweetlove et al. 2014). These analytical approaches have shown their potential power in plant metabolomic studies in many common plant species including staple food crops such as tomato, rice, wheat and maize for various purposes (Hu et al. 2014; Francki et al. 2016; Rao et al. 2014; Bénard et al. 2015). However, because of the intrinsic limitation of each analytical platform, combined approaches are increasingly used in metabolomics analysis (Figs. 1 and 2).

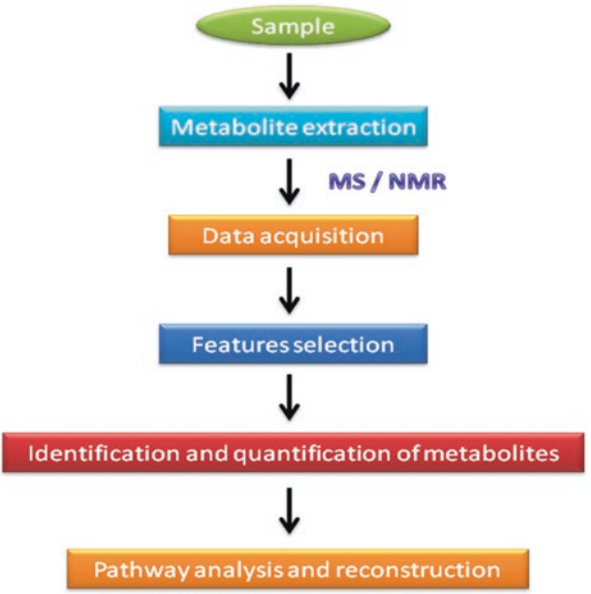
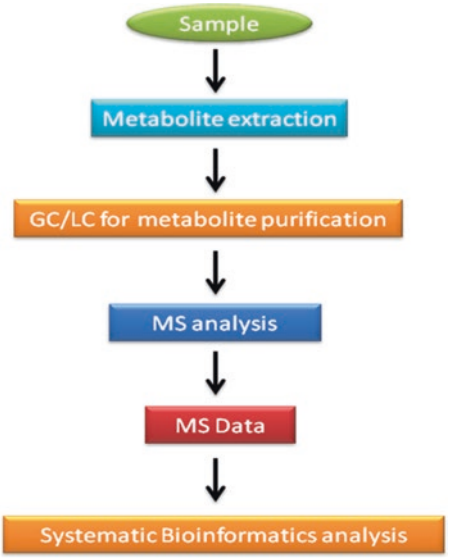


Fig. 1 Steps involved in untargeted metabolomics (Source: https://www.creative-proteomics.com/services/untargeted-metabolomics.htm)

Fig. 2 Steps involved in targeted metabolomics (Source: https://www.creative-proteomics.com/services/targeted-metabolomics.htm)



1 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry is the most commonly used technique for plant metabolomics research. Polar metabolites are derivatised to make them volatile and then separated by GC. The crucial advantage of this technology is that it has long been used for metabolite profiling, and thus there are stable protocols for machine setup and maintenance, and chromatogram evaluation and interpretation (Fernie et al. 2004; Lisec et al. 2006; Halket et al. 2005). The short running time and relatively low running cost are also strong advantages of GC-MS. However the use of GC-MS is limited for thermally stable volatile compounds, so the analysis of high molecular weight compounds (˃1 kDa) difficult. GC-MS facilitates the identification and robust quantification of a few hundred metabolites in plant samples such as sugars, sugar alcohols, amino acids, organic acids and polyamines, resulting in fairly comprehensive coverage of the central pathways of primary metabolism.

2 Liquid Chromatography (LC)-MS

LC does not require prior sample treatment and separates the components in a liquid phase and hence does not have the limitation due to volatilization of compounds. The choice of columns, including reversed phase, ion exchange and hydrophobic interaction columns, provides the separation of metabolites based on different chemical properties. Therefore, LC has the potential to analyse a wide variety of metabolites in plants. The technique becomes more powerful by the recent development of ultra-performance liquid chromatography (UPLC) which has higher resolution, sensitivity and throughput than conventional high-performance liquid chromatography (HPLC) (Rogachev and Aharoni 2012). Electrospray ionisation (ESI) is widely used for ionisation to connect LC and MS. Many types of MS including quadrupole (Q), TOF, qTOF, triple quadrupole (QqQ), ion trap (IT), linear trap quadrupole (LTQ)-Orbitrap and Fourier transform ion cyclotron resonance (FT-ICR)-MS are used depending on the sensitivity, mass-resolution and dynamic range required (Allwood and Goodacre 2010; Lei et al. 2011). The combination of these techniques allows us to identify and quantify a large variety of metabolites even if they have high molecular mass, great polarity and low thermostability.

3 Capillary Electrophoresis (CE)-MS

In capillary electrophoresis, polar and charged compounds are separated on the basis of their charge-to-mass ratio. CE is a more powerful technique than LC with respect to separation efficiency and is able to separate a diverse range of chemical compounds (Ramautar et al. 2009, 2011). ESI is commonly used for ionisation as in

LC-MS, with TOF-MS being the most commonly used detector in CE-MS-based metabolomics studies. This combination provides high mass accuracy and high resolution. The high scan speed of TOF-MS makes this instrument very suitable for full scan analyses in metabolomics. CE-MS requires only a small amount of sample for analysis; only nanolitres of sample are introduced into the capillary. It can produce analysis within seconds with high electric fields and short separation lengths. It is highly preferred in the metabolic analysis of volume-restricted samples. This leads to low concentration sensitivity requiring enrichment of metabolites within the samples (Monton and Soga 2007). Another drawback of CE is the poor migration time reproducibility and lack of reference libraries, which may only be partially overcome by the prediction of migration time (Sugimoto et al. 2010). Since CE and LC can both separate a large variety of metabolites via fundamentally different mechanisms, they are often used in combination to provide a wider coverage of metabolites (Urano et al. 2009; Williams et al. 2007; Soga and Imaizumi 2001). But, the use of CE-MS in plant studies remains relatively rare.

