Specific and unspecific responses of plants to cold and drought stress

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Different environmental stresses to a plant may result in similar responses at the cellular and molecular level. This is due to the fact that the impacts of the stressors trigger similar strains and downstream signal transduction chains. A good example for an unspecific response is the reaction to stressors which induce water deficiency e.g. drought, salinity and cold, especially frost. The stabilizing effect of liquid water on the membrane bilayer can be supported by compatible solutes and special proteins. At the metabolic level, osmotic adjustment by synthesis of low-molecular osmolytes (carbohydrates, betains, proline) can counteract cellular dehydration and turgor loss. Taking the example of *Pinus sylvestris*, changes at the level of membrane composition, and concomitantly of photosynthetic capacity during frost hardening is shown. Additionally the effect of photoperiod as measured via the phytochrome system and the effect of subfreezing temperatures on the incidence of frost hardening is discussed. Extremely hydrophilic proteins such as dehydrins are common products protecting not only the biomembranes in ripening seeds (late embryogenesis abundant proteins) but accumulate also in the shoots and roots during cold adaptation, especially in drought tolerant plants. Dehydrins are characterized by conserved amino acid motifs, called the K-, Y- or S-segments. Accumulation of dehydrins can be induced not only by drought, but also by cold, salinity, treatment with abscisic acid and methyl jasmonate. Positive effects of the overexpression of a wild chickpea (*Cicer pinnatifidum*) dehydrin in tobacco plants on the dehydration tolerance is shown. The presentation discusses the perception of cold and drought, the subsequent signal transduction and expression of genes and their products. Differences and similarities between the plant responses to both stressors are also discussed.

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1. Introduction

Cold, drought and salinity are those environmental stressors which affect plants in many respects and which, due to their wide-spread occurrence cause the most fatal economic losses in agriculture. No wonder that the effects of these stressors have been addressed in a countless number of studies ranging from the molecular to the whole plant level, from a description of the damages to mechanisms of tolerance and hardening. All three forms of abiotic stress affect the water relations of a plant on the cellular as well as whole plant level causing specific as well as unspecific reactions, damages and adaptation reactions. With respect to the specific examples, this communication focuses on stress by cold and drought (figure 1), which are considered to share more common features with each other than with the salinity syndrome.

In plant stress analysis, primary and secondary damage and primary and secondary stresses have been differentiated, the latter emerging as a consequence of the former. An example for secondary stress and damage is that caused by ROS (reactive oxygen species, Randy 1995), which arises from imbalances of electron transport rate and the metabolic consumer activity of the reductive power. The secondary stress results from the impacts of cold or of excess of light energy, which are the primary stressors (Huner *et al* 1998). Stressors like cold or drought induce various primary effects on the cellular level, termed strains (Levitt 1980), which in turn lead to uncontrolled (damage) or controlled effects (adaptation) on that level. Perception of the abiotic stressor

Keywords. Adaptation; *Arabidopsis*; molecular response to stress; stressors: cold, forst, drought; tobacco

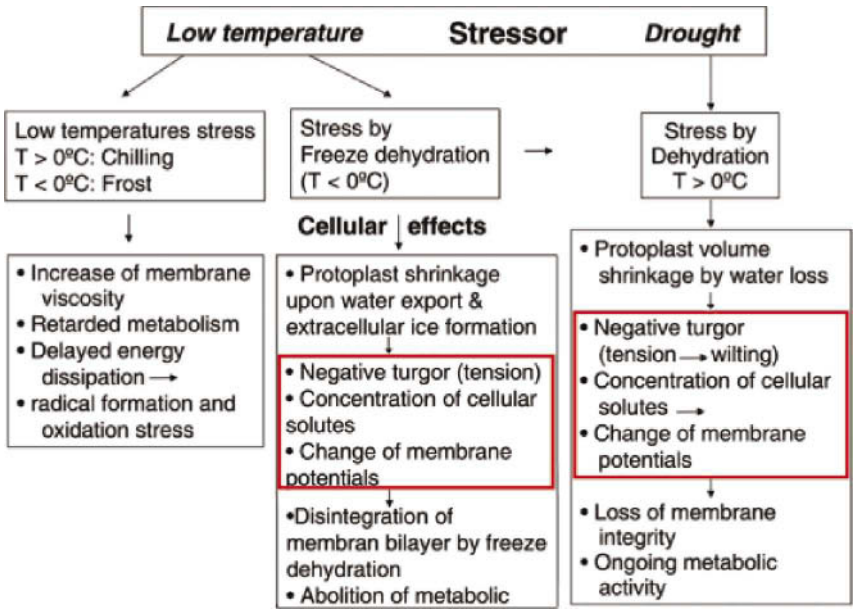

Figure 1. Strains produced by cold and drought in plant tissues.

