Chapter 10
Metabolomics on Combined Abiotic Stress Effects in Crops

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10.1 Sampling Defines the Picture

When metabolite profiling methods are applied to elucidate the response of plants to abiotic stresses, the strategy employed when taking samples from the plant has a major effect on the result. Decisions on the material sampled for the analysis and the timing of the analysis with respect to the diurnal cycle, the developmental stage of the plant, and the duration of the stress will affect the metabolite pattern significantly.

10.1.1 Organs

Leaves are the organs most frequently sampled for analysis, as they contain the tissues, in which photosynthesis as a basis for biomass production takes place. Leaf metabolism, however, changes significantly throughout the life cycle of a leaf, as the leaf or part of a leaf changes from a sink to a source to senescing tissue [2, 44, 67, 94, 110, 141]. Likewise, the leaf's response to stress may change with age [115]. Leaves that developed after stress application differ in their response from leaves that developed before the onset of stress [45, 87]. Sun and shade leaves of the same age, grown in parts of the canopy receiving different amounts and qualities of light, differ morphologically and physiologically [22, 69]. Surprisingly, transcript-level differences between sun and shade leaves were small compared to seasonal effects [91]. Furthermore, leaf samples taken from small species often

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contain stem material, when complete rosettes instead of leaf blades (with or without petioles) are sampled. As leaf age and leaf position affect metabolite composition, careful definition of the sampled leaf specimen with respect to its age and canopy position is required to ensure reproducibility of sampling and thus comparability of samples taken by different persons or at different times.

Flowers and flower organs are sampled in studies dealing with stress effects on fertility and seed development. Relevant stresses are high or low temperature or drought during or after flowering [73, 146]. The metabolic patterns of flowers change during their development [10]. Metabolite profiling on fruits is mainly performed on fruit crops such as tomato, pepper, apple, and grape. These studies focus on genetic and environmental effects on the sensory and nutritional quality of the harvest and postharvest product [26, 38, 83, 97, 113, 126, 127]. Root sampling is almost exclusively restricted to material grown on agar plates or in hydro- or aeroponics [5, 102, 119, 136]. Obtaining representative root samples from soil-grown material is laborious; thus, metabolite profiles of soil-grown roots are less frequently measured. The effort is, however, worthwhile to gain insight into root response to drought or mycorrhiza, which are best studied in soil-based systems [39, 40, 105].

10.1.2 Timing

10.1.2.1 Developmental Stage

The developmental stage of the plant influences the metabolic pattern in addition to the effect of the individual organ's age [42, 68, 92, 141]. The leaf metabolism changes especially at the onset of flowering as remobilisation of nitrogen and carbohydrates from leaves is enhanced due to remobilisation at the onset of flowering and during fruit growth [30, 66]. Thus, if genotypes differ substantially in their development speed, comparing them at the same age after sowing can be very misleading. In Arabidopsis, accessions vary significantly in the age of bolting, which may lead to comparing vegetative plants with plants that have already bolted. Ideally, samples are taken at a defined developmental stage, identified by the Zadok or BBCH scale [80, 92].

10.1.2.2 Diurnal Rhythm

Leaf metabolism changes with a diurnal rhythm, which is regulated by the plant's clock and entrained by light and temperature [52, 112]. In consequence, the concentrations of many metabolites change during the diurnal cycle. Diurnal changes are substantial in metabolites that serve as intermediate storage products of

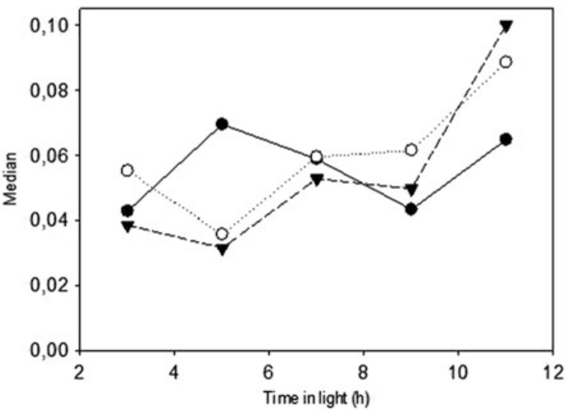

Fig. 10.1 Median of the relative change of concentration for 123 metabolites measured in leaves of *Arabidopsis thaliana* that were cultivated at 50 (*dot*), 150 (*circle*), or 550 (*triangle*) µmol m^{-2} s^{-1} (data from [35] #2999). The relative change was calculated as absolute $[(c(t) - c(t + 2h))/\text{mean}(c(t), c(t + 2h))]$ and plotted against the midpoint of the time interval. Four outliers (relative change >1) were removed from the dataset

photoassimilates, such as starch, sucrose, and raffinose family oligosaccharides. Likewise, amino acid concentrations change diurnally [35, 132, 147]. Light intensity affects the amplitude of diurnal changes in starch and total amino acid concentration. Furthermore, the shape of the curve, the time, at which the concentrations of, for example, glucose or glutamine peak, depends on the light intensity [35]. As the process of sampling takes a certain time, circadian change of metabolite concentration can influence the result if samples are not taken randomly. To identify the ideal time for sampling, the change of metabolite concentration within 2 h was calculated for 123 metabolites (see Fig. 10.1). The median change of metabolite concentration was between 3 and 7 % for the time between 2 and 10 h after the onset of light but tended to increase at the end of the day.