4 Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance spectroscopy is an entirely different analytical technique than that of MS-based techniques being based on atomic interaction. In a strong magnetic field, atoms with non-zero magnetic moment including ^{1}H, ^{13}C, ^{14}N, ^{15}N and ^{31}P absorb and re-emit electromagnetic radiation. The radiation is characterized by its frequency, intensity, fine structure and magnetic relaxation properties, all of which reflect the precise environment of the detected nucleus. Therefore, atoms in a molecule give a specific spectrum of radiation that can be used for identification and quantification of metabolites within a complex biological sample. The sensitivity of this method is much lower than that of MS-based techniques but the structural information content, reproducibility and quantitative aspects can be superior to them. The preparation of the sample is simple and even nondestructive measurement is possible. NMR can further generate kinetic measurements in vivo and examine metabolic responses on the same plant rather than on a set of similar plants (Terskikh et al. 2005). The difference in the sub cellular pHs of the vacuole and the rest of the cell results in distinctive signals from an identical metabolite and thus allows quantification at the subcellular level (Ratcliffe and Shachar-Hill 2005; Eisenreich and Bacher 2007). Thus analysing the metabolite composition of a tissue extract, determining the structure of a novel metabolite, demonstrating the existence of a particular metabolic pathway in vivo, isotope labelling experiment and localising the distribution of a metabolite in a tissue are all possible by NMR. These properties of NMR make it the ideal tool for broad-range profiling of abundant metabolites whilst studying changes in non-annotated profiles is highly useful for metabolite fingerprinting of extensive sample collections (Lommen et al. 1998; Dixon et al. 2006).

The NMR based metabolite detection is based on the magnetic properties of nuclei of atoms under magnetic field. The NMR is a non-destructive method extensively used to identify metabolites with smaller molecular weight (<50 kDa) for diverse applications like metabolite fingerprinting, profiling, metabolic flux and extracting the atomic structural information of compound present in the biological samples (Winning et al. 2009). The drawback of technique is its poor sensitivity owing to a limited coverage of low-abundance biomarkers which poses a major limitation that in turn restricts its extensive use.

Unlike NMR, a wide coverage of metabolome data can be attained by greater sensitivity of MS techniques, leading to the identification of novel metabolic biomarker, and molecules that can facilitate the reconstruction of metabolic pathways and networks. With the advances in the ionization methods such as atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI) and MALDI-TOF, MS has achieved greater accuracy (Issaq et al. 2009). MS is usually combined with chromatography techniques such as gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE), Fourier-transform ion cyclotron resonance (FT-ICR) and field asymmetric waveform ion mobility spectrometry (FAIMS) to enhance the throughput. Despite the low sensitivity and large sample requirement of NMR, its capacity of identifying physical properties of ligands, binding sites on protein, uncovering structures of protein ligand complexes and direct binding of target protein retains its use over MS.

Metabolomics is increasingly becoming common in plant physiology and biochemistry, and to date has been applied to a staggering number of studies. In this chapter we will see the application of metabolomic profiling to understand the reasons for plant stress resilience to abiotic stress.

5 Water Stress

One of the major threats in crop production is the limitation of water and is projected to get considerably worse in the coming years (Cominelli et al. 2009). For this reason considerable research effort has been expended to understand the response to this crucial and common stress. These studies have revealed an important role for metabolic regulation including regulation of photosynthesis and accumulation of osmolytes in the drought stress response (Chaves and Oliveira 2004; Verslues and Juenger 2011). Accumulation of many metabolites including amino acids such as proline, raffinose family oligosaccharides, γ-amino butyrate (GABA) and tricarboxylic acid (TCA) cycle metabolites was observed in *Arabidopsis* leaves under drought condition. These accumulated metabolites are known to respond to drought stress in plants (Urano et al. 2010). Analysis of wheat leaves in response to water deficient conditions, indicated amino acids, organic acids and sugars as the main metabolites changed in abundance upon water deficiency. Bahar *cv*, the drought susceptible spring-wheat cultivar showed increased levels in proline, methionine, arginine, lysine, aromatic and branched chain amino acids. Auxin production was sustained by

tryptophan accumulation via shikimate pathway and glutamate reduction is reasonably linked to polyamine synthesis. But the metabolome of drought tolerant Kavir *cv* was affected only to a lesser extent with only two pathways changed significantly, one of them being purine metabolism (Michaletti et al. 2018). Profiling of soybean leaf metabolites under control, drought and heat stress conditions was conducted in a controlled environment. Analyses of non-targeted metabolomic data showed that in response to drought and heat stress, carbohydrates, amino acids, lipids, cofactors, nucleotides, peptides and secondary metabolites were differentially accumulated in the leaves. The metabolites for various cellular processes, such as glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway and starch biosynthesis, that regulate carbohydrate metabolism, amino acid metabolism, peptide metabolism and purine and pyrimidine biosynthesis, were found to be affected by drought as well as heat stress (Das et al. 2017)

