Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks

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Received 7 October 2011; Revised 21 December 2011; Accepted 22 December 2011

Abstract

Plants regularly face adverse growth conditions, such as drought, salinity, chilling, freezing, and high temperatures. These stresses can delay growth and development, reduce productivity, and, in extreme cases, cause plant death. Plant stress responses are dynamic and involve complex cross-talk between different regulatory levels, including adjustment of metabolism and gene expression for physiological and morphological adaptation. In this review, information about metabolic regulation in response to drought, extreme temperature, and salinity stress is summarized and the signalling events involved in mediating stress-induced metabolic changes are presented.

Key words: Abiotic stress, metabolism, protein kinase, signal transduction, transcription factor.

Plants in adverse environments

Plants frequently encounter unfavourable growth conditions. Climatic factors, such as extreme temperatures (heat, cold, freezing), drought (deficient precipitation, drying winds), and contamination of soils by high salt concentration, are major abiotic environmental stressors that limit plant growth and development, and thus agronomical yield, and play a major role in determining the geographic distribution of plant species. These different adverse but not necessarily lethal conditions are generally known as stress.

Environmental stress can disrupt cellular structures and impair key physiological functions (Larcher, 2003). Drought, salinity, and low temperature stress impose an osmotic stress that can lead to turgor loss. Membranes may become disorganized, proteins may undergo loss of activity or be denatured, and often excess levels of reactive oxygen species (ROS) are produced leading to oxidative damage. As a consequence, inhibition of photosynthesis, metabolic dysfunction, and damage of cellular structures contribute to growth perturbances, reduced fertility, and premature senescence.

Different plant species are highly variable with respect to their optimum environments, and a harsh environmental condition, which is harmful for one plant species, might not be stressful for another (Larcher, 2003; Munns and Tester, 2008). This is also reflected in the multitude of different stress-response mechanisms. Two major strategies can be distinguished: stress avoidance and stress tolerance (Levitt, 1980). Stress avoidance includes a variety of protective mechanisms that delay or prevent the negative impact of a stress factor on a plant. For example, cacti have constitutively adapted their morphology, physiology, and metabolism to hot and arid climates. Adaptation is stable and inherited. On the other hand, stress tolerance is the potential of a plant to acclimate to a stressful condition. For example, in summer, trees and herbaceous plants in northern latitudes cannot withstand freezing. Exposure to chilling temperatures, however, induces hardening and acclimated plants survive winter temperatures far below freezing. Plants can increase their resistance to various stresses including heat, saline, and drought conditions in response to a period of gradual exposure to these constraints. Acclimation is plastic and reversible. The physiological modifications induced during acclimation are diverse and are usually lost when the adverse environmental condition does not persist.

Responses to environmental stresses occur at all levels of organization. Cellular responses to stress include adjustments of the membrane system, modifications of the cell wall architecture, and changes in cell cycle and cell division. In addition, plants alter metabolism in various ways, including
production of compatible solutes (e.g. proline, raffinose, and glycine betaine) that are able to stabilize proteins and cellular structures and/or to maintain cell turgor by osmotic adjustment, and redox metabolism to remove excess levels of ROS and re-establish the cellular redox balance (Bartels and Sunkar, 2005; Valliyodan and Nguyen, 2006; Munns and Tester, 2008; Janska et al., 2010). At the molecular level, gene expression is modified upon stress (Chinnusamy et al., 2007; Shinozaki and Yamaguchi-Shinozaki, 2007) and epigenetic regulation plays an important role in the regulation of gene expression in response to environmental stress (Hauser et al., 2011; Khraiwesh et al., 2011). Stress-inducible genes comprise genes involved in direct protection from stress, including the synthesis of osmoprotectants, detoxifying enzymes, and transporters, as well as genes that encode regulatory proteins such as transcription factors, protein kinases, and phosphatases.

In this review, the focus is on metabolic adjustments in response to high salt stress, drought, and extreme temperatures and our current knowledge of signal transduction components that regulate metabolite levels under these stress conditions will be summarized. Figures 1 and 2 give an overview of the biosynthesis and degradation pathways of important metabolites connected with stress tolerance and will guide you through the next paragraphs.

**Fig. 1.** Schematic overview of amino acid, polyamine, and glycine betaine metabolism. Plants with enhanced or reduced activity of the indicated enzymes show altered tolerance to abiotic stress. ApGSMT, *Aphanthece halophytica* glycine sarcosine methyl transferase (EC 2.1.1.156); ApDMT, *Aphanthece halophytica* dimethylglycine methyltransferase (EC 2.1.1.157); codA, choline oxidase (EC 1.1.3.17); betA, choline dehydrogenase (EC 1.1.99.1); betB, betaine aldehyde dehydrogenase (EC 1.2.1.8); GAD, glutamate decarboxylase (EC 4.1.1.15); GABA-T, 4-aminobutyrate aminotransferase (EC 2.6.1.19); SSADH, succinic semialdehyde dehydrogenase (EC 1.2.1.16); P5CS, 1-pyrroline-5-carboxylate synthetase (EC 2.7.2.11 and EC 1.2.1.41); P5CR, pyrroline-5-carboxylate reductase (EC 1.5.1.2); ProDH, proline dehydrogenase (EC 1.5.99.8); P5CDH, 1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.12); δ-OAT, ornithine δ-aminotransferase (EC 2.6.1.13); ADC, arginine decarboxylase (EC 4.1.1.19); ODC, ornithine decarboxylase (EC 4.1.1.17); SPDS, spermidine synthase (EC 2.5.1.16); SPMS, spermine synthase (EC 2.1.5.22); PAO, polyamine oxidase (EC 1.5.3.11); DAO, diamine oxidase (EC 1.4.3.22).
Amino acids