A most important finding for abiotic stress research is the interaction between diurnal rhythm and environmental response. The plant's response to the same stimulus depends on the time within the diurnal cycle (gating), which may result from an effect of the clock on the signalling pathway [147]. The diurnal change in the plant's response may also be the consequence of an interaction between a clock-regulated pathway (e.g., stomatal opening), and the effect of an environmental stimulus (e.g., increased vapour pressure deficit (VPD) outside the leaf) on leaf tissue, for example, change in VPD in the leaf aerenchym. An interaction between diurnal rhythm and the response to drought has been found in potato [7]. In 20 % of (detected) leaf metabolites, their concentration was simultaneously affected by drought, CO_2 concentration, and the diurnal cycle. Drought increased the amplitude of diurnal changes for hexoses and starch compared to control samples [7].

10.1.2.3 Duration of Stress

In many studies, response to stress is measured at a predefined time point after the application of stress. Measurements after a few minutes to several hours of stress generally aim at the discovery of components of the signal transduction pathway triggered by the stress [108]. Measurements after hours or days of stress target the effect of stress on the metabolism in the 'shocked' and in the 'acclimated' or 'damaged and declining' stage. When temperatures or light conditions were abruptly changed from optimal to stress levels, metabolite levels changed in less than an hour and more rapidly than transcript levels [20]. Moderate temperature decrease resulted in larger changes in transcript levels after 6 h than after 78 h. In contrast, metabolites changed rapidly in few pathways and more slowly for a larger group of metabolites, among them stress-responsive metabolites such as proline, raffinose, and polyamines [133]. The kinetic component of the response thus has a significant effect on the results. When observing the kinetics of transcript and metabolite response to temperature and light stress within the first 24 h after the change of conditions, the change of many metabolite concentrations is not continuous [20]. When *Arabidopsis thaliana* is transferred to 32 °C, the maltose concentration first increases (first 3 to 5 time points) and then decreases below the initial concentration. In contrast, the concentration of several amino acids, among them valine, asparagine, and ornithine, first decreases and then increases compared to time zero. In consequence, in studies where stress effects on metabolites are studied within 24 h, the effect of the diurnal cycle on metabolite concentrations and potential effects of the stress on the diurnal cycle have to be taken into account [33]. However, even after several days of stress, the diurnal cycle interacts with stress response [7]. When leaf metabolites in potato were determined predawn and midday after 5 or 11 days of drought stress, the time of day affected the magnitude of a metabolite's change but not its direction [7]. In contrast to many other stresses, drought stress cannot be imposed suddenly unless done in a fairly artificial way, for example, by removing the plant from its medium or by adding an osmolyte. When drought stress is imposed by withholding water from a plant in a pot or in soil, the amount of available water and the soil water potential decrease with time. The speed of change depends on the ratio between available water at the beginning of the experiment and the evapotranspiration rate of the pot–plant system. The latter is affected by the VPD of the atmosphere, the temperature of the plant leaf, and the surface of the plant and the pot. Thus plants of very different size will experience different drought stress when grown in the same pot size, which may account for the seemingly high drought tolerance of some dwarf mutants. The amount of water available at the beginning of the experiment depends on the size of the pot, the water capacity of the substrate, and its relative water content. The availability of the water depends on the soil water potential, which depends on the soil type and its water content. Large plants in relatively small pots will experience an onset of drought that may be much faster than in soil-grown plants. Furthermore, plants grown in dim light may change their leaf water content much more slowly than plants grown in high light, as leaf transpiration increases with light intensity [84].

Realistic climatic conditions are also required to assess the salt tolerance of a species, as salt accumulation is affected by transpiration [87]. Thus, ideally, the plant's water status during the experiment or when samples are taken is characterised by measuring the leaf water potential.

10.2 Drought Stress Combinations

When drought stress effects on metabolism and metabolite concentrations are studied, drought stress is predominantly applied in controlled environments where all parameters except water supply are kept constant. Under field conditions, drought stress is generally combined with other biotic and abiotic stresses [82]. Although many of these stressors combine randomly, the combinations of drought with salt, heat, and cold stress occur frequently due to constraints caused by environmental physics. Together, drought, salt, and extreme temperatures account for 70 % of yield loss in global crop production [17].

10.2.1 Drought and Salt

In arid climates, drought stress is often combined with salt stress. Under arid conditions, annual evapotranspiration exceeds annual precipitation. Thus, soil water moves predominantly towards the surface and thereby transports soluble soil compounds such as sodium, potassium, chloride, and sulphate towards the uppermost soil layer. In consequence, the osmotic potential of the soil solute decreases and salts may precipitate within the soil or at the soil surface (Fig. 10.2). This process can make arable land unfit for plant cultivation, especially over saline

Fig. 10.2 Salt precipitates on the soil surface in arid regions of the Himalaya (Nepal, October 2012)

outcrops or close to the sea. Salinisation is aggravated by insufficient irrigation or extensive use of fresh water from rivers [36, 46].

As soil salinisation took place in arid climates before man invented agriculture, so called xero-halophytes have evolved. Xero-halophytes are adapted to the combination of low matrix potentials resulting from low soil water content, low osmotic potential resulting from high solute concentrations in the soil water, and toxic concentrations of sodium and chloride [87, 104]. These plants thus evolved efficient strategies to exclude and sequester toxic ions. The challenges posed by low osmotic potentials of the soil solution and low matrix potentials in dry soils differ. In a wet saline soil, water moves easily towards the root surface. Thus, as long as the plant can reduce its water potential below the soil water potential, water uptake is not impaired. However, extensive water uptake increases the salt load of the shoot. In dry saline soils, water conductance is considerably reduced, resulting in depletion zones around the roots that force the root to grow towards new water sources. However, this concept has been challenged [27]. When water flow resistance in soil is high, maintaining root growth is paramount, which means that root turgour has to be maintained by osmotic adjustment and photosynthesis has to continue supplying chemical energy to fuel root growth. The same holds true for a plant on a dry soil without increased salt levels.

