

Plant stress adaptations – making metabolism move

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Persistently sub-optimal environmental conditions constitute stress. Perception and signaling lead to protein expression changes, the activation of new biochemical pathways, and repression of others which are characteristic of the unstressed state. Protective metabolic adaptations alter physiological reactions of the whole plant. Paramount among the mechanisms are oxygen radical scavenging, maintenance of ion uptake and water balance, and reactions altering carbon and nitrogen allocation, such that reducing power is defused. Elements of the stress signaling pathways and proteins that lead to stress protection have recently become known.

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Abbreviations

ASX	ascorbate peroxidase
DMSP	dimethylsulfoniopropionate
HKT	high affinity K ⁺ transporter
HOG	phosphorylation cascade ('high osmolarity glycerol')
LEA	late embryogenesis-abundant
ROS	reactive oxygen species
SOD	superoxide dismutase

Introduction

Plants experience shade or high light levels, sub-zero, low or high temperatures, drought, flooding, high salinity, inorganic nutrient imbalance, infection, predation, and natural or man-made toxic compounds — all of which can be stressful if they persist. Setting aside toxic stresses, for example excess of heavy metal ions or permanent lack of water in a true desert, we will focus on plant adaptive responses to resource stresses that generate osmotic imbalance, such as part-day low temperature, temporary lack of rain, or fluctuating sodium salinity. Osmotic stress perception and signaling, which has come into the focus of research on environmental stresses [1•,2•,3•,4–6,7•], is translated into biochemical reactions, metabolic adjustments and altered physiological state, thus re-programming the progression of development. Relevant to the topic of stress-mediated adjustments of metabolism is the recognition that stress responses are elicited through several pathways and that these pathways are cross-wired [1•,2•,3•,4,5]. At least four signal transduction chains exist in plants for responding to drought, salinity and low temperature. An abscisic acid (ABA)-dependent pathway responds to drought and

salinity signals. This pathway is itself complex, because some ABA-inducible stress responses depend on protein synthesis, but others utilize existing components of the signaling transduction chain [1•,2•,7•]. A second signaling pathway, which does not depend on abscisic acid, shows yet another bifurcation with differential responses of genes that are either affected by cold, salinity and drought, or by salinity and drought only. The receptors that sense drought or salinity are not yet identified — they may be similar to yeast osmo-sensors [4]. Emphasis here is on the biochemical mechanisms elicited by plant counterparts of ubiquitous signal transduction pathways, similar to, for example, the yeast HOG (phosphorylation cascade)-pathway determining carbohydrate allocation changes under stress, and similar to the yeast phosphorylation-relay in which the protein phosphatase calcineurin plays an important role [5], controlling water and ion uptake and ion exclusion or export during environmental stress [5,6].