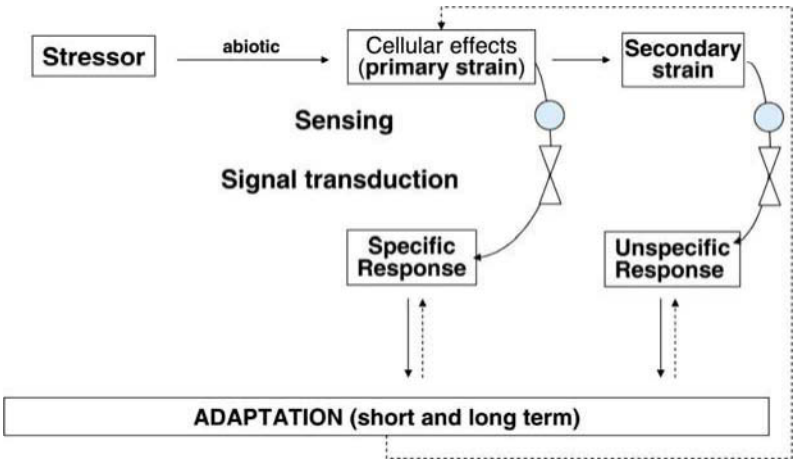

Figure 2. The general stress concept emphasizing the incidence of a specific primary and a less specific secondary strain by a specific stressor. The dashed arrows indicate adaptive reactions.

via the strains and the subsequent signal transduction cascade are responsible for the stressor-specific responses of a plant, whereas accompanying or secondary strains often induce less specific responses (figure 2). Both types of responses cause adaptation to and tolerance of environmental stresses. Subsequently the molecular effects of that stressor are suppressed and signal transduction pathways are modified. Adaptations include short-term (biochemical), but also long-term processes, like morphological changes of the habit of a plant.

2. Physiological characterization of stress by drought and frost

In contrast to plasmolysis, loss of cellular water, mainly from the vacuole, leads to a shrinkage of the whole cell, not only of the protoplast, as the pores in the cell wall are very small (4–5 nm in diameter, Carpita *et al* 1979) resulting in a tremendous matrix potential ($\tau \sim -60$ to -76 MPa) which air has to overcome to permeate the cell wall (Zhu and Beck 1991). Water loss beyond the turgor loss point ($P = 0$),