Accumulation of amino acids has been observed in many studies on plants exposed to abiotic stress (Barnett and Naylor, 1966; Draper, 1972; Handa et al., 1983; Rhodes et al., 1986; Fougere et al., 1991; Kaplan et al., 2004; Brosche et al., 2005; Zuther et al., 2007; Kempa et al., 2008; Sanchez et al., 2008; Usadel et al., 2008; Lugan et al., 2010). This increase might stem from amino acid production and/or from enhanced stress-induced protein breakdown. While the overall accumulation of amino acids upon stress might indicate cell damage in some species (Widodo et al., 2009), increased levels of specific amino acids have a beneficial effect during stress acclimation.

Proline

For many years, the capacity to accumulate proline has been correlated with stress tolerance (Barnett and Naylor, 1966; Singh et al., 1972; Stewart and Lee, 1974). Proline is considered to act as an osmolyte, a ROS scavenger, and a molecular chaperone stabilizing the structure of proteins, thereby protecting cells from damage caused by stress (Hare and Cress, 1997; Rhodes et al., 1986; Verbruggen and Hermans, 2008; Szabados and Savoure, 2010). Proline accumulates in many plant species in response to different environmental stresses including drought, high salinity, and heavy metals. Interestingly, heat stress did not lead to proline accumulation in tobacco and Arabidopsis plants and induced proline accumulation rendered plants more

Fig. 2. Schematic overview of starch, fructan, sugar, and polyol metabolism. Plants with enhanced or reduced activity of the indicated enzymes show altered tolerance to abiotic stress. SEX1, α-glucan water dikinase (EC 2.7.9.4); St. phos, starch phosphorylase (2.4.1.1); BMY, β-amylase (EC 3.2.1.2); DPE2, glucanotransferase (EC 2.4.1.25); 1-SST, sucrose:sucrose 1-fructosyltransferase (EC 2.4.1.99); 6-SFT, sucrose:fructan 6-fructosyltransferase (EC 2.4.1.10); FBF, fructan beta-fructosidase (EC 3.2.1.80); mtID, mannitol-1-phosphate dehydrogenase (EC 1.1.1.17); S6PDH, sorbitol-6-phosphate dehydrogenase (EC 1.1.1.200); SDH, sorbitol dehydrogenase (EC 1.1.1.14); Pase, unspecific phosphatase; MIPS, inositol-1-phosphate synthase (EC 5.5.1.4); IMP, inositol-1-phosphate phosphatase (EC 3.1.3.25); IMT, inositol methyltransferase (EC 2.1.1.40); GoIS, galactinol synthase (EC 2.4.1.123); RS, raffinose synthase (EC 2.4.1.82), StS, stachyose synthase (EC 2.4.1.67); TPS, trehalose-6-phosphate synthase (EC 2.4.1.15); TPP, trehalose-6-phosphate phosphatase (EC 3.1.3.12).
sensitive to heat (Rizhsky et al., 2004; Dobra et al., 2010; Lv et al., 2011). Proline levels are determined by the balance between biosynthesis and catabolism (Szabados and Savoure, 2010). Proline is produced in the cytosol or chloroplasts from glutamate, which is reduced to glutamate-semialdehyde (GSA) by Δ1-pyrroline-5-carboxylate synthetase (P5CS). GSA can spontaneously convert to pyrroline-5-carboxylate (P5C), which is then further reduced by P5C reductase (P5CR) to proline. Proline is degraded in mitochondria by proline dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH) to glutamate. Stress conditions stimulate proline synthesis while proline catabolism is enhanced during recovery from stress. Over-expression of P5CS in tobacco and petunia led to increased proline accumulation and enhanced salt and drought tolerance (Kishor et al., 1995; Hong et al., 2000; Yamada et al., 2005), whereas Arabidopsis P5CS1 knock-out plants were impaired in stress-induced proline synthesis and were hypersensitive to salinity (Szekely et al., 2008). Consistently, ProDH antisense Arabidopsis accumulated more proline and showed enhanced tolerance to freezing and high salinity (Nanjo et al., 1999). It is discussed that, in an alternative pathway, mitochondrial P5C can be produced by δ-ornithine aminotransferase (δ-OAT) from ornithine (Miller et al., 2009). Over-expression of Arabidopsis δ-OAT has been shown to enhance proline levels and to increase the stress tolerance of rice and tobacco (Roosen et al., 2002; Qu et al., 2005) even though Arabidopsis plants deficient in δ-OAT accumulated proline in response to stress and showed a salt stress tolerance similar to the wild type (Funck et al., 2008).

GABA

The non-protein amino acid γ-aminobutyric acid (GABA) rapidly accumulates to high levels under different adverse environmental conditions (Shelp et al., 1999; Rhodes et al., 1986; Kinnersley and Turano, 2000; Kaplan and Guy, 2004; Kempa et al., 2008; Renault et al., 2010).