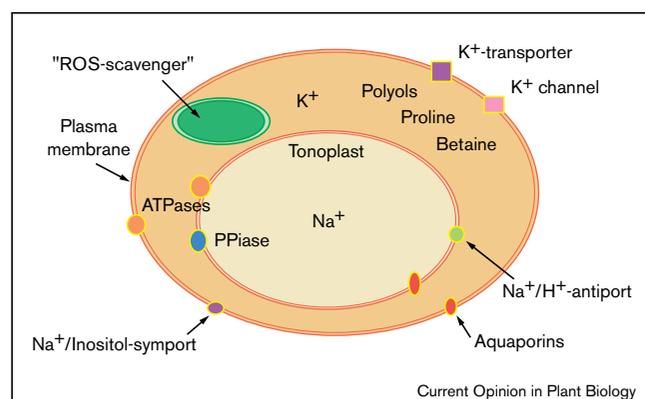
Metabolism under stress

Drought, salinity and low temperature affect uptake and conductance of water. Environmental factors that affect water supply lead to changes in stomatal opening which can, if stress persists, set in motion a chain of events originating from changes in the concentration of leaf-internal carbon dioxide, consecutively affecting the carbon reduction cycle, light reactions, energy charge, and proton pumping [8–12,13•]. Other pathways are affected as a result of increased shuttling of carbon through the photorespiratory cycle [9]. Eventually, carbon and nitrogen allocation and storage require readjustment; reactions that lead to the consumption of reducing power become favored, and development and growth may become altered [8–12]. During the past few years, the complex interrelationship of biochemical pathways that change during stress has become appreciated, although we are far from understanding this complexity; several review articles are available [2•,8–12]. In Figure 1 mechanisms for which experimental evidence indicates an important contribution to metabolic adjustments under stress at the cell level are illustrated with the names of proteins, enzymes and metabolites. The significance of these mechanisms is supported by gene discovery, with stress-dependent regulation of the corresponding transcripts, or by biochemical analyses. Further support comes from experiments with transgenic plants (which might be termed 'transgenovars', e.g., *N. tabacum* tgv) expressing proteins encoded by such transcripts.

The importance of ROS scavenging

In photosynthetic organisms, the inevitable production of reactive oxygen species (ROS) leads to singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals, but ROS are also formed in processes which are not related to

Figure 1



Enzymes, proteins, metabolites important in plant cellular stress responses. Stresses lead to increased production of reactive oxygen species (ROS) which are counteracted by changes in the activity and or amount of ROS scavenging systems [13••]. Stress leads to increased proton pumping across plasma membrane (P-ATPase) and tonoplast membrane (PPI-ase, V-ATPase) [8–10,29]. Compartmentation of sodium during salt stress is accomplished by a tonoplast Na^+/H^+ -antiport system [29] and potassium levels in the cytosol are maintained to some degree. Compensating the osmotic pressure generated by vacuolar sodium, cytosolic amounts of a variety of metabolites increase (exemplified by polyol, glycinebetaine, and proline) [9,10]. The mechanism of entry of sodium into the cytosol is not known, potassium transporters or channels might be responsible [24••,25], but uptake by a sodium/proton symporter also seems possible [48•]. The stress-dependent regulation of aquaporins indicates their involvement as water channels during stress responses and some may also function in metabolite or ion transport.

photosynthesis-specific reactions [13••,14••]. In addition, ROS serve as signaling molecules [13••,15•], for example in the recognition of attack by fungal pathogens [15•]. Mechanisms of ROS detoxification exist in all plants — enzymatic (for example, superoxide dismutase (SOD), ascorbate peroxidase (ASX), glutathione cycle (GST/GPX) and non-enzymatic (flavonones, anthocyanins, carotenoids, ascorbic acid, etc.)—and these suffice under normal conditions. Following stress, ROS increase and upregulation of mRNA transcript and protein levels or accelerated turnover of components of detoxification systems have been shown [13••,16,17]. To some extent, the transgenic enhancement of ROS scavenging components has been shown to positively affect plant performance during stress [16–18], but protection has not been observed in all experiments [13••]. It would certainly be premature to consider the protection provided by the over-expression of SOD, ASX, or enzymes of the ascorbate/glutathione cycle as the final word. Protection has typically been observed in strictly controlled environments, and protective effects have often been marginal. Many reasons can be given [13••], but one consideration may suffice—for example, in the case of ASX, we can expect at least six different isoforms which are located in mitochondria, chloroplasts (several, in different sub-compartments/membranes), soluble in the cytosol, and in the cytoplasmic endomembrane system [19••]. A similarly complex distribution has been

seen for SOD isoforms [13••] which are found in the cytosol (copper/zinc-SOD), mitochondria (manganese-SOD) and plastids (iron-SOD and copper/zinc-SOD). Thus, it seems transgenic modifications of single enzymes are likely to have a minimal effect because of the multitude of compartments that require protection. In addition, in most transgenic experiments little attention has been paid to the ‘when’, ‘where’, and ‘how much’ aspects of transgene expression — significantly more attention needs to be directed to the promoter elements that drive these transgenes [10,20•].