Too much water, in situations like flooding or water-logging of the rhizosphere causes problems because of the reduced oxygen availability (hypoxia/anoxia). ATP has to be produced by fermentation under anoxic conditions, resulting in cytosolic acidification and the accumulation of toxic products. The accumulation of amino acids, alanine, proline and GABA, and the phosphoesters, glucose-6-phosphate and glycerol-3-phosphate, were observed in the analyses of metabolic responses in Arabidopsis roots under anoxic conditions. Changes in the levels of minor sugars and various organic acids were also observed. There is a general tendency for an increase in the levels of the intermediates both of sucrose degradation and the TCA cycle, and in the levels of most amino acids when oxygen is decreased to 4%, whereas they are decreased when the oxygen is further decreased to 1%, indicating the inhibition and reactivation of metabolic activities. Together with the transcriptomic data showing a general downregulation of energy-consuming processes, the results demonstrated a large-scale reprogramming of metabolism under oxygen-limited conditions van Dongen et al. (2009). The accumulation of alanine under anoxic conditions in *Lotus japonicus*, which is highly tolerant to water logging was studied by Rocha et al. (2010). Succinate, alanine and the direct co-substrates for alanine synthesis, glutamate and GABA, were highly accumulated in the roots of *L. japonicus*, whereas the majority of amino acids that are derived from TCA cycle intermediate decreased during water logging. The results are in agreement with the metabolic equilibriums that are expected to drive the metabolic flux from glycolysis, via alanine synthesis and oxoglutarate to succinate, which prevents the accumulation of pyruvate activating fermentation and leading to ATP production by succinyl-CoA ligase.

6 Temperature Stress

Plant cells are seriously damaged by ice formation and dysfunction of cellular membranes when exposed to freezing environments (Guy 1990). Many plant species increase their freezing tolerance when exposed to non-freezing low

temperature by a process known as 'cold acclimation'. The molecular basis of this process has been extensively studied, and the role of particular metabolites including compatible solutes (Wanner and Junttila 1999) and the transcriptional regulatory network has been elucidated (Thomashow 2010; Medina et al. 2011). The metabolomic studies of cold acclimation were first performed by Cook et al. and Kaplan et al. in 2004. Metabolomic changes during cold acclimation in two ecotypes of *Arabidopsis thaliana*, Wassilewskija-2 (Ws-2) and Cape verde islands-1 (Cvi-1), which are relatively freezing tolerant and sensitive, respectively was compared by Cook et al. (2004). Ws-2 plants showed extensive alteration in metabolome in response to low temperature. Seventy-five percent of metabolites monitored were found to increase in cold-acclimated plants including metabolites known to increase in Arabidopsis plants upon exposure to low temperature, like the amino acid proline and the sugars glucose, fructose, inositol, galactinol, raffinose and sucrose. Novel changes like the increase of trehalose, ascorbate, putrescine, citrulline and some TCA cycle intermediates was also found. Considerable overlap was found in the metabolite changes that occurred in the two ecotypes in response to low temperature; however, quantitative differences were evident. Metabolome analysis of Arabidopsis over the time course following the shift to cold and heat conditions was conducted by Kaplan et al. (2004). Surprisingly the majority of heat shock responses were shared with cold shock including the increase of pool sizes of amino acids derived from pyruvate and oxaloacetate, polyamine precursors and compatible solutes. The results of this study were analysed together with following transcript profiling data (Kaplan et al. 2007), which revealed that the regulation of GABA shunt and proline accumulation under cold conditions are achieved by transcriptional and post-transcriptional manners, respectively.

7 Light Stress

Too high light irradiance represents an abiotic stress factor for plants since light is a highly energetic substrate driving photosynthesis that can induce secondary destructive processes at the same time. Metabolite profiling of Arabidopsis leaves for 6 days after transition to high light was conducted by Wulff-Zottele et al. (2010). Most of the metabolites of the glycolysis, TCA cycle and oxidative pentose phosphate pathway were altered in their content, indicating that plants exposed to high light undergo a metabolic shift and enhance the Calvin–Benson cycle to fix more carbon. Elevation of glycine in addition indicated the activation of photorespiratory pathways. The early metabolic response against high light was studied by Caldana et al. (2011) as a part of a more comprehensive study. The photorespiratory intermediates such as glycine and glycolate, were found to be accumulated in the early phase (5–60 min after transition). Interestingly the response during the mid-phase (80–360 min) shares similar properties with low temperature treatment, which includes the accumulation of shikimate, phenylalanine and fructose, and the decrease of succinate; Arabidopsis plants were treated with UV light and the

subsequent metabolic effect of UV light stress was analysed by Kusano et al. (2011). Arabidopsis showed an apparent biphasic response to UV-B stress, characterised by major changes in the levels of primary metabolites, including ascorbate derivatives. By contrast, mid- to late-term responses were observed in the classically defined UV-B protectants, such as flavonoids and phenolics. The results suggested that in early stages of exposure to UV-B, the plant cell is 'primed' at the level of primary metabolism by a mechanism that involves reprogramming of the metabolism to efficiently divert carbon towards the aromatic amino acid precursors of the phenylpropanoid pathway. It also suggested the importance of ascorbate in the short-term response to UV-B.