The demand on root water uptake can be alleviated by increased water use efficiency of the shoot, which is relevant for drought and salinity tolerance and can be achieved by an increase of cuticula resistance and reduced stomata density [109]. The reduction of the plant's water potential below the soil water potential relies on the accumulation of so-called compatible solutes. Compatible solutes do not interfere with metabolism at high concentrations and act as chaperons or scavengers of reactive oxygen species (ROS). Among these compounds are amino acids, especially proline, quaternary ammonium compounds including glycine betaine, soluble carbohydrates such as sugars and sugar alcohols, and raffinose family oligosaccharides [14, 41, 49, 144]. The most commonly accumulated compounds proline and glycine betaine accumulate under salt and drought stress. Salt tolerance and drought tolerance increase with the intracellular level of compatible solutes [13, 55, 60, 75, 139]. Studies on short-term response of plants to NaCl or dry soil in a system, where soil water potential was 'clamped' with a pressure bomb, indicated that reduced leaf expansion rate under stress is not brought about by altered water pressure, but by hormonal signals [85]. The plant's response to salt stress and drought are very similar [85], but not identical. Drought and salt stress lead to accumulation of aspartate, fumarate, and sucrose in *Medicago truncatula* roots, but proline and glucose accumulation was restricted to drought-treated plants [117]. Thus, salt-resistant plants are not necessarily drought resistant and vice versa.

10.2.2 Drought and Cold

The water vapour deficit at given atmospheric water content and thus the evapotranspiration rate increase with temperature. Thus, even very low annual precipitation rates of 250 mm result in humid climate conditions in high latitudes where average air temperatures are low [64]. However, there are situations where low water availability is combined with air or soil temperatures low enough to cause cell damage. The temperature limits, below which plant tissue is damaged, are highly species-specific and can be reduced by hardening (see Priming). Many tropical plant species such as rice, cucurbits, and tomato suffer from so-called chilling damage at temperatures well above freezing [65]. Species from Mediterranean climates or tropical mountains tolerate temperatures down to a few degrees Celsius below zero as long as they can prevent ice formation inside the cell by accumulation of osmolytes. These can prevent freezing down to temperatures of −5 °C. Some species that grow in regions, where temperatures change between freezing and thawing each day, acquire meta-stable states, in which supercooled cell sap remains liquid down to temperatures of <−30 °C [143]. Species from temperate regions and high latitudes tolerate freezing of leaf tissue and wood, in the extreme down to temperatures of below −40 °C and show a pronounced change of cold tolerance during the year [65].

In cold and arid climates, plants are subjected to low temperatures and low humidity or low precipitation. Cold arid and semi-arid regions are found in northern Asia, especially in the Himalaya, in northwest China, northern Iran, and Afghanistan, but also on the Colorado plateau and in Alberta (Canada; [32, 50, 74, 114]). In many cold and dry areas, all (trans-Himalaya) or a substantial percentage (Colorado) of precipitation falls as snow during the cold months, alleviating low temperature effects. Plants thus are most likely affected by a combination of cold and drought when they are not or no longer covered by snow. When frozen leaves are exposed to the sun, their water potential can be higher than that of the surrounding air, which results in water loss by evaporation. At the same time, water uptake from the soil and water transport in the xylem is restricted by high water flow resistance in frozen soil and xylem [129]. In consequence, leaf cells can be damaged by low leaf water content. Evergreen trees adapted to very low winter temperatures reduce water loss from the leaves by producing thick cuticula. In chilling-sensitive plants such as tomato or pumpkin, chilling stress is often combined with drought stress as temperature-dependent inhibition of water uptake by the roots and transport in the xylem occurs, when temperatures fall below 15 °C [64].

10.2.3 Drought and Heat

Drought stress is frequently combined with heat stress as a large amount of energy is required to convert liquid water into water vapour. This large latent energy of water renders a wet evaporation object that is exposed to radiation cooler than a dry object. Both on the global scale as on the level of a single plant, evaporation of water consumes most of the solar energy that reaches the atmosphere, respectively, the plant, compared to less than 3 % used by photosynthesis [37]. Thus, if water is scarce, plant temperature tends to increase. This may explain why when both stresses are combined experimentally, the metabolic response tends to be more similar to the drought response than to the heat response [90].