Excellent evidence for a protective effect of ROS scavenging systems has recently been provided by the overexpression of an enzyme with combined activities of glutathione S-transferase, GST, and glutathione peroxidase, GPX [18]. By doubling the GST/GPX activity in transgenic tobacco, the seedlings and plants showed significantly faster growth than wild-type during chilling and salt stress episodes. The increased enzyme activities resulted in higher amounts of oxidized glutathione in the stressed plants, indicating that the oxidized form could provide an increased sink for reducing power.

Functions of accumulating ions and metabolites

A general stress response in all kingdoms is the accumulation of ions (potassium, sodium and calcium) and increased amounts of metabolites which are a part of normal metabolism and which are considered compatible solutes. Examples are sugars, sugar alcohols, low-complexity carbohydrates (e.g., fructans, raffinose series), tertiary amines, sulfonium compounds and amino acids [8,9,21–23•]. Table 1 lists transgenic experiments, mostly with tobacco, with genes that lead to the synthesis of these compounds (and to the synthesis of a late embryogenesis-abundant [LEA] protein). In all cases some protective effect has been observed with the expressed transgenes.

The accumulation of potassium in the vacuole is a preferred strategy which lowers the osmotic potential of the cell. Several potassium channels and transporters which seem to work at different external concentrations have been discovered [24••]. Channels seem to constitute a low-affinity uptake system operating in the millimolar range, while high-affinity transporters operate at micromolar concentrations of external potassium. The regulation of potassium-transport during stress, the cellular location of transporters, and the extent to which the uptake systems discriminate between sodium and potassium during salinity stress is intensely debated [24••,25,26••]. Although the functional characteristics of the wheat HTK (high affinity K^+ -transporter) have been clearly documented by expression of the transporter in yeast [25], the degree to which this transport system is involved in plants, and in which cells or tissues it is located, remain controversial. New transporters and routes for potassium uptake (including ATPases and amino acid- or

Table 1**Transgenically expressed proteins with effects on water deficit, salinity stress, or oxygen radical protection.**

Gene (Source species)	Enzyme	Host species	Notes
<i>MnSOD</i> (<i>N. plumbaginifolia</i>)	Manganese-superoxide dismutase	<i>N. tabacum</i> <i>M. sativa</i>	Organelle targeted expression leading to reduced damage by reactive oxygen species.
<i>MtID</i> (<i>E. coli</i>)	Mannitol 1-P dehydrogenase	<i>N. tabacum</i> <i>A. thaliana</i> <i>N. tabacum</i>	Sodium tolerance at early growth; enhanced seed germination in sodium chloride; reactive oxygen species scavenging in chloroplasts. Protection of calvin-cycle enzymes.
<i>Hva1</i> (<i>H. vulgare</i>)	HVA1-late embryogenesis abundant protein	<i>O. sativa</i>	Maintenance of higher growth rate by stressed plants [55].
<i>Imt1</i> (<i>M. crystallinum</i>)	Myo-inositol O-methyltransferase	<i>N. tabacum</i>	Stress-induced accumulation of D-ononitol based on substrate availability.
<i>SacB</i> (<i>B. subtilis</i>)	Levansucrase	<i>N. tabacum</i>	Fructan accumulation; higher growth rate during drought.
<i>Tps1</i> (<i>S. cerevisiae</i>)	Trehalose synthase	<i>N. tabacum</i>	Increased drought tolerance at low concentration.
<i>CodA</i> (<i>A. globiformis</i>)	Choline oxidase	<i>A. thaliana</i>	Glycine betaine accumulation: enhanced low temperature and salinity tolerance.
<i>P5CS</i> (<i>V. aconitifolia</i>)	Pyrroline 5-carboxylate synthase	<i>N. tabacum</i>	Proline accumulation lowering osmotic potential.
<i>FeSOD</i> (<i>A. thaliana</i>)	Iron-superoxidase dismutase	<i>N. tabacum</i>	Photosystem II and membrane protection; methyl viologen resistance.
<i>Gst/Gpx</i> (<i>N. tabacum</i>)	Glutathione-S-transferase /glutathione peroxidase	<i>N. tabacum</i>	Increased oxidized glutathione enhanced seedling growth.