8 Ion Stress

High levels of salinity in the soil inhibit the growth and development of crops and cause serious problems for world food production (Munns, 2005). Both hyperionic and hyperosmotic stress effects were caused due to high concentrations of NaCl, which results in the decline of turgor, disordered metabolism and the inhibition of uptake of essential ions, as well as other problems in plant cells (Kim et al. 2007; Tester and Davenport 2003). Metabolite profiling of salt-treated *Arabidopsis thaliana* and its relative *Thellungiella halophila* (salt cress), which shows extreme tolerance to a variety of abiotic stresses, like low humidity, freezing and high salinity was studied by Gong et al. (2005). There was a dramatic increase in proline, inositols, hexoses and complex sugars in both the species. The concentrations of metabolites were often several-fold higher in Thellungiella and stress exacerbated the differences in some metabolites. The difference in metabolites between Arabidopsis and Thellungiella under salt and osmotic stresses was assessed for a broader range of metabolites (Lugan et al. 2010). A shift from nonpolar to polar metabolites in both species was observed by the analysis of global physicochemical properties of metabolites, but the shift was much more pronounced in Thellungiella. Such a shift may contribute to keep the water potential during dehydration. The cellular level metabolic response under salinity stress using Arabidopsis T87 cultured cells was studied by Kim et al. (2007). The methylation cycle for the supply of methyl groups, the phenylpropanoid pathway for lignin production and glycine betaine biosynthesis were found to be synergetically induced as a short-term response against salt-stress treatment. The results also suggest the co-induction of glycolysis and sucrose metabolism as well as co-reduction of the methylation cycle as long-term responses to salt stress. Due to the importance of salinity stress in agriculture, there are many metabolomic studies to assess the metabolic effect of salinity in a variety of crop and related plant species including tomato (Shulaev et al. 2008; Johnson et al. 2003), grapevine (Cramer et al. 2007), poplar (Brosché et al. 2005), sea lavender (*Limonium latifolium*; Gagneul et al. 2007) and rice (Zuther et al. 2007).

Heavy metals such as cadmium (Cd), caesium (Cs), lead (Pb), zinc (Zn), nickel (Ni) and chromium (Cr) are major pollutants of the soil causing stress on plants. At inappropriate concentration even the essential nutrients such as copper (Cu), iron (Fe) and manganese (Mn) can cause heavy metal stresses. Generally heavy metals induce enzyme inhibition, cellular oxidation and metabolic perturbation, resulting in growth retardation and in extreme instances can cause plant death (Sharma and Dietz 2009). Increased levels of alanine, β-alanine, proline, serine, putrescine, sucrose and other metabolites with compatible solute-like properties, notably GABA, raffinose and trehalose were found in Arabidopsis plants treated with Cd (Sun et al. 2010). The concentrations of α-tocopherol, campesterol, β-sitosterol and isoflavone (antioxidants) also increased significantly. When taken together these results indicate an important role of antioxidant defences in the mechanisms of resistance to cadmium stress. Transcriptomic and metabolomic analysis of rice roots treated with Cr was conducted by Dubey et al. (2010). Under these conditions proline accumulated to a threefold level than those of the control as did ornithine, which can be used in its synthesis. The content of several other metabolites including lactate, fructose, uracil and alanine increased following exposure to Cr stress. The observations suggest that the modulation of the sucrose degradation pathway involving the three main fermentation pathways was operating as a rescue mechanism when respiration is arrested.

9 Oxidative Stress

Oxidative stress is a key component of most abiotic stresses and a major limiting factor of plant growth in the field (Mittler 2006) which is the result of the overproduction of reactive oxygen species (ROS) in plant cells when plant metabolism is perturbed by various stresses. This consequently leads to oxidative damages of cellular components such as DNA, proteins and lipids (Moller et al. 2007). The metabolic network of plant cells must be reconfigured either to bypass damaged enzymes or to support adaptive responses in order to cope with oxidative stress. Baxter et al. treated, heterotrophic Arabidopsis cells with menadione, which enhances the ROS production via electron transport chains and changes in metabolite abundance, and ^{13}C-labelling kinetics were quantified. Sugar phosphates related to glycolysis and oxidative pentose phosphate pathways (OPPP) were found to be accumulated, suggesting the rerouting of glycolytic carbon flow into the OPPP possibly to provide NADPH for antioxidative effort. In addition the decrease of ascorbate and accumulation of its degradation product, threonate, indicated the activation of antioxidative pathways in menadione-treated cells. The reduced glycolytic activity probably leads to the decrease of levels of amino acids derived from glycolytic intermediates. The decrease of amino acids linked to TCA cycle intermediates and decrease of malate indicated a perturbation of TCA cycle (Baxter et al. 2007).

10 Combination of Stresses

Adverse environmental conditions in nature usually are made of several different factors, where one stress is usually accompanied or followed by another (Král'ová et al. 2012). In order to clearly define the contribution of individual stress, a controlled variable method was introduced and plants were subjected to a single primary stress factor to simplify the system (Chaves et al. 2009). But, in nature, plants often encounter not only one single stress, it will be followed by other stresses. It is most convenient both for experiments and discussion at the single stress level, but the plants are actually subjected to a combination of abiotic stress conditions in their natural habitat. Even some abiotic stresses are already combinations of stresses. For example high salt concentration causes osmotic and ion stresses, and flooding results in hypoxic and shading stresses. Although the metabolic responses of plants under a single abiotic stress have been analysed extensively as discussed, there are only few studies regarding to the effect of stress combinations on plant metabolism. When maize plants are subjected to water stress and salinity stress either separately or at the same time, levels of citrate, fumarate, phenylalanine, valine, leucine, isolecuine in leaves change significantly only under combined stresses, clearly explaining a crosstalk effect in multiple stresses (Sun et al. 2015). Heat and drought stresses becoming big challenges in the era of global warming to sustain grain yields. An experiment on rice floral organ development when subjected to combined stresses, analyses on metabolomics and transcriptomic features indicated that sugar starvation is the determinant of the failure of reproductive success under heat and drought stress in rice (Li et al. 2015). GC–MS profiling combined with transcriptomic analysis in Arabidopsis leaves revealed a synergistic stress response for the joint treatment of darkness and high temperature, which is stopped/lowered by low temperature. Protein degradation occurs rapidly and the amino acid catabolism is the main cellular energy supply in the absence of photosynthesis, as evidenced by the conditional connections between amino acid metabolism and the Kreb's cycle (Caldana et al. 2011). In rice combined cold and dehydration stresses resulted in the upregulation of carbohydrate metabolism associated genes, which are consistent with the buildup of glucose, fructose and sucrose in the aerial parts of the plant (Maruyama et al. 2014). Sugars such as sucrose, raffinose, maltose and glucose frequently accumulate in plant cells when subjected to combined stresses, perhaps protecting plants via osmotic adjustment from oxidative damage that usually follows most stress conditions (Rizhsky et al. 2004; Wulff-Zottele et al. 2010). Combined stresses normally result in a more extreme condition than that of each individual stress alone, and hence has profound effects on central metabolisms such as sugars and their phosphates and sulphur-containing compounds (Caldana et al. 2011; Rizhsky et al. 2004; Wulff-Zottele et al. 2010).