Plants that are subjected to drought stress reduce transpiration by closing stomata. As energy consumption by phase transition from liquid to vapour decreases, absorbed radiation is converted into heat and results in a temperature increase of the leaf. In consequence, leaf temperatures can be 10 °C higher than air temperature [21]. Many species limit this effect by changing the leaf surface exposed to radiation and thus the amount of intercepted radiation by leaf rolling or altering the angle between leaf surface and direct radiation. The radiation that is not intercepted by the plant canopy will reach the soil surface and will increase the soil temperature. This increase depends on the soil's albedo, its moisture content, and evaporation rate. Thus temperatures increase very rapidly at the surface of dry, dark, sandy soil (e.g., derived from volcanic ash or dry peat) and much slower in wet clay soils. In consequence, the soil surface can be much hotter than the air during the day. This fact is very important for the germination and establishment of seedlings under drought stress. Rain or irrigation water can transport heat to lower soil layers, which results in an increase of root temperatures that cannot be compensated by water evaporation. These environmental physics constraints do not only define the selection pressure, to which plants in hot arid environments were subjected. Environmental physics also affects the degree of stress a plant is subjected to in a drought or heat stress experiment under controlled conditions. In consequence, plants kept at the same, high air temperature (e.g., *Arabidopsis thaliana* at 30 °C) can experience leaf temperatures much lower than 30 °C when grown in moist soil and at moderate light intensities, whereas leaf temperatures can be much higher than 30 °C when plants are grown in dry soil, in the dark, or at high light intensities, or in a very moist atmosphere [56]. Furthermore, small pots with a dark surface or closed containers (Petri dishes) exposed to sunlight can heat up to temperatures well above the atmospheric temperature. Thus, judging the degree of stress, to which an experimental plant was exposed, requires detailed meta-information on atmosphere and soil conditions. Without these often under-reported meta-data, comparison of metabolite profiles of different drought and heat experiments is very difficult.

10.3 Stress Duration and Intensity Affect the Metabolic Response

The plant's physiological and molecular response to adverse abiotic conditions depends on the duration and the intensity of the stress [87]. Short-term stress treatments study the plant's response within a few minutes to several hours after the onset of stress. These studies mainly focus on the identification of components of stress perception, signal cascading, and subsequent regulation of gene expression [15, 89, 125, 145]. In long-term stress treatment, timing and duration of the stress are adjusted to the developmental phase and the time at which the stress typically hits the species or—especially in agricultural research—at which stress causes the largest yield loss. In temperate regions, droughts typically last a few weeks and are followed by periods of ample precipitation. Under these conditions, fitness or yield depends on the genotype's ability to recover from stress. In semi-arid climates, wet seasons are often followed by several months of drought, during which the plant may die (terminal stress) with or without completing its life cycle. Under these conditions, yield or fitness depends on the species' ability to make the most out of available water. In Indian pearl millet varieties, variation in grain yield under terminal drought depends on small temporal differences in water uptake and use during the plant's life cycle [134].

The intensity of a stress treatment needs to be judged with respect to the stress resistance of the respective species. Stress resistance can be quantified by the cardinal points of stress intensity, which specify the highest or lowest value of an environmental parameter acceptable to the respective species. In the case of temperature stress, these are the minimum and maximum temperatures of survival, growth, photosynthesis, and so on. Resistance to chemical stress can be given by the toxic concentration, at which survival or growth declines in a logit-like manner. This logit curve can be parameterised by the no observed effect concentration (NOEC), which is the highest concentration, at which no effects on growth or survival are observed, or the lethal dosis, at which 50 (LD50) or 100 % (LD100) of the specimens die. Although the NOEC is an ideal concentration to study stress effects without secondary effects, it is rarely used, as its determination requires an even higher number of replicates than LD50 measurements.

In many short-term stress treatments, short stress duration is combined with high stress intensity and fast increase of stress intensity (shock treatment; [121]. Short-term drought stress treatments are often dehydration treatment, in which the plant is removed from its water supply (e.g., out of hydroponics) or the root system is treated with PEG. Only 10 % of genes with significant change in expression upon dehydration shock also show altered expression after several days of moderate drought stress [121]. In conclusion, dehydration shock treatments seemed to be suitable as a fast screening method with the necessity to validate the obtained results under more realistic conditions [121]. For salt-tolerance studies, the predictive value of assays based on the survival in short, hard treatment for performance under agricultural conditions has been disputed. In short treatments, the salt effect is restricted to the osmotic effect, whereas salt toxicity effects develop considerably later [86, 87].

10.3.1 Response Types A: Tolerance, Resistance, and Avoidance

The plant's response to stress is quantified by determining parameters related to fitness (for wild plants) or yield (for crops). As growth and seed yield determination are laborious, proxies such as shoot biomass, photosynthesis, or leaf survival are used to assess resistance. However, it has to be taken into account that shoot biomass is not necessarily correlated to seed production or yield even in a population of closely related genotypes as shown by the wide variation in harvest index [78]. Stress response is classified as tolerant or sensitive, which are the extremes of a response continuum. In a tolerant plant, fitness and yield change less with increasing stress intensity than in a sensitive plant. As illustrated in Fig. 10.3, high tolerance is thus not necessarily linked to a high yield under stress conditions. Stress tolerance often occurs in crop cultivars of low yield potential, which means that they show low yield under optimal conditions. There are even indications for a negative correlation between yield potential and stress tolerance (yield penalty), although it has not yet been shown that tolerance implies metabolic costs [13, 87, 116].