Although documented effects of overexpression indicate protection, the mechanisms leading to enhanced tolerance under controlled growth conditions are not understood. A note of caution has recently been voiced [54]; the accumulation of mannitol in a transgenic tobacco line was shown to reduce growth by up to 40%. Such reduction in growth might lead to less sodium uptake which might be misinterpreted as an increase in tolerance. References can be found in [10,55,56].

sugar-transporters) are continually being found [27•,28•], indicating a surprising number and variety of different systems for the regulation of potassium acquisition.

For osmotic adjustment during salt stress, the uptake of abundantly available sodium provides an advantage, if sodium can effectively be partitioned and confined to the vacuole. This strategy is used by halophytic plants, but even plants that are generally considered sodium excluders will take up and partition sodium during prolonged stress. Exactly through which transport systems, and along which route sodium enters the root and vascular system, and how it is directed to the vacuole of mesophyll cells is not clear. It may be that the high-affinity HKT-type potassium-transporters constitute major ports of entry, because the wheat HKT, when expressed in yeast, has been shown to discriminate ineffectively between potassium and sodium [25]. Sodium uptake through HKT-type transporters may be a mechanism for loading sodium into root cells at low external potassium concentrations. The mechanisms that lead to long-distance transport of sodium and loading into mesophyll cells are not known. Confinement of sodium to vacuoles is

accomplished by sodium/proton antiporters which have been characterized only physiologically [29].

In contrast to ion accumulation which provides for a lower osmotic potential cheaply, functions of other accumulators prove more difficult to assess. Increased sugars and amino acids provide osmotic potential and some protection, but these metabolites are also part of normal metabolism with which their accumulation might interfere. Sensing systems that can alter partitioning and tissue allocation as well as gene expression have been described for reducing sugars [30,31,32•]. Their accumulation during stress might be pathological. Similarly, the accumulation of proline in a large number of species may result from metabolic disturbance. We view proline accumulation as a consequence of altered nitrogen allocation. Proline overexpression does provide for a lowering of the osmotic potential in transgenic plants when proline feedback inhibition of the rate-limiting enzyme is abolished [22], but this engineered situation is not reflected during the normal accumulation of proline under stress. In stressed non-transformed tobacco plants, for example, proline amounts vary in a diurnal cycle [33]. It is difficult to

imagine how five-fold day/night fluctuations in proline concentration might provide protection considering that cellular sodium levels do not change accordingly [33]. It seems that proline's true function in osmotic stress protection is still to be determined. This notion is supported by the analysis of a highly salt-sensitive *Arabidopsis* mutant with a defective potassium uptake system, which accumulates more proline than wild-type without becoming more stress-resistant [34].

To what extent have transgenic plants provided insights? Engineered expression has been reported for genes that lead to the accumulation of proline, trehalose, polyols, fructan, ectoine (in bacteria), LEA proteins, glycinebetaine, ROS scavenging enzymes and support systems (Table 1, [10,21–23]). In most reports, some protective effect has been observed, but not enough to call the marginal increases of tolerance under defined conditions an unqualified success that could be transposed to growing plants under natural stress conditions. Published results from field tests are missing, but such analyses are underway — not with transgenic plants but in a comparison between glycinebetaine-deficient and glycinebetaine-containing maize breeding lines ([35], Rhodes D, personal communication).

Multiple functions?