GABA is mainly synthesized from glutamate in the cytosol by glutamate decarboxylase (GAD) and then transported to the mitochondria. GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) convert GABA into succinate that feeds into the TCA-cycle (Shelp et al., 1999; Fait et al., 2008). GABA metabolism has been associated with carbon-nitrogen balance and ROS scavenging (Bouche and Fromm, 2004; Song et al., 2010; Liu et al., 2011). A functional GABA shunt is important for stress tolerance. Salt stress enhances the activity of enzymes involved in GABA metabolism (Renault et al., 2010). Arabidopsis mutants defective in GABA-T were hypersensitive to ionic stress and showed increased levels of amino acid (including GABA), while carbohydrate levels were decreased (Renault et al., 2010). Disruption of the SSADH gene led to the accumulation of ROS associated with dwarfism and hypersensitivity to UV-B and heat stress (Bouche et al., 2003).

Amines

Polyamines

Polyamines (PA) are small aliphatic molecules positively charged at cellular pH. Various stresses, such as drought, salinity and cold, modulate PA levels, and high PA levels have been positively correlated with stress tolerance (Yang et al., 2007; Cuevas et al., 2008; Groppa and Benavides, 2008; Usadel et al., 2008; Kovacs et al., 2010; Quinet et al., 2010; Alcazar et al., 2011).

Putrescine, spermidine, and spermine are the most common PAs in higher plants. Putrescine is produced from either ornithine or arginine by ornithine decarboxylase (ODC) and arginine decarboxylase (ADC), respectively. Putrescine is converted to spermidine by spermidine synthase (SPDS) and then to spermine by spermine synthase (SPMS). Spermidine and spermine are substrates of polyamine-oxidases (PAOs), which catalyse the back-conversion to putrescine.

PAs have been implicated in protecting membranes and alleviating oxidative stress (Groppa and Benavides, 2008; Alcazar et al., 2011; Hussain et al., 2011) but their specific function in stress tolerance is not well understood. Analyses of transgenic plants and of mutants involved in PA metabolism clearly showed a positive role of PAs in stress tolerance. Plants deficient in ADC1 or ADC2 had reduced putrescine levels and were hypersensitive to stress (Urano et al., 2004; Cuevas et al., 2008), whereas constitutive or stress-induced over-expression of ADC led to higher putrescine levels and enhanced drought and freezing tolerance (Capell et al., 2004; Alcazar et al., 2010; Alet et al., 2011). Arabidopsis plants over-expressing SPDS produced higher amounts spermidine and were more resistant to drought, salt, and cold stress (Kasukabe et al., 2004). Plants deficient in SPMS were unable to synthesise spermine and are hypersensitive to salinity (Yamaguchi et al., 2006). Furthermore, the introduction of ornithine decarboxylase (ODC) from mouse enhanced polyamine levels and the tolerance of tobacco to salt stress (Kumriaa and Rajam, 2002).

Glycine betaine

Glycine betaine (GB) is a quaternary ammonium compound that occurs in a wide variety of plants, but its distribution among plants is sporadic (Cromwell and Rennie, 1953). Arabidopsis and many crop species do not accumulate GB. In plants that produce GB naturally, abiotic stress, such as cold, drought, and salt stress, enhances GB accumulation (Rhodes and Hanson, 1993; Chen and Murata, 2011).

GB can be synthesized from choline and glycine (Chen and Murata, 2011). Introduction of the GB biosynthesis pathway genes into non-accumulators improved their ability to tolerate abiotic stress conditions (Hayashi et al., 1997; Alia et al., 1998; Sakamoto et al., 1998, 2000; Holmstrom et al., 2000; Park et al., 2004; Waditee et al., 2005; Bansal et al., 2011), pointing to the beneficial role of GB in stress tolerance. Targeting GB production to the chloroplasts led to a higher tolerance of plants against stress than in the cytosol (Park et al., 2007). In salt-tolerant plant species, GB
can also accumulate to osmotically significant levels (Rhodes and Hanson, 1993). It has been suggested that GB protects photosystem II, stabilizes membranes, and mitigates oxidative damage (Chen and Murata, 2011).

**Carbohydrates**

**Fructans**

Plants accumulate carbohydrates such as starch and fructans as storage substances that can be mobilized during periods of limited energy supply or enhanced energetic demands. While most plant species use starch as their main storage carbohydrate, several angiosperms, mainly from regions with seasonal cold and dry periods, accumulate fructans (Hendry, 1993). Accumulation of fructans might be advantageous, due to their high water solubility, their resistance to crystallization at freezing temperatures, and the fact that fructan synthesis functions normally under low temperatures (Vijn and Smeekens, 1999; Livingston et al., 2009). Furthermore, fructans can stabilize membranes (Valluru and Van den Ende, 2008) and might indirectly contribute to osmotic adjustment upon freezing and dehydration by the release of hexose sugars (Spollen and Nelson, 1994; Olien and Clark, 1995).

Fructans are branched fructose polymers that are synthesized in the vacuole by fructosyltransferases, including 1-SST and 6-SFT, which transfer fructose from sucrose to the growing fructan chain (Vijn and Smeekens, 1999; Livingston et al., 2009). The introduction of fructosyltransferases to fructan non-accumulating tobacco and rice plants stimulated fructan production associated with enhanced tolerance to low-temperature stress and drought (Pilonsmits et al., 2007; Kawakami et al., 2008).