In its strict definition [71], stress tolerance is one strategy, and stress avoidance the other strategy to achieve stress resistance. Whereas a stress-tolerating organism tolerates stress in the sense defined above, a stress avoider employs a mechanism to

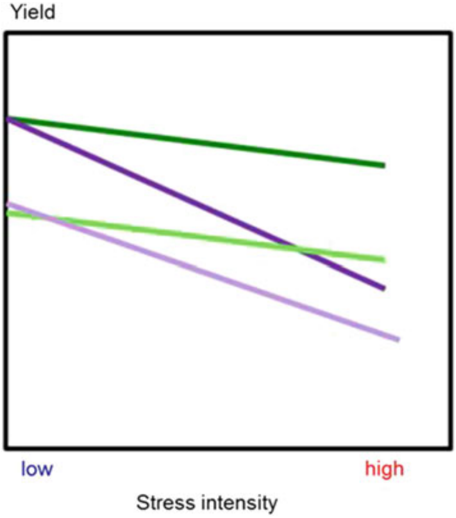

Fig. 10.3 Relationship between yield and stress intensity. In highly tolerant genotypes (*green*) yield is less affected by stress intensity than in sensitive genotypes (*violet*), but tolerance can be combined with high (*dark green*) or low (*light green*) yield potential

prevent the organism from being confronted with the stress. Classical examples are winter annuals and bulb plants in climates with dry hot summers and moist mild winters (Mediterranean climate), which are in a dormant state as seeds or bulbs when heat and drought stress prevail [64]. Likewise, summer annuals avoid cold and drought stress during winter by germinating in spring and terminating their life cycle within a few months. However, when looking more closely at the mechanism employed by plants that grow and produce seeds during the stress period, it becomes obvious that avoidance and tolerance are not sharply distinct classes but rather extremes in a continuum. With respect to drought tolerance this can be illustrated by deep-rooting trees on one end and dehydration-tolerant resurrection plants on the other end of the strategy continuum. In arid climates, some tree species grow deep roots to tap into deeper water layers containing water from precipitation during wet seasons. This strategy is discussed as one possible adaptation mechanism in crops for semi-arid environments [122]. Obviously, this mechanism is only sustainable if the reservoirs these plants tap into are refilled by precipitation eventually. In some cases, the rain that feeds these water reservoirs fell in distant areas or even a few years ago; in the worst case, the water reservoirs are historic. This mechanism resembles the strategy employed by water-storing plants such as succulent cacti and euphorbia but also the savannah tree baobab, which take up water during rainy seasons and store it within bulky shoot organs. Water-storing and deep rooting plants may thus suffer less from water shortage than co-occurring plants without these adaptations. However, they have to invest carbon and energy into the production and maintenance of heterotrophic tissues. In ecosystems similar to those of water-storing avoiders, desiccation-tolerant resurrection plants survive dry periods by dehydration. These plants tolerate dehydration of the shoot down to 10 % of their saturated water content [12]. This group contains a number of higher plants, including some grass species, and most mosses. The cytoplasm of their leaf cells tolerates extreme dehydration in a dormant state due to various protective mechanisms. This strategy resembles the process in ripening seeds, which also acquire the ability to survive dehydration and thus avoid times of water shortage and extreme temperatures. In contrast to seeds, leaves of resurrection plants can resume net-carbon fixation within very short times after rehydration.

The majority of plants and almost all crops can neither draw on large internal or external water stores nor do they tolerate complete dehydration of their leaf cytoplasm. These plants experience stress when water uptake and transport into the shoot no longer match the water loss by transpiration. In this case, many physiological and metabolic changes can be observed, some of which are adjustments to prevent damage, and others are the consequence of cell damage. Unfortunately, it is not trivial to distinguish between both. An illustrative example for this problem is the role of proline accumulation in stress response. Proline accumulates in the leaves of many plant species, when plants are subjected to drought, salt, oxidative stress, or extreme temperatures [120]. Proline concentration in unstressed mature leaves is fairly low (<2 mmol kg^{-1}), but proline accumulates by a factor of >20 to concentrations above 50 mmol kg^{-1} when plants are subjected to drought or extreme temperatures [58, 88]. Thus, proline has been suggested to act as a protective compound (see below) within a stress- tolerance mechanism [120, 137]. However, when comparing sensitive and tolerant cultivars of crop species, proline concentrations under stress are reported to be higher in sensitive than in tolerant genotypes. This pattern suggests that proline accumulation is linked to damage, either as a consequence of damage (like scattered glass after a car accident) or part of the mechanism to prevent further damage (in the image of the accident: the warning triangle at the roadside). The association of proline accumulation pattern and tolerance in single genotypes that vary widely in their genetic background is inadequate to prove causality, as tolerance and metabolic change may be chance associations. To unravel the role of compounds in stress tolerance, metabolite concentrations are observed in time-series or in genetically similar genotypes, in which metabolite concentrations are modified by—ideally inducible and tissue-specific—overexpression or repression of genes coding for relevant enzymes [87]. Modification of proline concentration by constitutive overexpression of Delta (1)-pyrroline-5-carboxylate synthase (P5CS) resulted in proline accumulation and affected salt and osmolyte tolerance in a species-specific manner [137]. Similar effects were observed when proline concentrations were increased by inhibition of proline degradation [120, 137]. Thus, although proline is accumulated in response to various stresses and likely to be involved in the tolerance mechanism, proline accumulation is no requirement for tolerance [120]. A similar conclusion was drawn for raffinose, which increases during cold acclimation, but is, as mutant studies showed, not required for freezing tolerance [148]. Thus, compounds that are accumulated by plants under stress are likely to be involved in the tolerance mechanism without being a requirement for tolerance.