Osmotic adjustment through metabolite accumulation, ROS scavenging, adjustments in carbon/nitrogen balance, the 'burning' of excess reducing power, and alternative carbon or nitrogen storage have frequently been proposed as possible functions of the diverse reactions characterizing plant stress responses. Why should we not assume multiple functions for each or at least some of the accumulating metabolites? Newer data support this multiple function notion. Mannitol may accumulate in some species to osmotically significant amounts which lowers the osmotic potential of cells. This may be allowed because mannitol and other polyols seem not to interfere with the normal sugar-sensing systems in plants. In addition, mannitol provides protection even at low concentrations due to a specific role in scavenging of hydroxyl radicals that are produced in a Fenton-reaction between free Fe^{2+} , which is present in sufficiently high concentrations in plant cells, and hydrogen peroxide [13••,14••,36•]. *In vitro* and *in vivo* experiments indicate that glycinebetaine also could have such dual function. It stabilizes, first, the native structure of proteins and protects membranes. Effects of glycinebetaine on the osmotic potential have been shown in near-isogenic corn lines which are distinguished only by glycinebetaine content [35] and by gene transfer of a bifunctional choline oxidase, converting choline into glycine betaine, into *Arabidopsis* [37]. It may further serve as an end-product that accepts excess methyl groups from

a stress-related increase in photorespiration, although this function is still hypothetical [9].

Yeast and *Arabidopsis* as models

Saccharomyces cerevisiae, whose entire genome has been sequenced, is the ideal model for investigating the responses of plants to osmotic stress at the cell organization level — at least in the absence of the DNA sequence of a whole plant genome. The *Arabidopsis* genome sequence will, however, become available by 2001 and possibly 20% of the genomic DNA will have been published by the end of 1998 [38•]. This sequence, complemented by a set of mutants covering every *Arabidopsis* gene, and the techniques available for manipulating *Arabidopsis* will be powerful tools for finding all stress-related plant genes. One of the first benefits from such a sequence will be the possibility of using micro-array techniques for genome-wide monitoring of all genes that are expressed under any condition which is already extensively being used in yeast studies [39,40]. Meanwhile, the complementation of yeast mutants, or of knock-out strains in which specific genes for a mechanism already studied in yeast have been deleted, is a most economical way for finding corresponding, homologous plant stress response mechanisms. The power of yeast complementation has been documented by, for example, the detection of potassium-transport systems [25] or amino acid transporters [41]. In addition, strains have been constructed which lack glycerol production, a natural yeast salinity stress response [42••,43••]. For studying the function of accumulating metabolites, other than glycerol, such transgenic yeast strains will provide more insight than transformed plants in the short term. Once we have learned about functions in yeast, the search for plant mutant phenotypes, physiological and biochemical analyses in non-transformed plants and transgenic plant studies can become more focused.

New models, new pathways, new functions

Other models that complement *Arabidopsis* and yeast are members of the extremely dehydration-tolerant 'resurrection' plants, with representatives in the ferns, mosses, and angiosperms [11], the salt-tolerant alga *Dunaliella salina* [44] and the halophytic angiosperm *Mesembryanthemum crystallinum* (iceplant) [45••].

Apart from the already well-known compounds, several new metabolites have been studied in recent years. One is ectoine, a zwitter-ionic tetrahydropyrimidine of which different derivatives are known. *In vitro* experiments document strong protective effects of ectoine on enzyme activity in the presence of sodium. Stress-dependent uptake and accumulation of externally provided ectoine have been detected in bacteria inhabiting extreme habitats [46•,47]. It can be expected that the protective effect of ectoine biosynthesis and accumulation will soon be tested in transgenic plants.

Better known are derivatives of *myo*-inositol which appear in a great variety of mono- or di-methylated forms in many species of diverse evolutionary history, including oak (Hamamelididae), soybean (Rosidae) and iceplant (Caryophyllidae). Best known is the pathway from the iceplant where the genes and proteins of this pathway have been characterized [45••,48•]. Originating from glucose-6-phosphate, the ubiquitous pathway to *myo*-inositol is extended leading to two methyl-inositols, ononitol and pinitol. The pathway, which seems to be either absent or not expressed in most plants, is stress-regulated in the iceplant and, upon stress, becomes the major route for carbon metabolism during the initial stress adaptation period. The very high accumulation of pinitol in the cytoplasm which parallels vacuolar sodium concentrations constitutes probably the clearest example of osmotic adjustment [45••], but even this pathway, however, seems to have, at least, two functions. Inositol and its methylated derivative ononitol are, in a stress-dependent fashion, transported to the roots and then recycled back to the leaves through the xylem. Xylem transport of inositol is positively correlated with sodium transport. In the leaves, sodium enters the vacuole and ononitol/pinitol are confined to the cytosol [48•]. It seems that this cycle effectively synchronizes long-distance sodium transport with leaf photosynthetic capacity and vacuolar space in leaf cells. We suggest that this mechanism includes a sodium/inositol symporter similar to systems described in other organisms [49].