A combination of drought and heat stress was applied to Arabidopsis plants and the metabolic profile was analysed by Rizhsky et al. (2004). The metabolite profile of plants subjected to a combination of drought and heat stress was more similar to that of plants subjected to drought than to that of control plants or plants subjected

to heat stress. High levels of sucrose and other sugars instead of proline was accumulated by the plants subjected to combined stresses, which is accumulated to a very high level in plants subjected to drought but not under stress combination. They concluded that sucrose replaces proline as the major osmo protectant in plants subjected to combined stress because the toxic effect of high level of proline is enhanced under heat stress. The effect of the combination of high light irradiance and S depletion, which can occur in the field simultaneously was analysed by Wulff-Zottele et al. (2010; Buri et al. 2000). The combination of high light and S depletion gives rise to similar metabolic pool modifications such as in high light. Proline was accumulated in a differential time course under high light and stress combination. Other metabolites such as raffinose and putrescine replaced proline during the delay of proline accumulation in the plants subjected to high light and S depletion. The replacement of proline with those sugars is similar to that observed under the combination of drought and heat stress (Rizhsky et al. 2004).

11 Toward the Elucidation of Molecular Mechanisms Underlying Abiotic Stress Tolerance

A wealth of metabolomics data concerning the plant stress response has been accumulated and a large number of metabolic pathways are suggested to be regulated under various abiotic stress. But there are relatively few pathways and metabolites have been experimentally proven to function in abiotic stress tolerance. A metabolite profile does not tell exactly whether the related metabolic pathway is up- or downregulated since both upregulation of upstream reaction and downregulation of downstream reactions can lead to the accumulation of a metabolite. This can be solved by comparing the metabolomic data with those from transcriptomic or proteomic analysis or activities of specific enzymes (Cramer et al. 2011). Gene to metabolite regulatory networks of glucosinolate synthesis and primary metabolism under sulphur- and nitrogen-limited conditions was revealed by Hirai et al. by applying integrated analysis of transcriptome and metabolome data (Hirai et al. 2004).

Successful demonstration of connections between genes and metabolites, elucidating a wide range of signal output from ABA under dehydration (Urano et al. 2009) and the DREB1/CBF transcription factors in response to low temperature was made possible because of integrated analyses of the transcriptome and the metabolome (Maruyama et al. 2014). This approach is proven to be useful to elucidate the regulation of the pathway and also the involvement of transcriptional regulation of the pathway. The studies using proteomics together with metabolomics are relatively rare in the plant stress response field. One example is the study by which showed the importance of starch and raffinose family oligosaccharide metabolism during temperature stress by the metabolomic and proteomic analysis of the starch-deficient Arabidopsis mutant lacking phosphoglucomutase (pgm mutant) was studied by Wienkoop et al. (2008).

To summarise, experiments to date have allowed us to catalogue a vast array of metabolic changes in response to abiotic stress. Without over generalising, since some of the metabolic changes are very well understood at a mechanistic level, our understanding of the causes and effects of these changes remains in some cases is rather negligible. The metabolic changes are considered to be divided into three phases in responses to stress, including a direct effect of environmental changes, transient adaptation to stress conditions and the new steady state established under prolonged stress conditions. Each phase adopts a different duration depending on the type and the severity of the stress. A detailed time course experiment is therefore necessary to distinguish to which phase the metabolic changes are related. It is also very important that the results already obtained should be integrated with those from isotope feeding experiments, comprehensive phytohormone measurements. Better dissection of the plant metabolic regulatory networks and their functions in the responses to complex abiotic stresses can only be achieved by integrated multiple-omics techniques (Caldana et al. 2011; Maruyama et al. 2014; Urano et al. 2010; Kanani et al. 2010). Transcriptomic and proteomic studies will also deepen our understanding of these crucial survival processes. Once obtained such information will provide an immense knowledge and base for various approaches to ensure food security.