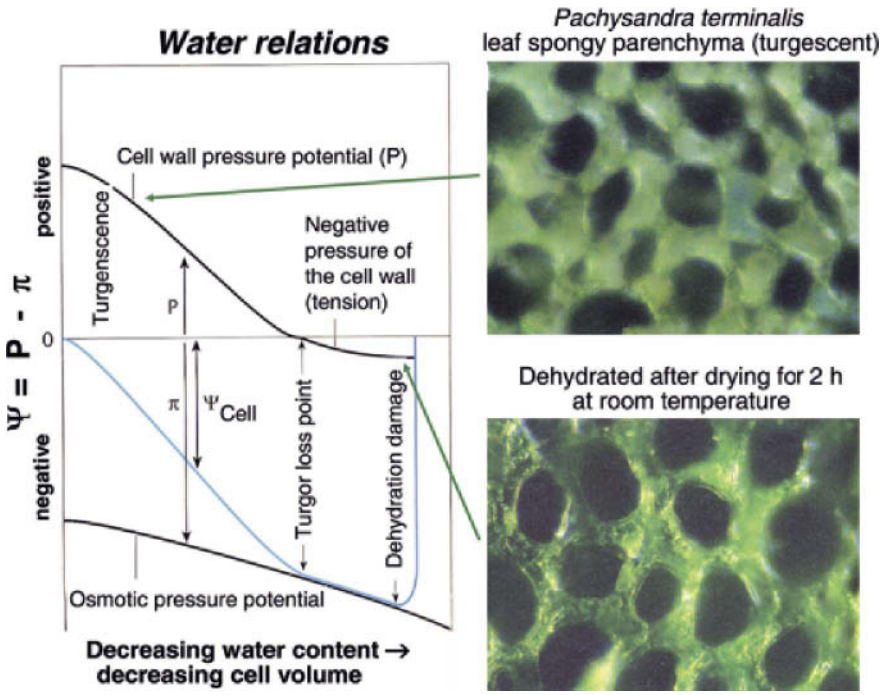

Figure 3. *Left:* Water potential diagram after Larcher (2001) showing the transition of the water relations of a turgescent to a drying leaf tissue. Note the reversion of the positive into the negative pressure potential upon falling below the turgor loss point. *Right:* Spongy leaf parenchyma of *Pachysandra terminalis* in the turgescent (up) and the dehydrated (bottom) stage.

therefore, results in a negative pressure potential (tension) forcing the cell wall to bend inwards thus increasing the water potential of the cell (figure 3). This cell wall mediated tension contributes to retention of more water in the cell than by the osmotic potential (π) alone. Low temperature can affect plants in at least twofold manner: by the effects of low temperature alone and by dehydration of the cells and tissues due to the crystallization of the cellular water (Beck *et al* 2004; Pearce 2001). During freezing of freezing tolerant species, cellular water moves from the protoplast to the intercellular spaces resulting in a glassy appearance of the respective plant organ. Surpassing the nucleation point, ice forms in the intracellular spaces which, due to its higher (numerically lower) water potential attracts more water from the cell until an equilibrium between the water potential of the remaining concentrated cellular solutions and the water potential of the extra-cellular ice is attained (Hansen and Beck 1988). Intracellular ice formation, however, is detrimental since ice crystals do not exert the hydrophobic forces of liquid water which are necessary to maintain the lipid bilayer. Therefore, contact of an ice crystal with a biomembrane must lead to a disintegration of the bilayer. Whether the so-called antifreeze proteins (Griffith and Yaish 2004) which associate with small ice-crystals, forming a hydrophobic coat (Antikainen *et al* 1993) can mitigate the membrane destroying effect of ice crystals is questionable as these proteins are exported from the protoplast and accumulate at the outer periphery of the cell wall (Griffith *et al* 2003). Therefore freezing resistance of a plant means extra cellular ice formation associated with tolerance of protoplast dehydration at low temperatures. The portion of cellular water, which is exported from the cell to crystallize as extra-cellular ice is considerable. It can be determined by NMR spectroscopy which shows the physicochemical state of liquid water as a sharp peak while ice produces only a weak and very broad signal. So-called freezing curves have been produced with this technique showing the extent of freeze dehydration in response to subfreezing temperatures. The theory therefore has been presented elsewhere (Hansen and Beck 1988, Zhu and Beck 1991).

Figure 4 presents such a freezing curve for frost hardy leaves of ivy (*Hedera helix*) indicating that at –10°C about 80% of the cellular water is deposited as extracellular icecaps on the surface of the cells. This leads to a tremendous shrinkage of the cell volume, and in soft tissue like leaf spongy parenchyma to a constriction of the cell and folding of the cell wall, termed freeze cytorrhysis (Sakai and Larcher 1987). However, in rigid tissues, such deformation of the cell walls is not possible and the enormous tension produced by the export of the cellular water may lead to the incidence of water vapour bubbles in the vacuole which again disappear upon warming and melting of the extracellular ice and re-import of the water into the cells as the water vapour dissolves readily in the liquid phase. Of course, metabolic

activity decreases to a minimum or is completely abolished not only due to dehydration but also to an effect of the low temperature. However, in supercooling tissues, i.e. in tissues that avoid extracellular and intracellular ice formation at moderate subfreezing temperatures, a low metabolic activity is still detectable.

Low, but non-freezing temperatures, are injurious to sensitive plant tissues which can not increase the fluidity of the biomembranes by an increase of the proportion of unsaturated fatty acids to accommodate to lower temperature. The result is considered as membrane leakiness (Verlues *et al* 2005), which, however, may be enhanced by the temperature-dependent inactivation of ion pumps or channels. Additionally low temperatures retard metabolic processes and thus promote radical formation and oxidative stress by delayed dissipation of photosynthetic energy (Vogg *et al* 1998).