**Starch, mono- and disaccharides**

Starch is composed of glucose polymers arranged into osmotically inert granules. It serves as the main carbohydrate store in most plants and can be rapidly mobilized to provide soluble sugars. Its metabolism is very sensitive to changes in the environment. In addition to diurnal fluctuations in starch levels, salt and drought stress generally leads to a depletion of starch content and to the accumulation of soluble sugars in leaves (Todaka et al., 2000; Kaplan and Guy, 2004; Basu et al., 2007; Kempa et al., 2008). Sugars that accumulate in response to stress can function as osmolytes to maintain cell turgor and have the ability to protect membranes and proteins from stress damage (Madden et al., 1985; Kaplan and Guy, 2004).

Starch is produced in plastids from excess sugars during photosynthesis and involves ADP-glucose pyrophosphorylase (AGPase), starch synthases, branching enzymes, and debranching enzymes. Phosphorylation of starch granules by glucan-water dikinase (GWD) and phosphoglucan-water dikinase (P WD) stimulates starch degradation. β-amylases produce maltose from glucans. In the cytosol, maltose is converted to glucose and, subsequently, fructose and sucrose are formed (Tetlow et al., 2004; Kotting et al., 2010).

Hydrolysis of starch by the β-amylolytic pathway represents the predominant pathway of starch degradation in leaves under normal growth conditions and may also be involved in stress-induced starch hydrolysis. Arabidopsis sexl (starch excess l) mutants, that are impaired in GWD activity, were compromised in cold-induced malto-oligosaccharide, glucose, and fructose accumulation during the early stages of cold acclimation and sexl plants that have been briefly pre-exposed to cold showed a reduced freezing tolerance (Yano et al., 2005). Osmotic stress was shown to enhance total β-amylase activity and to reduce light-stimulated starch accumulation in wild-type Arabidopsis but not in bami1 (bmy7) mutants, which appeared to be hypersensitive to osmotic stress (Valerio et al., 2011). Similarly, BMY8 (BAM3) antisense plants accumulated high starch levels, were impaired in cold-induced maltose, glucose, fructose, and sucrose accumulation, and showed a reduced tolerance of photosystem II to low temperature stress (Kaplan and Guy, 2005). Data by Zeeman et al. (2004) also suggest a role of the phosphorolytic starch degradation pathway during stress. Arabidopsis plants deficient in plastidal α-glucan phosphorylase showed enhanced formation of lesions surrounded by cells with high starch levels after exposure to low air humidity or salt stress.

**Trehalose**

The non-reducing disaccharide trehalose accumulates to high amounts in some desiccation-tolerant plants, for example, Myrothamnus flabellifolius (Bianchi et al., 1993; Drennan et al., 1993). At sufficient levels, trehalose can function as an osmolyte and stabilize proteins and membranes (Paul et al., 2008). In most angiosperms however, trehalose is present in trace amounts and abiotic stress increases the levels of trehalose only moderately (Fougere et al., 1991; Garg et al., 2002; Kaplan et al., 2004; Panikulangara et al., 2004; Rizhsky et al., 2004; Guy et al., 2008; Kempa et al., 2008).

In plants, trehalose is synthesized in a two-step process (Paul et al., 2008). Trehalose-6-phosphate synthase (TPS) generates trehalose-6-phosphate (T6P) from UDP-glucose and glucose-6-phosphate followed by dephosphorylation to trehalose by trehalose-6-phosphate phosphatase (TPP). Transgenic expression of trehalose biosynthesis genes showed that enhanced trehalose metabolism can positively regulate tolerance to abiotic stress, even though only a limited increase in trehalose content could be observed, excluding a direct protective role of trehalose in these plants. Heterologous expression of genes involved in the trehalose pathway from E. coli or Saccharomyces cerevisiae enhanced tolerance to drought, salt, and low temperature stress in several plant species (Iordachescu and Imai, 2008). Over-expression of different isoforms of TPS from rice conferred enhanced resistance to salinity, cold, and/or drought (Li et al., 2011). Arabidopsis plants constitutively over-expressing AtTPS1 showed a small increase in trehalose and T6P levels and were more tolerant to drought (Avonce et al., 2004). Consistently, loss of TPS5 function, a TPS with a TPP domain, reduced the basal thermotolerance of Arabidopsis (Suzuki et al., 2008).
TPP activity and trehalose levels transiently increased in response to cold stress in rice (Panikulangara et al., 2004) and over-expression of OsTPP1 rendered rice more tolerant to salinity and cold even though no increase in the trehalose content could be observed (Ge et al., 2008). These genetically engineered plants provide evidence that enhanced trehalose metabolism can positively regulate stress tolerance, but the precise role of T6P and trehalose during abiotic stress remains to be further elucidated.