10.3.2 Response Types B: Disruption, Adjustment, Senescence, and Chaos

Physiological studies concentrate on the abiotic stress effects on leaves in light as the site and situation, in which carbon is fixed as a basis of growth. Drought, extreme temperatures, and salt result directly or indirectly in a change of water status and temperature of the leaf towards an unfavourable range [14]. Under drought conditions, water potentials of soil and atmosphere decrease. Heat decreases air water potential; frost immobilises water in the soil and increases water flow resistance in the shoot [21, 84, 129]. As the plant is basically clamped between soil and atmosphere in a water-flow continuum, decreased soil or atmosphere water potentials rapidly lead to a decreased water potential of the leaf [95]. By closing stomata, the plant can increase the resistance of water flow between leaf and atmosphere to reduce water loss and increase leaf water potential at the cost of increasing leaf temperatures. Decrease of water potential of plant cells results in a loss of turgour pressure, which is a prerequisite for cell expansion in growing tissues and thus required for any morphological adaptation of the plant. As

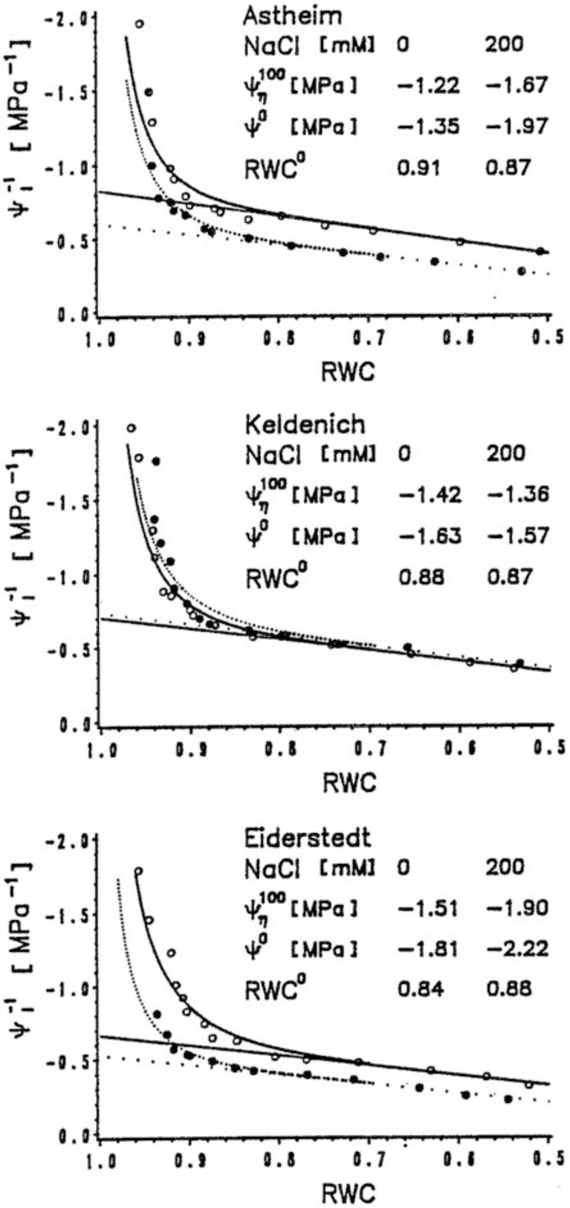

Fig. 10.4 Pressure volume curve of leaves from three different populations of *Armeria maritima* grown at salt concentrations of 0 (*open circles*) or 200 mmol l^{-1} NaCl (*dots*) [58]. Curve fitting and calculation of osmotic potential Ψ at 100 and 0 % turgour according to Andersen et al. [3]

explained above (see Drought and Salt), high water flow resistance in dry soils requires maintenance or even increase of root growth to obtain the water required to compensate for water losses from the plant to the atmosphere. By accumulating osmolytes within the cell, the plant maintains turgour and thus continues growth at lower tissue water potentials (see Fig. 10.4).

Several metabolite groups have been identified as so-called compatible solutes that can be accumulated to high concentrations either in the vacuole or in the cytoplasm without affecting metabolism by adverse effects on enzymes. Proline, quaternary ammonium compounds, polyamines, polyols, and sucrose and oligosaccharides were grouped among compatible solutes based on the observation that (a) they accumulate when leaves are subjected to abiotic stress, and (b) they can be present in high concentrations without inhibiting enzyme activities or bacterial growth in vitro [6, 14, 48, 49, 144]. Furthermore, many of the above-mentioned compounds have been supposed to protect macromolecules and membranes from damage under stress conditions [1, 6, 47, 51, 101, 137].

10.3.2.1 Oxidative Stress

Stomatal closure results in reduced CO_2 uptake and low CO_2/O_2 ratios, which decreases photosynthesis and increases photorespiration. Photosynthesis itself is a process which is most sensitive to abiotic stress [100]. High leaf temperatures as a consequence of stomatal closure or high external temperatures damage the oxygen-evolving complex and cofactors in PS II, enhance oxygenase activity of Rubisco, and damage the ATP generating system [1]. In consequence, most abiotic stresses lead to oxidative stress [90]. Some of the metabolites accumulated under drought, heat, or salt stress were shown to act as antioxidants or to protect enzymes that detoxify ROS. Proline scavenges ROS by acting as singlet oxygen quencher and by stabilising among others superoxide dismutase and catalase [120]. ROS scavenger functions have also been assumed for mannitol or polyols in general [14]. However, when Arabidopsis is transferred to heat or high light conditions, no rapid accumulation of proline, mannitol, or myo-inositol is observed within the first 6 h [20], although all these metabolites have been shown to accumulate after prolonged heat, salt, or drought stress [18, 34, 77]. Another compound with protective function against ROS is tryptophan. It is assumed to protect proteins, especially the D1 protein required for the repair of PS II. In cereals, which do not accumulate proline, tryptophan concentrations increase under drought within a few days [16]. In addition, oxidative stress causes a change of carbon flow away from glycolysis to the oxidative pentose phosphate pathway and a perturbation of the tricarboxylic acid (TCA) cycle [90]. This is assumed to reduce the levels of amino acids that are derived from intermediates of glycolysis or the TCA cycle. The TCA-cycle-derived amino acids aspartate (Asp) and glutamate (Glu) indeed decrease under drought and salt stress. However, glycolysis-intermediate-derived amino acids leucine (Leu), isoleucine (Ile), and valine (Val) increase under drought, salt, and heat stress [90]. The pattern of amino acid changes thus resembles less the situation under oxidative stress than the changes observed under C-starvation.