Drought on the other hand means water loss and dehydration at normal or even elevated temperatures. Shrinking of cells leads to loss of turgor, osmotic stress and a potential change of membrane potentials. Upon severe water loss from the cells, membrane disintegration and abolition of metabolic processes occur (Mahajan and Tuteja 2006).

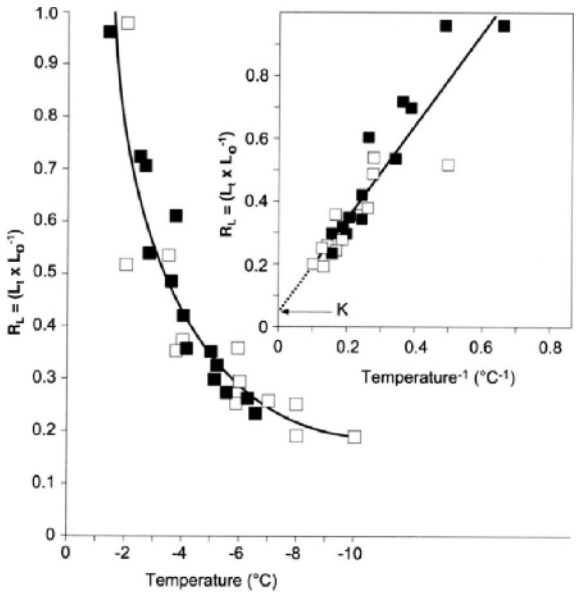

Figure 4. Freezing curve of frost tolerant *Hedera helix* leaves. The curve represents the portion of liquid tissue water (R_L) from total amount of leaf water (L_0) in relation to subfreezing temperatures. *Insert:* Reciprocal plot of R_L versus T^{-1}, transforming the hyperbolic freezing curve into a straight line allowing the determination of the *K*-value. *K* = portion of cellular water, which theoretically cannot freeze (here 4.9 %).

3. Cold perception, signal transduction and response

Although the low-temperature sensor of plants has not yet been definitively identified, findings during the last few years suggest that a decrease of the membrane fluidity represents the sensed parameter (figure 5). The cyanobacterium *Synechocystis* PCC6803 possesses at least two sensors for low temperatures, one of which is a classical histidine kinase and consists of the protein components Hik33 and Hik19 (Browse and Xin 2001). Because no homologues to

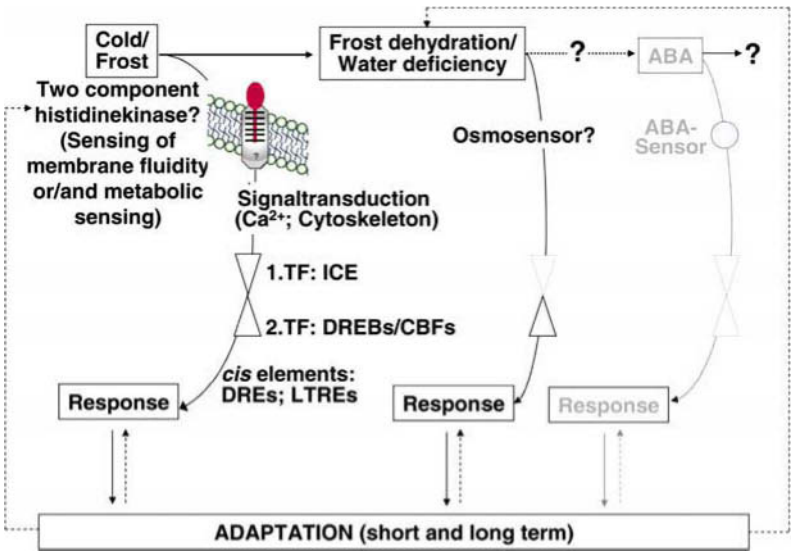

Figure 5. Signalling in the cold strain adaptation response. TF, transcription factor; DREBs, dehydration responsive element binding proteins; CBFs, cold responsive binding factors; LTREs, low-temperature responsive elements; ICE, tanscription factor inducer.