**Raffinose family oligosaccharides**

Raffinose family oligosaccharides (RFO) such as raffinose, stachyose, and verbascose accumulate in various plant species during seed desiccation (Peterbauer and Richter, 2001) and in leaves of plants experiencing environmental stress like cold, heat, drought or high salinity (Castonguay and Nadeau, 1998; Gilmour et al., 2000; Taji et al., 2002; Cook et al., 2004; Kaplan et al., 2004; Peters et al., 2007; Kempa et al., 2008; Usadel et al., 2008). RFOs have been implicated in membrane protection and radical scavenging (Hincha, 2003; Nishizawa et al., 2008). Biosynthesis of RFO is initiated by the formation of galactinol from myo-inositol and UDP-galactose by galactinol synthase (GolS). Sequential addition of galactose units, provided by galactinol, to sucrose leads to the formation of raffinose and higher order RFO (Peterbauer and Richter, 2001).

The role of the RFO pathway in acquiring stress tolerance is not yet fully understood. *Arabidopsis* plants over-expressing *Arabidopsis* GolS1 or GolS2 accumulated high levels of galactinol and raffinose and were more tolerant to drought and salinity stress (Taji et al., 2002; Nishizawa et al., 2008). Similarly, constitutive and stress-inducible expression of GolS1 from the resurrection plant *Boea hygrometrica* in tobacco conferred enhanced drought tolerance (Wang et al., 2009). By contrast, increased raffinose content in *Arabidopsis* plants constitutively expressing cucumber GolS did not enhance freezing tolerance (Zuther et al., 2004) and neither go1s1 knock-out plants nor knock-out mutants in the raffinose synthase gene, that were both impaired in raffinose accumulation, showed any obvious increase in sensitivity to heat or freezing stress, respectively (Panikulangara et al., 2004; Zuther et al., 2004).

**Polyols**

Polyols are implicated in stabilizing macromolecules and in scavenging hydroxyl radicals, thereby preventing oxidative damage of membranes and enzymes (Smirnoff and Cumbes, 1989; Shen et al., 1997). Accumulation of the straight-chain polyols, mannitol and sorbitol, has been correlated with stress tolerance in several plant species (Stoop et al., 1996). Expression of mannitol-1-phosphate dehydrogenase (mtID) from *E. coli*, which catalyses the reversible conversion of fructose-6-phosphate to mannitol-1-phosphate in *Arabidopsis*, tobacco, poplar, and wheat induced mannitol accumulation and enhanced tolerance to salinity and/or water deficit (Tarczynski et al., 1993; Thomas et al., 1995; Abebe et al., 2003; Chen et al., 2005). Similarly, photosystem II was less affected by salinity in persimmon trees that accumulated sorbitol by over-expression of sorbitol-6-phosphate dehydrogenase (S6PDH) from apple (Gao et al., 2001).

The cyclic polyols myo-inositol, and its methylated derivatives D-ononitol and D-pinitol, accumulate upon salt stress in several halotolerant plant species (Adams et al., 1992; Vernon and Bohnert, 1992; Ishitani et al., 1996; Sengupta et al., 2008). L-myo-inositol-1-phosphate synthase (MIPS) forms myo-inositol-1-P from glucose-6-P, which is dephosphorylated by myo-inositol 1-phosphate phosphatase (IMP) to form myo-inositol. Inositol O-methyltransferase (IMT) methylates inositol to form D-ononitol, which is epimerized to D-pinitol. In line with a stress-protective role, over-expression of MIPS and IMT from halotolerant plants increased cyclic polyols levels and salt stress tolerance of tobacco (Sheveleva et al., 1997; Majee et al., 2004; Patra et al., 2010).

**Complexity of the metabolic response**

Metabolic adjustments in response to unfavourable conditions are dynamic and multifaceted and not only depend on the type and strength of the stress, but also on the cultivar and the plant species. Traditionally, metabolic studies focused on single metabolites or groups of metabolites. Recent advances in metabolic profiling allow increasingly comprehensive metabolite analyses, illustrating the complexity of metabolic adjustments to stress. The metabolic profiles of different plant species, including rice, poplar, *Vitis vinifera*, *Thellungiella halophila*, and *Arabidopsis thaliana*, have been analysed after exposure to drought (Rizhsky et al., 2004; Cramer et al., 2007; Urano et al., 2009), salinity (Gong et al., 2005; Cramer et al., 2007; Gagneul et al., 2007; Kempa et al., 2008; Sanchez et al., 2008; Janz et al., 2010; Lungan et al., 2010), and temperature stress (Cook et al., 2004; Kaplan et al., 2004, 2007; Rizhsky et al., 2004; Usadel et al., 2008; Espinoza et al., 2010; Caldana et al., 2011).

Plants respond to stress by a progressive adjustment of their metabolism with sustained, transient, early- and late-responsive metabolic alterations. For example, raffinose and proline accumulate to high levels over the course of several days of salt exposure, drought, or cold, whereas central carbohydrate metabolism changes rapidly in a complex, time-dependent manner.