10.3.2.2 Energy Crisis?

Carbohydrate limitation results in a concentration increase of Ile, Leu, Val, arginine (Arg), phenylalanine (Phe), tryptophan, tyrosine (Tyr), and proline as a consequence of protein degradation. The induction of genes coding for protein and amino acid degradation under carbohydrate starvation and abiotic stress suggests that proteins and amino acids are used to fuel energy metabolism [29, 93, 98, 111]. Under drought, released branched chain amino acids are used by the alternative respiration pathway [90, 142]. The central carbohydrate metabolism is also considered a most important component in the adjustment of metabolome to low temperature stress [47]. Caldana et al. furthermore showed that transfer of *A. thaliana* into low light or the dark elicits a metabolic change similar to that after transfer to high temperatures [20]. Several facts thus suggest an energy shortage under abiotic stress.

The hypothesis is consistent with the observation that N-metabolism is inhibited by C-starvation and by abiotic stress, as energy for N reduction is saved when energy is limited and reduced N is released from degraded amino acids. Under C-starvation and abiotic stress, changes in Glu, glutamine (Gln), Asp, and asparagine (Asn) indicate the inhibition of N assimilation [35, 90, 93]. Altogether, these findings led to the hypothesis of an energy crisis as the common denominator of abiotic stresses [66]. Abiotic stress may cause carbon and energy limitation as a result of decreased photosynthesis in consequence of stomatal closure or damage to the photosynthetic apparatus. When drought requires enhanced root growth to forage for additional soil water, C-allocation to the root meristems has to be increased. Salt stress increases respiratory metabolism [62], and the synthesis for heat shock proteins and osmolytes in response to extreme temperatures requires C-resources. Thus, energy and carbon demand may increase under abiotic stress, while C fixation is limited. The change of TCA cycle intermediates under drought, salt, and temperature stress [28, 43, 106], the upregulation of TCA enzyme activity [62], and the induction of the respective genes [29] support the assumption that energy metabolism is enhanced by abiotic stress. The carbohydrate catabolism is fueled by starch degradation leading to increased concentrations of soluble carbohydrates under salt and extreme temperature stress. Drought stress leads to increased or decreased concentrations of mono- or disaccharides depending on stress duration and intensity. However, increased concentrations of soluble carbohydrates under salt stress and extreme temperature stresses contrast with the finding that concentrations of mono- and disaccharides, organic acids, and polyols decrease under carbon limitation. It may be speculated that in those experiments where soluble carbohydrate concentrations decreased, carbon export from the leaves was reduced to allow maintenance metabolism of the source leaf.

10.3.2.3 Senescence

In contrast, a high concentration of soluble carbohydrate in the leaves may indicate a remobilisation of resources from the leaf to provide sink tissue with energy, carbon, and nitrogen. Remobilisation means that macromolecules are degraded to export their monomers, for example, sugar or amino acids via the phloem system. Sugars such as raffinose or sucrose that are found to accumulate in leaves under stress are major transport forms of carbohydrates. The amino acids Glu and Asp and their respective amides are the main nitrogen transport form [63, 135]. Remobilisation typically occurs during senescence. This process occurs as leaf tissue ages but is indeed enhanced during drought, salt, and extreme temperature stress. Starch and protein concentrations decrease during leaf senescence and stress. The concentrations of the amino acids Val, Leu, and Ile and the polyol concentrations (myo-inositol, sorbitol, galactinol) increase with the age of a leaf [141] and under abiotic stress. Likewise, the concentrations of soluble carbohydrates (sucrose, fructose, glucose, trehalose) rise with age as under salt and extreme temperature stress. In contrast, hexoses get depleted in stress hypersensitive autophagy mutants, whereas glutamate and glutathione increase [79]. The change in proline concentration with age is less consistent than the stress-induced increase. The Gln/Glu ratio and the Asn/Asp ratios increase with age, whereas the concentrations of all four amino acids as well as alanine and arginine generally decrease with age to peak again in the final stage. Low Gln/Glu ratios are markers for nitrogen limitation, as Gln and Asn concentrations decrease in nitrogen-limited *Arabidopsis thaliana* [70, 130]. Furthermore low Gln, Glu, Asp, and Ala concentrations indicate an inhibition of nitrogen metabolism, or, if co-occurring with a decreased level of minor amino acids (e.g., Val, Ile, Arg), restart of protein synthesis, for example, after growth inhibition in consequence of C starvation [93]. Taking the observations together, there are no indications for nitrogen limitation or carbon starvation in senescing leaves; carbohydrate and amino acid concentrations are high, likewise ready for export to sink organs. This metabolic pattern of senescing leaves—high abundance of nitrogen, carbohydrates, and amino acids—resembles frequently reported metabolic patterns in leaves under stress.