Hik33 and *Hik19* could be detected in plants, alternative concepts of cold-sensing have been proposed. One of them suggests that the increase of membrane viscosity affects Ca^{2+} channels, inducing an increase of the cytosolic calcium-concentration, which in turn activates a MAP kinase or other signal-transducing cascades (Monroy and Dhindsa 1995). Additional signals may originate from the effect of cold on metabolic processes resulting in imbalances of otherwise well-compensated reactions. Imbalances between the absorption of light energy and its metabolic consumption change the redox state of photosynthetic electron-transport components which could function as a sensor (Emsminger *et al* 2006). Interestingly, plant temperature sensing appears to depend on the cooling rate dT/dt, rather than on the subfreezing temperature to which it is subjected (Plieth *et al* 1999). Calcium flux is clearly involved in the cold signal transduction pathway. However, its role is still incompletely understood. Nevertheless, actin cytoskeleton re-organization seems to be an integral component between membrane rigidification and calcium flux (Örvar *et al* 2000). Inositol 1,4,5-trisphospate is likewise involved as a secondary messenger in the transduction of the cold signal (Viswanathan and Zhu 2002). Cold-induced reactive oxygen species may activate a MAP-kinase cascade. At the end, cold induces the expression of C-repeat binding transcription factors (CRBs), which downstream activate the expression of genes that confer cold tolerance. Using two-hybrid systems, several DRE- and CRT-binding proteins, which act as transcription factors, have been detected (Browse and Xin 2001; Nakashima and Yamaguchi-Shinozaki 2006). The expression of CRBs is regulated by the transcription factor inducer ICE1. ICE1 is probably negatively controlled by HOS1, which is a ring finger protein that has been identified as an E3 ubiquitin conjugating enzyme (Viswanathan *et al* 2006). Cold responsive genes (COR-genes) are regulated through specific *cis*-acting promoter elements called C-repeat/dehydration-responsive elements (CRT/DRE) or low-temperature-responsive elements (LTREs).

Transcriptome profiling of approximately 8000 *Arabidopsis* genes showed that multiple regulatory pathways are involved in the response to cold. Expression of more than 300 genes was affected by low temperature, with transcripts for 218 genes increasing and those for 88 genes decreasing threefold or more during the 7-day experiment (Fowler and Thomashow 2002). Several categories of genes which respond to the stress could be differentiated (figure 6): Genes which encode protective but metabolically inactive polypeptides, such as dehydrins, chaperones (including proteases), antifreeze proteins or ice-nucleation-active proteins, genes for metabolic pathways leading to the synthesis of low molecular osmolytes which increase stress tolerance, radical scavengers or compounds with both functions, and regulatory proteins such as transcription factors, protein kinases, phospholipase C or 14-3-3 proteins. Most of the cold-upregulated genes are expressed only at a slightly higher level after the cold pulse, but the dehydrins are the only ones whose genes are strongly induced by cold (Browse and Xin 2001).