Some metabolic changes are common to salt, drought, and temperature stress, whereas others are specific. For example, levels of amino acids, sugars, and sugar alcohols typically increase in response to different stress conditions. Notably, proline accumulates upon drought, salt, and low temperature but not upon high temperature stress (Kaplan et al., 2004; Rizhsky et al., 2004; Gong et al., 2005; Cramer et al., 2007; Gagneul et al., 2007; Kempa et al., 2008; Sanchez et al., 2008; Usadel et al., 2008; Urano et al., 2009; Lungan et al., 2010). In most studies, organic acids and TCA-cycle intermediates decreased in glycophytes after salt
stress (Gong et al., 2005; Gagneul et al., 2007; Zuther et al., 2007; Sanchez et al., 2008), but increased in response to temperature or drought stress (Kaplan et al., 2004; Usadel et al., 2008; Urano et al., 2009). A direct comparison of the metabolic profile of Arabidopsis thaliana acclimated to either low or high temperatures showed a significant overlap, but also major differences in metabolic adjustments (Kaplan and Guy, 2004). A greater number of metabolite levels changed specifically in response to cold than to heat, pointing towards a strong impact of cold on plant metabolism. Similarly, metabolic rearrangements after drought were more profound than after heat stress and the metabolic profile of Arabidopsis plants exposed to a combination of drought and heat was more similar to that of drought-stressed than heat-stressed plants. Exposure of drought-stressed plants to heat also induced unique metabolic profiles of cultivars or species with different salt tolerance (Kempa et al., 2008; Urano et al., 2009). A direct comparison of the metabolic profile of stress-tolerant accessions with different freezing tolerances point to a crucial role of compatible solutes, including proline and raffinose, in freezing tolerance (Hannah et al., 2006; Zuther et al., 2007; Janz et al., 2010; Korn et al., 2010; Lugan et al., 2010). Generally, stress-tolerant plants have higher levels of stress-related metabolites under normal growth conditions and/or accumulate larger amounts of protective metabolites, such as proline and soluble sugars, under unfavourable conditions, indicating that their metabolism is prepared for adverse growth conditions. For example, analyses of the metabolic profile of Arabidopsis accessions with different freezing tolerances point to a crucial role of compatible solutes, including proline and raffinose, in freezing tolerance (Hannah et al., 2006; Korn et al., 2010). Similarly, comparison of the metabolome of cultivars or species with different salt tolerance indicates a beneficial role of compatible solutes under salinity conditions (Gong et al., 2005; Zuther et al., 2007; Janz et al., 2010; Lugan et al., 2010). Interestingly, metabolism of salt-tolerant plants appeared to be pre-adapted to saline environments by constitutively high levels of some protective metabolites such as proline and raffinose. Consistent with classical studies, these recent metabolomic analyses illustrate that plants have developed a whole range of strategies to adapt their metabolism to unfavourable growth conditions and that enhanced stress resistance is not restricted to a single compound or mechanism. Different plant species accumulate different metabolites (e.g. trehalose, proline, glycine betaine) and there is no absolute requirement for the accumulation of a specific metabolite for acclimation to stress. In some cases, the flux through a metabolic pathway, rather than the accumulation of a specific metabolite per se, might contribute to stress tolerance.

Several metabolites/metabolic pathways that contribute to stress acclimation also play a role in development. Appropriate proline levels have been shown to be important for embryo and flower development (Nanjo et al., 1999; Samach et al., 2000; Mattioli et al., 2008, 2009; Szekely et al., 2008). GABA regulates pollen tube growth and guidance (Wilhelmi and Preuss, 1996; Palanivelu et al., 2003). Polyamines regulate several developmental processes including embryogenesis, meristem, flower, and gametophyte development (Hanzawa et al., 2000; Imai et al., 2004; Alcazar et al., 2005; Gupta and Kaur, 2005; Deeb et al., 2010; Zhang et al., 2011b). Trehalose metabolism is essential for proper embryo maturation as well as for vegetative and inflorescence development (Eastmond et al., 2002; van Diik et al., 2004; Satoh-Nagasawa et al., 2006).

Signal transduction and transcriptional control involved in stress-induced metabolic changes

Acclimation of plants to changes in their environment requires a new state of cellular homeostasis achieved by a delicate balance between multiple pathways. Hormones, secondary messengers, phosphatases, and protein kinases are crucial components within the stress-induced signalling network that regulates a multitude of biochemical and physiological processes (Hirayama and Shinozaki, 2010).

As highlighted in the previous paragraphs, metabolic adjustments to stress are vital for acquiring stress tolerance. Transcriptional analyses of stress signalling mutants showed that, in numerous mutants with altered stress tolerance, genes involved in the synthesis of stress-associated metabolites are altered, and thus might be directly or indirectly involved in metabolic adjustment upon stress. However, due to post-transcriptional and post-translational modifications, compartmentalization, metabolite stability, substrate availability, etc. changes in the abundance of transcripts are not necessarily translated into changes in metabolite levels. A start has been made to link signal transduction with the metabolite response upon drought, salt, and temperature stress conditions including targeted analyses of metabolites and metabolic profiling of mutants in signalling components.

ABA and metabolic adjustments

Abscisic acid (ABA) is an integral regulator of abiotic stress signalling (Cutler et al., 2010; Hubbard et al., 2010; Raghavendra et al., 2010; Umezawa et al., 2010). ABA quickly accumulates in response to different environmental stress conditions and ABA-deficient plants have an altered stress response. ABA promotes stomatal closure, inhibits stomatal opening to reduce water loss by transpiration, induces the expression of numerous stress-related genes, and recent studies indicate a role in regulation of stress-induced metabolic adjustments.