References

Allwood JW, Goodacre R (2010) An introduction to liquid chromatography–mass spectrometry instrumentation applied in plant metabolomic analyses. Phytochem Anal 21:33–47

Baxter CJ, Redestig H, Schauer N, Repsilber D, Patil KR, Nielsen J, Selbig J, Liu J, Fernie AR, Sweetlove LJ (2007) The metabolic response of heterotrophic Arabidopsis cells to oxidative stress. Plant Physiol 143:312–325

Bénard C, Bernillon S, Biais B, Osorio S, Maucourt M, Ballias P, Deborde C, Colombié S, Cabasson C, Jacob D (2015) Metabolomic profiling in tomato reveals diel compositional changes in fruit affected by source–sink relationships. J Exp Bot 66:3391–3404

Brosché M, Vinocur B, Alatalo ER, Lamminmäki A, Teichmann T, Ottow EA, Djilianov D, Afif D, Bogeat-Triboulot MB, Altman A, Polle A, Dreyer E, Rudd S, Paulin L, Auvinen P, Kangasjärvi J (2005) Gene expression and metabolite profiling of Populus euphratica growing in the Negev desert. Genome Biol 6:R101

Buri M, Masunaga T, Wakatsuki T (2000) Sulfur and zinc levels as limiting factors to rice production in West Africa lowlands. Geoderma 94:23–42

Caldana C, Degenkolbe T, Cuadros-Inostroza A, Klie S, Sulpice R, Leisse A, Steinhauser D, Fernie AR, Willmitzer L, Hannah MA (2011) High-density kinetic analysis of the metabolomic and transcriptomic response of Arabidopsis to eight environmental conditions. Plant J 67:869–884

Chaves MM, Oliveira MM (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J Exp Bot 55:2365–2384

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560

Cominelli E, Galbiati M, Tonelli C, Bowler C (2009) Water: the invisible problem. Access to fresh water is considered to be a universal and free human right, but dwindling resources and a burgeoning population are increasing its economic value. EMBO Rep 10:671–676

Cook D, Fowler S, Fiehn O, Thomashow MF (2004) A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of Arabidopsis. Proc Natl Acad Sci U S A 101:15243–15248

Cramer GR, Ergül A, Grimplet J, Tillett RL, Tattersall EA, Bohlman MC, Vincent D, Sonderegger J, Evans J, Osborne C, Quilici D, Schlauch KA, Schooley DA, Cushman JC (2007) Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Funct Integr Genomics 7:111–134

Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol 11:163

Das A, Rushton PJ, Rohila JS (2017) Metabolomic profiling of soybeans (Glycine max L.) reveals the importance of sugar and nitrogen metabolism under drought and heatstress. Plant (Basel) 6:21

Dixon RA, Gang DR, Charlton AJ, Fiehn O, Kuiper HA, Reynolds TL, Tjeerdema RS, Jeffery EH, German JB, Ridley WP, Seiber JN (2006) Applications of metabolomics in agriculture. J Agric Food Chem 54:8984–8994

Dubey S, Misra P, Dwivedi S, Chatterjee S, Bag SK, Mantri S, Asif MH, Rai A, Kumar S, Shri M, Tripathi P, Tripathi RD, Trivedi PK, Chakrabarty D, Tuli R (2010) Transcriptomic and metabolomic shifts in rice roots in response to Cr (VI) stress. BMC Genomics 11:648

Eisenreich W, Bacher A (2007) Advances of high-resolution NMR techniques in the structural and metabolic analysis of plant biochemistry. Phytochemistry 68:2799–2815

Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L (2004) Metabolite profiling: from diagnostics to systems biology. Nat Rev Mol Cell Biol 5:763–769

Francki MG, Hayton S, Gummer J, Rawlinson C, Trengove RD (2016) Metabolomic profiling and genomic analysis of wheat aneuploid lines to identify genes controlling biochemical pathways in mature grain. Plant Biotechnol J 14:649–660

Gagneul D, Aïnouche A, Duhazé C, Lugan R, Larher FR, Bouchereau A (2007) A reassessment of the function of the so-called compatible solutes in the halophytic plumbaginaceae Limonium latifolium. Plant Physiol 144:1598–1611

Gong Q, Li P, Ma S, Indu Rupassara S, Bohnert HJ (2005) Salinity stress adaptation competence in the extremophile Thellungiella halophila in comparison with its relative Arabidopsis thaliana. Plant J 44:826–839

Guy CL (1990) Cold acclimation and freezing stress tolerance: role of protein metabolism. Annu Rev Plant Physiol Plant Mol Biol 41:187–223

Halket JM, Waterman D, Przyborowska AM, Patel RK, Fraser PD, Bramley PM (2005) Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. J Exp Bot 56:219–243

Hall RD, Brouwer ID, Fitzgerald MA (2008) Plant metabolomics and its potential application for human nutrition. Physiol Plant 132:162–175

Hegeman AD (2010) Plant metabolomics—meeting the analytical challenges of comprehensive metabolite analysis. Brief Funct Genomics 9:139–148

Heyman HM, Dubery IA (2016) The potential of mass spectrometry imaging in plant metabolomics: a review. Phytochem Rev 15:297–316

Hirai MY, Yano M, Goodenowe DB, Kanaya S, Kimura T, Awazuhara M, Arita M, Fujiwara T, Saito K (2004) Integration of transcriptomics and metabolomics for understanding of global responses to nutritional stresses in Arabidopsis thaliana. Proc Natl Acad Sci U S A 101:10205–10210

Hu C, Shi J, Quan S, Cui B, Kleessen S, Nikoloski Z, Tohge T, Alexander D, Guo L, Lin H et al (2014) Metabolic variation between japonica and indica rice cultivars as revealed by non-targeted metabolomics. Sci Rep 4:50–67

Issaq HJ, Van QN, Waybright TJ, Muschik GM, Veenstra TD (2009) Analytical and statistical approaches to metabolomics research. J Sep Sci 32:2183–2199

Johnson HE, Broadhurst D, Goodacre R, Smith AR (2003) Metabolic fingerprinting of salt-stressed tomatoes. Phytochemistry 62:919–928