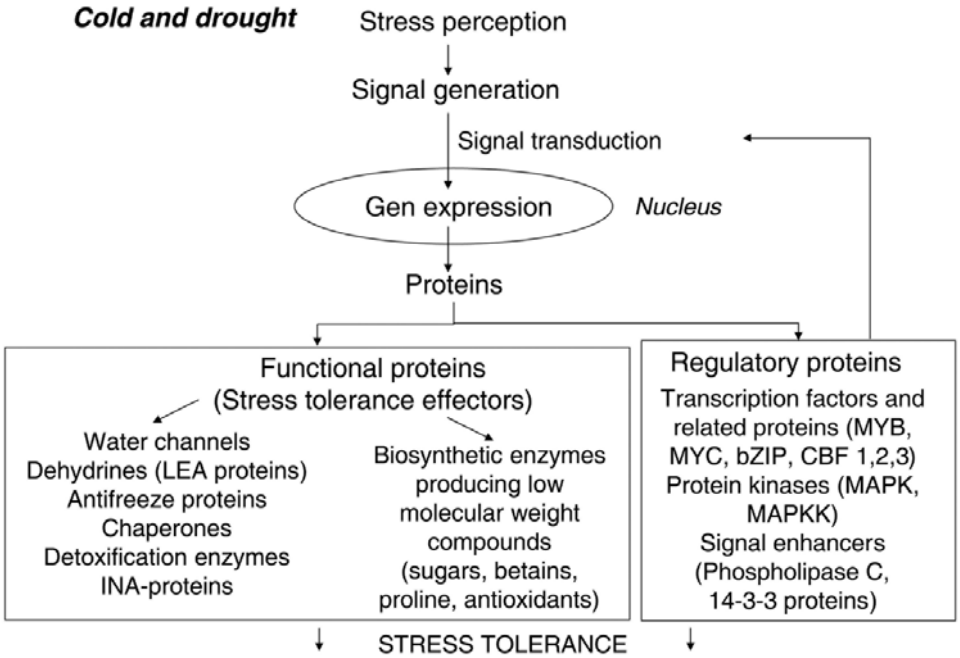

Figure 6. Stress tolerance factors produced in adaptive responses of a plant to cold and drought stress.

4. Drought perception, signal transduction and response

Drought stress, also termed water stress, seems to be sensed by a membrane-bound two-component histidine kinase, which is activated by high osmolarity. Increase of a cell's osmolarity upon water loss during drought therefore triggers the drought response signal chain. The active signal receptor activates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphat to yield the second messengers inositol 1,4,5-trisphosphat ($InsP_3$) and diacylglycerol (Mahajan and Tuteja 2006). $InsP_3$ releases calcium from internal stores and the Ca^{2+}-sensor (Calcineurin B-like protein, CBL) activates downstream protein kinases and phosphatases. Drought-inducible genes display characteristic promotor *cis*-acting-elements, the dehydration-responsive elements (DREs) which at least partially resemble those of the cold-induced genes (Bray 1997). In contrast to the cold response, in which ABA appears to play a minor role, abscisic acid triggers a major signalling pathway in drought stress response (figure 7). Activation of the abscisic acid responsive elements (ABREs) by several transcription factors such as the DRE-binding factors and bZIP-proteins leads to the expression of drought stress tolerance effectors such as dehydrins or enzymes catalyzing low molecular weight osmolytes. The signal transduction pathway of ABA involves cADP ribose, NAADP and Ca^{2+} as second messenger (Quatrano *et al* 1997).

5. Specific and unspecific signalling in cold and drought stress response

Calcium appears as a prime candidate in cold- or drought stress signal transduction resulting in a metabolic or structural mitigation of the effect of the stressor. Therefore, proteins, which sense changes in the cytoplasmic calcium concentrations, are important components of the signal transduction chain. The question is, at which level of the signalling chain the specific responses arise. On a short term, 30% of the *Arabidopsis* transcriptome responded to cold, salinity and water deficiency treatment in a rather specific way. Only < 5% of the responses were induced by all 3 stressors. This rate decreased to < 0.5% after about 1 day indicating a growing tendency for a specific reaction (Kreps *et al* 2002). Although a cross talk in the signalling pathways is evident from the well-known cross-protections, e.g. frost-hardening by salt treatment, selective as well as cooperative actions of transcription factors and the occurrence of several different *cis*-acting elements in one promoter must also exist (figure 8, Mahajan and Tuteja 2005).

6. Adaptation to cold and drought

Stressors like cold and drought induce specific and unspecific responses in a plant. The products of gene-expression mitigate the primary as well as secondary strains