Comparison of the metabolic profile of Arabidopsis plants treated with ABA, or exposed to high soil salinity, revealed ABA-induced and ABA-independent steps of salt stress-induced metabolic rearrangements (Kempa et al., 2008). Both ABA and salt stress led to a depletion of starch and the accumulation of maltose. However, subsequent carbon flux appears to be differentially regulated. While
glucose-6-phosphate and fructose-6-phosphate levels, and the glucose/fructose ratio, decreased under salt stress conditions, glucose-6-phosphate and fructose-6-phosphate levels and the glucose/fructose ratio increased in response to ABA treatment. Interestingly, ABA did not induce galactinol and raffinose accumulation (Kempa et al., 2008). In support of an ABA-independent induction of these sugars, Arabidopsis mutants deficient in stress-induced ABA accumulation were able to induce galactinol and raffinose under drought conditions (Urano et al., 2009). Similarly, stress-induced accumulation of citrate, malate, succinate (TCA cycle), and GABA is ABA-independent (Kempa et al., 2008; Urano et al., 2009). In contrast, accumulation of many osmotic stress-induced proteinogenic amino acids, including proline, were induced and depended on ABA (Kempa et al., 2008; Urano et al., 2009).

**Signalling towards proline accumulation**

Proline plays a multifunctional role in stress defence. Several protein kinases important for salt, drought, and/or cold tolerance, have been shown to regulate proline accumulation (Fig. 3). SNF-related protein kinases 2 (SnRK2s) are activated by osmotic stress (Boudsocq et al., 2004). Analyses of Arabidopsis mutants revealed that the ABA-responsive SnRKs 2.2, 2.3, and 2.6 are important for osmotic stress- and ABA-induced proline accumulation, whereas SnRK2.9 appears to play a negative role in ABA-induced proline accumulation (Fujii et al., 2011).

SnRK3s, which interact with calcineurin B-like (CBL) calcium binding proteins, and are thus also known as CBL interacting kinases (CIPKs), are also involved in modulating proline levels. In rice, over-expression of OsCIPK03 and OsCIPK12 enhanced tolerance to cold and drought, respectively, and increased the levels of proline under stress conditions. However, over-expression of the closely related OsCIPK15 enhanced salt tolerance without any significant influence on stress-induced proline content indicating that only a subset of SnRK3s are involved in signalling towards proline accumulation in response to stress (Xiang et al., 2007).

In addition to the above-mentioned calcium-responsive protein kinases, Arabidopsis calcium-dependent protein kinase 6 (CDPK6) (Xu et al., 2010) and soybean calmodulin GmCAM4 (Yoo et al., 2005) positively regulate stress tolerance and proline content in Arabidopsis, suggesting a central role for intracellular calcium signals in proline metabolism.

MAPK (mitogen activated protein kinase) cascades regulate numerous processes including abiotic stress responses. Several MAPKs are activated by cold, salt, and drought in monocot and dicot plants (Jonak et al., 1996; Hoyos and Zhang, 2000; Ichimura et al., 2000; Xiong and Yang, 2003) and genetic manipulation of MAPK signalling alters plant tolerance to these stresses (Xiong and Yang, 2003; Shou et al., 2004a, b; Teige et al., 2004). Recent data indicate a positive role for MAPK-based signalling in stress-induced proline accumulation (Kong et al., 2011; Zhang et al., 2011a).

While ABA and several stress-related protein kinases can stimulate proline accumulation, a maize protein phosphatase type 2C negatively regulated stress-induced proline accumulation and tolerance to hyperosmotic stress (Liu et al., 2009). PP2C type phosphatases PP2Cs are involved in regulating diverse processes including development and responses to environmental stress and have been shown to regulate stress-induced MAPK and SnRK2 protein kinases negatively (Schweighofer et al., 2004; Cutler et al., 2010; Hubbard et al., 2010; Raghavendra et al., 2010; Umezawa et al., 2010).

Phospholipid metabolism is involved in mediating hyperosmotic-stress responses (Testerink and Munnik, 2011). Based on a pharmacological approach PLC-based signalling has been implicated in salt-induced proline accumulation (Parre et al., 2007).

**Protein kinases involved in stress-induced changes in carbohydrate metabolism**

Carbohydrate metabolism is quickly modulated in response to adverse environments. Plant glycogen synthase kinase 3 (GSK-3)/shaggy-like kinases appear to connect stress signalling and carbon metabolism (Kempa et al., 2007). The Medicago sativa MsK4 is a positive regulator of salt stress tolerance. Plants over-expressing MsK4 have elevated levels of sugars including raffinose and galactinol, increased amounts of putrescine and amino acids under normal growth conditions. Remarkably, MsK4 associates with starch granules and over-expression of MsK4 in Arabidopsis increased the levels of starch and of several soluble sugars under salt stress conditions (Kempa et al., 2007). These data are also interesting from an evolutionary point of view as GSK-3 was originally identified as a regulator of the animal storage carbohydrate glycogen (Woodgett and Cohen, 1984).

OsCIPK03, OsCIPK12, and the MAPK kinase ZmMKK4, which promote proline accumulation under stress conditions,
also stimulated stress-induced increase in soluble sugar content (Xiang et al., 2007; Kong et al., 2011).

Further to the protein kinases mentioned, the negative regulator of drought tolerance, SAL1, has been associated with sugar and starch metabolism (Wilson et al., 2009).