Altogether, metabolic patterns in leaves subjected to abiotic stress show similarities to leaves subjected to carbon starvation, but even more so to aging leaves. An abiotic stress-induced energy crisis due to photosynthesis inhibition and potentially increased energy demand of roots may enhance senescence in source leaves to remobilise carbon and nitrogen. Stress-induced senescence of leaves has indeed been discussed as an adaptive feature for many decades [64]. Time-series analysis on the leaf and the sink organs depending on it may allow distinguishing whether stress-induced changes in the leaf metabolome reflect senescence or defence of a single leaf. This analysis should be enhanced by physiological performance measurement of the entire plant system. Detailed flow analysis may reveal whether the senescence of source leaves indeed enhances fitness of the entire plant and thus is of adaptive value or just reflects chaotic breakdown.

10.4 Priming

A plant that encounters abiotic stress will upon perceiving the environmental signal activate a signal cascade that alters gene expression and thus leads to changes in the plant's morphology, physiology, and biochemistry. These changes, that increase the plant's chance to survive stress, are called acclimation to distinguish them from evolutionary adaptation that is brought about by changes in gene sequences in subsequent generations due to natural selection. The concept of acclimation assumes that stress resistance mechanisms are not constitutively expressed, but have to be induced by an external signal [61]. The classic example of this acclimation concept is the cold acclimation process undergone by cold-resistant plants in autumn [47, 65]. In *Arabidopsis thaliana*, cold acclimation reduces the lower cardinal point of temperature (estimated as the LT50) by 3–5 °C [150]. Low temperature acclimation in the extremely cold-tolerant *Picea obovata* develops in three steps, preacclimation, early acclimation, and late acclimation to a fully acclimated state, during which the cardinal temperature decreases from −10 to below –35 °C. [4]. Resistance mechanisms are supposed to be induced rather than constitutively expressed, as they are assumed to incur costs or are incompatible with sexual reproduction [87, 116, 149]. This concept has, however, been challenged [13]. Especially species with high resistance towards abiotic stress constitutively express tolerance mechanisms that are induced by stress in less-resistant relatives [8, 33, 59, 60].

For those species that acclimate to abiotic stress, a stress memory mechanism has been postulated that primes the organism to subsequent stresses [11]. Priming is shown by treating the organisms with a mild stress, removing the stress for a certain time and then comparing the organisms' response to a more severe stress to the response of organisms that have not received the first mild stress treatment. When priming occurs, resistance to the later-occurring stress is increased in consequence of a faster and stronger response to stress signals [19, 96]. Mechanistically, priming-induced changes are linked to DNA modifications by methylations that alter DNA accessibility to RNA polymerase [31]. Priming has been shown for various abiotic stresses. When *Arabidopsis thaliana* seedlings are pretreated with high temperatures (60 min at 37 °C plus 45 min 44 °C), they will survive 80 min at 44 °C, whereas seedlings that have not received the priming treatment will die at that temperature [118]. In *Triticum aestivum*, exposure to moderate water deficits during vegetative growth increases grain yield when plants are subjected to drought or heat in the generative stage compared to plants that have not received the priming treatment [140]. Likewise, pretreatment with heat or waterlogging increased resistance to the respective stresses during grain filling [72, 140]. In all three cases, pretreatment alleviated the negative effect of abiotic stress on photosynthesis and membrane composition, whereas increased abscisic acid (ABA) concentrations modified the sink strength of developing grains.

Agriculturally, priming treatments can be employed in deficit irrigation schemes [103, 138]. In peanut, early to mid-season drought treatment maintains the amount and quality of peanut yield when plants are additionally subjected to late-season drought, while reducing water use in semi-arid areas [103]. For agricultural application, seed priming is of special interest as it can be applied in a standardised way by the seed producer and potentially induces cross-tolerance [24]. For priming, seeds are treated with water, but also with solutions of potassium dihydrogen phosphate, sodium molybdate dihydrate, mannitol, polyethylene glycol, potassium nitrate, and salicylic acid [81, 131]. Seed priming has been shown to enhance germination and root development under suboptimal temperatures and improve early drought resistance by modification of the antioxidant system [23, 124].

During plant growth, priming by moderate abiotic stress can be replaced by treatment with hormones or xenobiotics, a phenomenon that is highly interesting for agricultural applications. The senescence-delaying effect of strobilurin fungicides has been known for more than a decade. Pyraclostrobin treatment increases drought resistance of maize under field conditions [9]. Beta-aminobutyric acid (BABA) prime pathogen and abiotic stress resistance in plants by enhancing the salicylic (SA)- or the ABA-signalling pathway [25, 54, 76, 128]. Direct SA application has a positive effect on freezing tolerance of wheat [123] and drought tolerance of maize [99]. Exogenous application of ABA has been shown to elicit PSII protection from photoinhibition [53]. Furthermore, applications of beneficial microorganisms have been shown to improve growth and abiotic stress tolerance, presumably by priming stress acclimation [107, 117].

10.5 Conclusion

Although plant resistance to drought does not necessarily imply resistance to extreme temperatures or salt, the plant's responses to these stresses resemble each other in many aspects, namely accumulation of osmolytes, protection against ROS, adjustment of energy metabolism, and processes related to altered carbon allocation and senescence. This may result from the fact that many of these stresses occur in combination [82] in consequence of constraints imposed by environmental physics. The evolutionary selection of the plant's stress response under combined stress conditions may explain some of the similarities in signalling and regulation of stress response (crosstalk) elicited by different abiotic stresses [57].

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