Kanani H, Dutta B, Klapa MI (2010) Individual vs. combinatorial effect of elevated CO_2 conditions and salinity stress on Arabidopsis thaliana liquid cultures: comparing the early molecular response using time-series transcriptomic and metabolomic analyses. BMC Syst Biol 4:177

Kaplan F, Kopka J, Haskell DW, Zhao W, Schiller KC, Gatzke N, Sung DY, Guy CL (2004) Exploring the temperature-stress metabolome. Plant Physiol 136:4159–4168

Kaplan F, Kopka J, Sung DY, Zhao W, Popp M, Porat R, Guy CL (2007) Transcript and metabolite profiling during cold acclimation of Arabidopsis reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. Plant J 50:967–981

Khakimov B, Bak S, Engelsen SB (2014) High-throughput cereal metabolomics: current analytical technologies, challenges and perspectives. J Cereal Sci 59:393–418

Khush GS (2001) Green revolution: the way forward. Nat Rev Genet 2:815–822

Kim JK, Bamba T, Harada K, Fukusaki E, Kobayashi A (2007) Time-course metabolic profiling *in Arabidopsis thaliana* cell cultures after salt stress treatment. J Exp Bot 58:415–424

Král'ová K, Jampílek J, Ostrovský I (2012) Metabolomics-useful tool for study of plant responses to abiotic stresses. Ecol Chem Eng S 19:133–161

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. Plant J 70:39–50

Kusano M, Tohge T, Fukushima A, Kobayashi M, Hayashi N, Otsuki H, Kondou Y, Goto H, Kawashima M, Matsuda F, Niida R, Matsui M, Saito K, Fernie AR (2011) Metabolomics reveals comprehensive reprogramming involving two independent metabolic responses of Arabidopsis to UV-B light. Plant J 67:354–369

Langridge P, Fleury D (2011) Making the most of 'omics' for crop breeding. Trends Biotechnol 29:33–40

Lei Z, Huhman DV, Sumner LW (2011) Mass spectrometry strategies in metabolomics. J Biol Chem 286:25435–25442

Li X, Lawas LM, Malo R, Glaubitz U, Erban A, Mauleon R, Heuer S, Zuther E, Kopka J, Hincha DK (2015) Metabolic and transcriptomic signatures of rice floral organs reveal sugar starvation as a factor in reproductive failure under heat and drought stress. Plant Cell Environ 38:2171–2192

Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat Protoc 1:387–396

Lommen A, Weseman JM, Smith GO, Noteborn HPJM (1998) On the detection of environmental effects on complex matrices combining off-line liquid chromatography and 1H-NMR. Biodegradation 9:513–525

Lugan R, Niogret MF, Leport L, Guégan JP, Larher FR, Savouré A, Kopka J, Bouchereau A (2010) Metabolome and water homeostasis analysis of Thellungiella salsuginea suggests that dehydration tolerance is a key response to osmotic stress in this halophyte. Plant J 64:215–229

Maruyama K, Urano K, Yoshiwara K, Morishita Y, Sakurai N, Suzuki H, Kojima M, Sakakibara H, Shibata D, Saito K (2014) Integrated analysis of the effects of cold and dehydration on rice metabolites, phytohormones, and gene transcripts. Plant Physiol 164:1759–1771

Medina J, Catalá R, Salinas J (2011) The CBFs: three Arabidopsis transcription factors to cold acclimate. Plant Sci 180:3–11

Michaletti A, Naghavi MR, Toorchi M, Zolla L, Rinalducci S (2018) Metabolomics and proteomics reveal drought-stress responses of leaf tissues from spring-wheat. Sci Rep 8:5710

Misra BB, Assmann SM, Chen S (2014) Plant single-cell and single-cell-type metabolomics. Trends Plant Sci 19:637–646

Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19

Moller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481

Monton MR, Soga T (2007) Metabolome analysis by capillary Electrophoresis–mass spectrometry. J Chromatogr A 1168:237–246

Moussaieff A, Rogachev I, Brodsky L, Malitsky S, Toal TW, Belcher H, Yativ M, Brady SM, Benfey PN, Aharoni A (2013) High-resolution metabolic mapping of cell types in plant roots. Proc Natl Acad Sci U S A 110:1232–1241

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663

Nakabayashi R, Saito K (2015) Integrated metabolomics for abiotic stress responses in plants. Curr Opin Plant Biol 24:10–16

Okazaki Y, Saito K (2012) Recent advances of metabolomics in plant biotechnology. Plant Biotechnol Rep 6:1–15

Parry MA, Hawkesford MJ (2012) An integrated approach to crop genetic improvement. J Integr Plant Biol 54:250–259

Ramautar R, Somsen GW, de Jong GJ (2009) CE-MS in metabolomics. Electrophoresis 30:276–291

Ramautar R, Mayboroda OA, Somsen GW, de Jong GJ (2011) CE-MS for metabolomics: developments and applications in the period 2008–2010. Electrophoresis 32:52–65

Rao J, Cheng F, Hu C, Quan S, Lin H, Wang J, Chen G, Zhao X, Alexander D, Guo L et al (2014) Metabolic map of mature maize kernels. Metabolomics 10:775–787

Ratcliffe RG, Shachar-Hill Y (2005) Revealing metabolic phenotypes in plants: inputs from NMR analysis. Biol Rev Camb Philos Soc 80:27–43

Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R (2004) When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. Plant Physiol 134:1683–1696

Rocha M, Licausi F, Araújo WL, Nunes-Nesi A, Sodek L, Fernie AR, van Dongen JT (2010) Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of Lotus japonicus. Plant Physiol 152:1501–1513

Rogachev I, Aharoni A (2012) UPLC-MS-based metabolite analysis in tomato. Methods Mol Biol 860:129–144