Transcription factors regulating metabolite levels

Salinity, drought, and temperature stress have a strong impact on gene expression. Many genes coding for enzymes involved in cellular metabolism are differentially expressed upon stress, and modulation of some stress-related transcription factors have been shown to induce changes in stress-associated metabolite levels.

The CBF/DREB1 proteins (C-repeat binding factor or dehydration responsive element binding proteins) are members of the AP2/EREBP (Apetala2/ethylene-responsive element binding protein) family of transcription factors. CBF1/DREB1B, CBF2/DREB1C, and CBF3/DREB1A play an important role in the transcriptional response to osmotic stress (Shinozaki and Yamaguchi-Shinozaki, 2007; Thomashow, 2010). Over-expression of these transcription factors in Arabidopsis improved tolerance to freezing, drought, and/or salt stress (Liu et al., 1998; Kasuga et al., 1999; Gilmour et al., 2000, 2004) and CBF/DREB1 over-expressing plants accumulated higher levels of proline and soluble sugars (glucose, fructose, sucrose, and raffinose) when grown under normal growth conditions and during cold acclimation (Gilmour et al., 2000, 2004; Cook et al., 2004; Achard et al., 2008). The overall metabolic profile of CBF3/DREB1A over-expressers grown at normal growth temperatures resembled that of cold-exposed plants (Cook et al., 2004; Maruyama et al., 2009).

DREB2A (dehydration responsive element binding protein 2A) is activated by osmotic stress and over-expression of a constitutively active version of DREB2A, lacking the inhibitory domain, improved tolerance to dehydration but only slightly to freezing temperatures (Sakuma et al., 2006a). In line with this tolerance pattern, the metabolite profile of plants over-expressing constitutively active DREB2A was more similar to that of dehydration-exposed than that of cold-treated plants (Maruyama et al., 2009). While the global metabolic profile of plants over-expressing the constitutively active DREB2A resembled that of stressed plants, levels of galactinol, raffinose, and proline were not increased. Proline accumulates in response to drought, but not to heat or a combination of drought and heat (Rizhsky et al., 2004). Interestingly, DREB2A has also been shown to promote heat stress tolerance (Sakuma et al., 2006b).

Heat shock transcription factors (HSF) are central regulators of heat stress responsive genes. In plants, HSFs are encoded by a large gene family with different expression patterns and functions (von Koskull-Doring et al., 2007). Over expression of HsFA2 led to the constitutive accumulation of galactinol and raffinose and improved the tolerance of Arabidopsis plants to different environmental stresses (Nishizawa et al., 2006, 2008; Ogawa et al., 2007). Similarly, over-expression of HSF3/HsfA1b enhanced thermo tolerance (Prandl et al., 1998) and raffinose levels under normal and heat stress conditions (Panikulangara et al., 2004).

The NAC domain family of plant-specific transcriptional regulators are involved in developmental processes, as well as in hormonal control and stress defence (Olsen et al., 2005). OsNAC5 is induced by osmotic stress and ABA. Over-expression of OsNAC5 enhanced stress-induced proline and soluble sugar levels and tolerance to cold, salt, and drought stress (Takasaki et al., 2010; Song et al., 2011) whereas knock-down lines were impaired in proline and soluble sugar accumulation upon stress exposure and were more sensitive to stress (Song et al., 2011).

Levels of soluble sugars (glucose, fructose, and sucrose) and proline, as well as glycine betaine, were also elevated under normal and stress conditions in Arabidopsis plants over-expressing the rice Myb transcription factor, OsMyb4. Accumulation of these metabolites was concomitant with enhanced drought and freezing tolerance (Mattana et al., 2005).

Conclusion

During the last decade, our knowledge on the importance of metabolic adjustments to unfavourable growth conditions has increased considerably. Natural stress tolerance is a very complex process involving numerous metabolites and metabolic pathways. Analyses of metabolic adjustments of plants with different levels of stress tolerance and transgenic approaches provide important complementing evidence for better understanding the role of different metabolites in adjusting to harsh environments. Despite these substantial gains, several questions remain to be addressed. For example, the developmental stage of a plant is important for its potential to tolerate an adverse condition and cellular metabolism changes during development. Thus, it will be interesting to analyze how the developmental stage influences the metabolic adjustment to stress conditions.

Metabolic networks are highly dynamic. Metabolites can move between different cellular compartments. Since metabolic profiling only reveals the steady-state level of metabolites, detailed kinetics and flux analyses will be instrumental for a better understanding of metabolic fluctuations in response to stress. Metabolic analysis at the subcellular level in specific tissues is a further challenge.

Stress-induced signalling networks are well studied. However, the signalling processes required for homeostasis of basic cellular and metabolic processes in adverse environments are just starting to emerge. Better understanding of how environmental changes are communicated via cellular signal transduction to induce a co-ordinated metabolic response, and how the function of metabolic enzymes are adjusted by transcriptional and post-translational modifications, are of basic scientific interest and will contribute to meet the goal of increased plant stress tolerance and productivity in an ever-changing environment.
Acknowledgements

Work in our laboratory is supported by grants from the Austrian Science Fund P 20375-B03 and the EU FP7-ITN ‘COSI’. We apologize to authors whose papers have not been included due to space limitation in this review.

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