A similar case can be made for the occurrence of enzymes that lead to the biosynthesis of dimethylsulfoniopropionate (DMSP). The recent elucidation of the biochemical pathway of this compound in marine algae [50] provides evidence for its stress-alleviating function — DMSP seems to replace glycinebetaine in habitats that are nitrogen-limited. A second function of DMSP seems to be that it acts as a protectant against predation [51••]. In addition, the volatility of DMSP might indicate yet another function, namely in a capacity for ‘burning’ reducing power as it is synthesized and released into the water and atmosphere.

Continued water supply is a critical aspect for stresses that affect water uptake or transport through the vascular system. The recent discovery of proteins that act as water channels — termed aquaporins — and the stress-dependent regulation of the expression of several of these channels is an indication for their involvement in water uptake. The *Arabidopsis* genome includes at least 23 genes that encode proteins of the water channel family, several of which have been functionally characterized and are located in either the plasma membrane or the tonoplast membrane [52••]. It is not known whether all 23 are aquaporins, some may function in metabolite

or ion transport. Stress-dependent altered expression, both up and down, of several putative aquaporins has been reported in *Arabidopsis* and in other plants [52••]. Regulation of activity may be by phosphorylation of individual aquaporins, by changes in oligomerization, and possibly also by cycling through the endomembrane system, that is, removal from the plasma membrane or tonoplast during stress and either degradation and new synthesis of the aquaporins or re-insertion of existing proteins into the membrane as has been observed in animal systems [53]. The existence of specific channels for facilitated water movement in plants has been accepted only reluctantly, and working out the details of their functioning and possible contribution to stress protection requires more work.

Conclusions

The next few years will see rapid progress in our understanding of the molecular genetic basis of stress perception, in how plants and cells measure and quantify deviations from their innate ‘set-value of maximal comfort’, in how hormonal, metabolic, and biochemical stress responses change physiology and development. In addition, we expect that genome sequences and micro-array analysis will provide a complete inventory of the genes whose expression is affected by stress. The nature of many upregulated transcripts will be indicative of a function in protection.

The universality of stress responses is probably the most salient feature of analyses over the last five years. All plants react to the various abiotic stresses by a signal relay whose components and cross-wiring are similar to those described in yeast [3•-5]. The network of interactions between different inputs and signaling channels that is formed in a plant-specific way drives metabolic adjustments which include reactions that are common to all or nearly all plant species, such as changes in carbon allocation and nitrogen/ carbon balance, ROS scavenging, and adjustments in metabolism which affect the redox state. Different orders, families, and species evolved different pathways and accomplish protection through different biochemical adjustments. They are variations of general themes, exemplified by the accumulation of glycinebetaine, DMSP, ectoine, methyl-inositols, or amino acids.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Shinozaki K, Yamaguchi-Shinozaki K: **Gene expression and signal transduction in water-stress response.** *Plant Physiol* 1997, **115**:327-334.

An excellent overview of the current information on water stress/drought signaling pathways and the isolation of transcription factors that control or are involved in drought-specific gene expression. The authors discuss abscisic acid-dependent and -independent pathways. They also discuss possible sensors of osmotic stress in relationship to the information available from yeast and bacterial systems and the network of interdependent signaling events that promote stress signals and lead to metabolic adjustments in higher plants.