Scossa F, Brotman Y, de Abreu e Lima F, Willmitzer L, Nikoloski Z, Tonga T, Fernie AR (2016) Genomics-based strategies for the use of natural variation in the improvement of crop metabolism. Plant Sci 242:47–64

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14:43–50

Shulaev V, Cortes D, Miller G, Mittler R (2008) Metabolomics for plant stress response. Physiol Plant 132:199–208

Soga T, Imaizumi M (2001) Capillary electrophoresis method for the analysis of inorganic anions, organic acids, amino acids, nucleotides, carbohydrates and other anionic compounds. Electrophoresis 22:3418–3425

Sugimoto M, Hirayama A, Robert M, Abe S, Soga T, Tomita M (2010) Prediction of metabolite identity from accurate mass, migration time prediction and isotopic pattern information in CE-TOFMS data. Electrophoresis 31:2311–2318

Sun X, Zhang J, Zhang H, Ni Y, Zhang Q, Chen J, Guan Y (2010) The responses of *Arabidopsis thaliana* to cadmium exposure explored via metabolite profiling. Chemosphere 78:840–845

Sun C, Gao X, Fu J, Zhou J, Wu X (2015) Metabolic response of maize (Zea mays L.) plants to combined drought and salt stress. Plant Soil 388:99–117

Sweetlove LJ, Obata T, Fernie AR (2014) Systems analysis of metabolic phenotypes: what have we learnt? Trends Plant Sci 19:222–230

Terskikh VV, Feurtado JA, Borchardt S, Giblin M, Abrams SR, Kermode AR (2005) In vivo 13C NMR metabolite profiling: potential for understanding and assessing conifer seed quality. J Exp Bot 56:2253–2265

Tester M, Davenport R (2003) Na+ tolerance and Na+ transport in higher plants. Ann Bot 91:503–527

Thomashow MF (2010) Molecular basis of plant cold acclimation: insights gained from studying the CBF cold response pathway. Plant Physiol 154:571–577

Tohge T, de Souza LP, Fernie AR (2014) Genome-enabled plant metabolomics. J Chromatogr B 966:7–20

Toubiana D, Fernie AR, Nikoloski Z, Fait A (2013) Network analysis: tackling complex data to study plant metabolism. Trends Biotechnol 31:29–36

Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N, Suzuki H, Saito K, Shibata D, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2009) Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. Plant J 57:1065–1078

Urano K, Kurihara Y, Seki M, Shinozaki K (2010) 'Omics' analyses of regulatory networks in plant abiotic stress responses. Curr Opin Plant Biol 13:132–138

van Dam NM, Bouwmeester HJ (2016) Metabolomics in the rhizosphere: tapping into belowground chemical communication. Trends Plant Sci 21:256–265

van Dongen JT, Fröhlich A, Ramírez-Aguilar SJ, Schauer N, Fernie AR, Erban A, Kopka J, Clark J, Langer A, Geigenberger P (2009) Transcript and metabolite profiling of the adaptive response to mild decreases in oxygen concentration in the roots of Arabidopsis plants. Ann Bot 103:269–280

Verslues PE, Juenger TE (2011) Drought, metabolites, and Arabidopsis natural variation: a promising combination for understanding adaptation to water-limited environments. Curr Opin Plant Biol 14:240–245

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu JK (2006) Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J 45:523–539

Wang C, Hu S, Gardner C, Lübberstedt T (2017) Emerging avenues for utilization of exotic germplasm. Trends Plant Sci 22:624–637

Wanner LA, Junttila O (1999) Cold-induced freezing tolerance in Arabidopsis. Plant Physiol 120:391–400

Wienkoop S, Morgenthal K, Wolschin F, Scholz M, Selbig J, Weckwerth W (2008) Integration of metabolomic and proteomic phenotypes: analysis of data covariance dissects starch and RFO metabolism from low and high temperature compensation response in *Arabidopsis thaliana*. Mol Cell Proteomics 7:1725–1736

Williams BJ, Cameron CJ, Workman R, Broeckling CD, Sumner LW, Smith JT (2007) Amino acid profiling in plant cell cultures: an inter-laboratory comparison of CE-MS and GC–MS. Electrophoresis 28:1371–1379

Winning H, Roldan-Martln E, Dragsted LO, Viereck N, Poulsen M, Sanchez-Moreno C et al (2009) An exploratory NMR nutri-metabonomic investigation reveals dimethyl sulfone as a dietary biomarker for onion intake. Analyst 134:2344–2351

Wulff-Zottele C, Gatzke N, Kopka J, Orellana A, Hoefgen R, Fisahn J, Hesse H (2010) Photosynthesis and metabolism interact during acclimation of Arabidopsis thaliana to high irradiance and sulphur depletion. Plant Cell Environ 33:1974–1988

Wuolikainen A, Jonsson P, Ahnlund M, Antti H, Marklund SL, Moritz T et al (2016) Multi-platform mass spectrometry analysis of the CSF and plasma metabolomes of rigorously matched amyotrophic lateral sclerosis, Parkinson's disease and control subjects. Mol BioSyst 12:1287–1298

Xavier A, Hall B, Hearst AA, Cherkauer KA, Rainey KM (2017) Genetic architecture of phenomic-enabled canopy coverage in *Glycine max*. Genetics 206:1081–1089

Zuther E, Koehl K, Kopka J (2007) Comparative metabolome analysis of the salt response in breeding cultivars of rice. In: Jenks MA, Hasegawa PM, Jain SM (eds) Advances in molecular breeding toward drought and salt tolerant crops. Springer, Berlin, pp 285–315