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Similar to [1•], this review discusses signal transduction chains involved in water deficit signaling. In addition, a discussion of cellular adaptations to water deficit is included as is an evaluation of the possible functions of the proteins and pathways induced under stress.

3. Hirt H: **Multiple roles of MAP kinases in plant signal transduction.** *Trends Plant Sci* 1997, **2**:11-15.

A general discussion, not restricted to stress aspects, of the multiple functional components of mitogen activated protein (MAP) kinase pathways and their roles in intracellular signal transduction. Distinct stimuli derived from wounding, pathogens, and abiotic stress affect different MAP kinases. The relationship of the phospho-relay and the coupling to plant hormones, abscisic acid, ethylene, and auxins, and specific physiological reactions is discussed.

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7. Ishitani M, Xiong L, Stevenson B, Zhu J-K: **Genetic analysis of osmotic and cold stress signal transduction in Arabidopsis: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways.** *Plant Cell* 1997, **9**:1935-1949.

The presentation of a novel and efficient approach towards the generation of mutants in stress signaling pathways. The strategy is based on the promoter of a stress-responsive gene from *Arabidopsis* driving the expression of a luciferase coding region in transgenic *Arabidopsis*. A mutagenized seed population has been screened for plants showing altered luciferase expression resulting in the detection of a large number of mutant plants that are being studied for signal pathway genes involved in the response to abscisic acid, cold, drought and salinity stress. The constitutive, low, or high expression phenotypes (in comparison to the normal stress-inducible expression of the promoter) and effect of different stresses on these mutants allow for a genetic road-map about cross-talk and convergence of different stress signaling pathways.

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11. Ingram J, Bartels D: **The molecular basis of dehydration tolerance in plants.** *Annu Rev Plant Physiol Plant Mol Biol* 1996, **47**:377-403.

12. Jain RK, Selvaraj G: **Molecular genetic improvement of salt tolerance in plants.** *Biotechnol Annu Rev* 1997, **3**:245-267.

13. Noctor G, Foyer CH: **Ascorbate and glutathione: keeping active oxygen under control.** *Annu Rev Plant Physiol Plant Mol Biol* 1998, **49**:in press.

The complexity of antioxidative scavenging systems in plants, both enzymatic and low molecular weight components, is viewed from the aspects of their role in normal metabolism and their function when reactive oxygen species formation is increased under stress conditions. The current knowledge of the biosynthesis, compartmentation, and transport of ascorbate and glutathione is reviewed. Included is a critical evaluation of current engineering schemes that alter reactive oxygen species scavenging systems in transgenic plants.

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With emphasis on DNA damage by hydroxyl radicals generated via the Fenton reaction, this review provides an excellent overview of the consequences of reactive oxygen species generation in non-photosynthetic organisms.

15. Lamb C, Dixon RA: **The oxidative burst in plant disease resistance.** *Annu Rev Plant Physiol Plant Mol Biol* 1997, **48**:251-275.

Rapid generation of superoxide and the accumulation of hydrogen peroxide are features that distinguish the hypersensitive response after perception of a pathogen. Discussion covers the enzyme systems and events that generate a radical burst, long-distance signals and signal transduction which may lead to hypersensitivity and cell death.

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18. Roxas VP, Smith RK Jr, Allen ER, Allen RD: **Overexpression of glutathione S-transferase/ glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress.** *Nature Biotechnol* 1997, **15**:988-991.

19. Jespersen HM, Kjaersgard IV, Ostergard L, Welinder KG: **From sequence analysis of three novel ascorbate peroxidases from Arabidopsis thaliana to structure, function and evolution of seven types of ascorbate peroxidase.** *Biochem J* 1997, **326**:305-310.

Several components of the radical scavenging system have been enhanced in transgenic plants [16–18] and marginal growth improvement has been observed which seems to be restricted to either certain developmental stages or physiological conditions of the transgenics. Conceivably, single gene transfer and expression cannot produce global protection – multigene transfer with attention to cell-specificity and developmental control of transgene expression should provide additional clues as to which compartment or tissue requires protection. Also, the detection of multiple isoforms of ascorbate peroxidase enzymes [19] which are targeted to different intracellular compartments provides a strategy for transgenic functional analysis.

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Many transgenic plant studies use constitutively expressed promoters for transgene expression, but control of cell-, tissue- and organ-specific expression patterns, strength of expression, inducibility and developmental timing will be necessary in the future. Described is the transfer of a self-contained promoter element from *Aspergillus nidulans* into tobacco fused to a minimal plant promoter, which can be induced by ethanol.

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23. Holmström K, Mantyla E, Welin B, Mandal A, Palva ET, Tunnela OE, Londeborough J: **Drought tolerance in tobacco.** *Nature* 1996, **379**:683-684.

This paper, and [21,22] describe transgenic approaches for overexpression of enzymes leading to fructan, proline, or trehalose accumulation. Accumulation of these compounds is positively correlated with stress protection. With the exception of proline, however, accumulation is not osmotically significant, indicating that mechanisms – which remain unknown – other than simple osmotic adjustment governs their action.

24. Maathuis FJM, Ichida AM, Sanders D, Schroeder JI: **Roles of higher plant K⁺ Channels.** *Plant Physiol* 1997, **114**:1141-1149.

A comprehensive update on plant potassium channel structure, functioning and functions in diverse tissues, cells and physiological states. While the

review does not discuss a function of these channels in stress responses, their crucial involvement in potassium nutrition, stomatal movements, leaf movement, xylem transport and vacuolar potassium regulation provides an appreciation for their importance.

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- An *Arabidopsis* mutant is described that is extremely sensitive to sodium and lithium and is also unable to grow at low potassium levels. The phenotype with respect to growth deficiency on low potassium and low sodium is shown to be suppressed by high external calcium. Salt stress leads to hyperaccumulation of sodium and less uptake of potassium compared to wild-type. This mutant represents the first plant mutant with an altered response to calcium and illustrates the important relationship between salt tolerance and potassium nutrition.
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- See annotation [28*]
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- Two manuscripts [27*,28*] describing the detection and functional characterization of yet another class of potassium uptake systems in plants.
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- Sensing of the cellular carbohydrate status leads to the modulation of gene expression and changes in starch and sugar synthesis, storage and transport [30,31]. These processes are also affected by stress. Extremely high accumulation of the polyol sorbitol [32*] has several effects which are due to draining of substrates towards sorbitol biosynthesis, changes in reducing sugars and *myo*-inositol deficit. Phenotypic changes occur which are similar to those observed with antisense expression of extracellular invertases and pathogen attack.
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- Previous publications had indicated marginal growth protection under stress conditions in transgenic tobacco which contained elevated amounts of mannitol. These papers report on the effect of mannitol's presence in the chloroplast compartment. Results indicate that mannitol acts specifically in the scavenging of hydroxyl radicals produced through the Fenton-reaction from hydrogen peroxide and abundant free Fe²⁺ in chloroplasts. In addition, the protective effect seems most crucial for protection of SH-containing, regulated enzymes of the carbon reduction cycle under stress conditions when the photosystems and electron transport are not yet affected.
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- Compared to the classic models *Arabidopsis*, tomato, maize, rice and yeasts, the iceplant is little known and studied. Promoting this plant as another model can be justified considering the plant's developmental plasticity, with morphologically easily discernible growth phases, that are largely determined by the environment. The plant is the most extensively studied halophyte from physiological aspects, showing inducible Crassulacean acid metabolism and well-characterized pathways leading to stress tolerance (growth in 500 mM sodium chloride), possessing a small genome (twice that of *Arabidopsis*) with nine chromosomes (N), has been mutagenized, and can be transformed and regenerated.
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membranes – dependent on hormonal and environmental signals – is largely unexplored. It can be expected that the analysis of aquaporin functioning in the next few years will provide more insight into plant water relations than the physiological literature which considered water movement in plants under mechanical aspects.

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