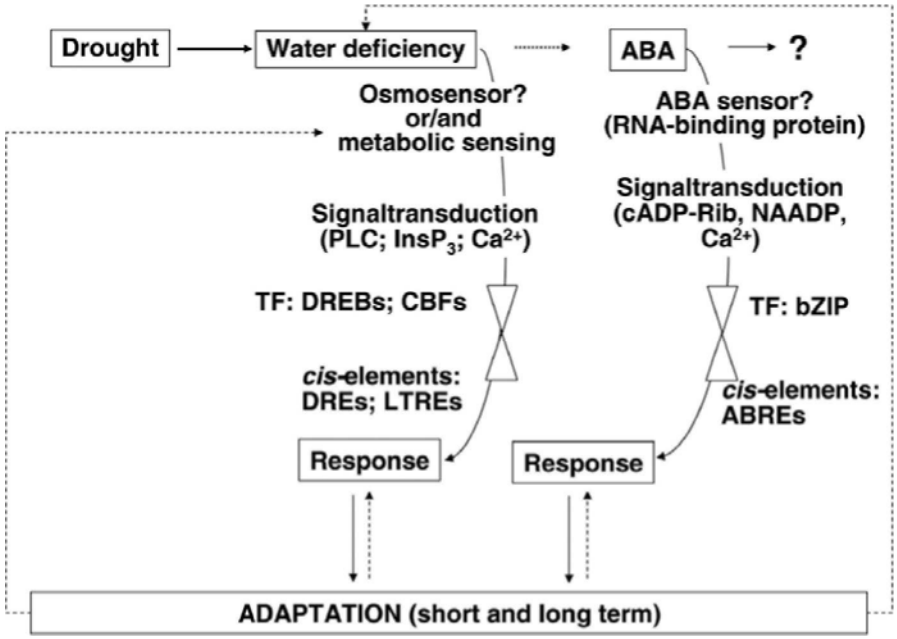

Figure 7. Signalling in the drought strain adaptation response. TF, transcription factor; DREB, dehydration responsive element binding factor; CBF, C-repeat binding factor; DRE, dehydration responsive element; LTRE, low temperature responsive element; ABRE, adscisic acid responsive element; PLC, Phospholipase C; $InsP_3$, Inositol 3,4,5-triphosphate.

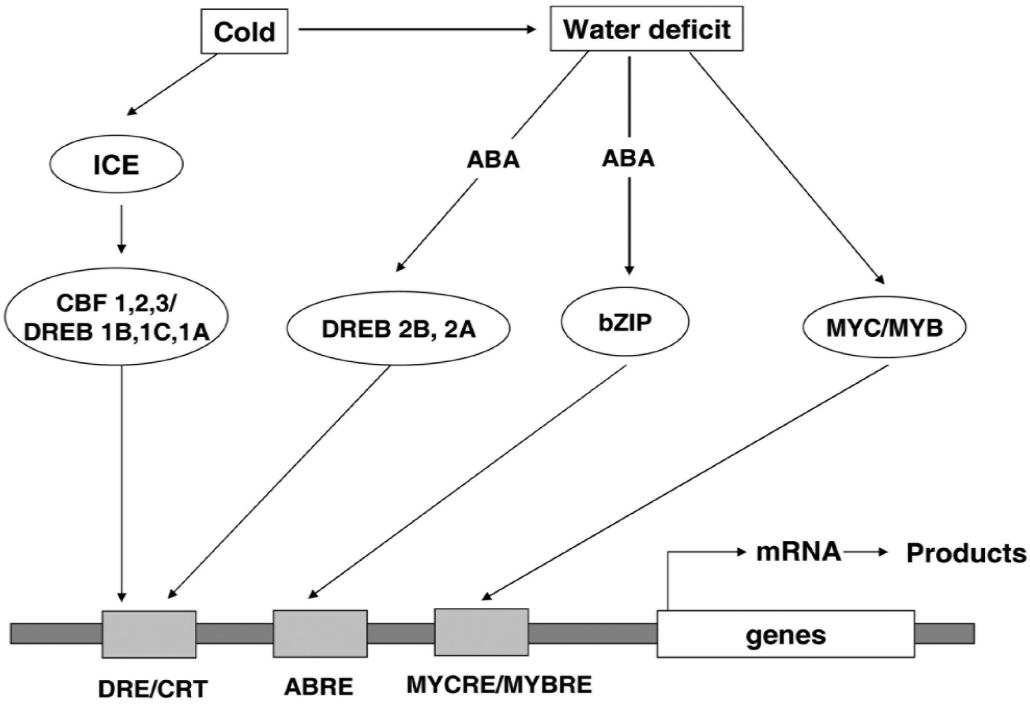

Figure 8. Involvement of various signal transduction elements in the adaptation response to cold and drought. ICE, transcription induction factor; ICE; transcription induction factor; MYC, MYB, transcription activators and MYCRE, MYBRE, MYC- and MYB-responsive elements in the promoter; other abbreviations as in figure 7. After Mahajan and Tuteja (2005), modified.

and thus implement the hardening process. As a consequence the stress tolerance increases, which is understood as adaptation or acclimation to the respective stressor. The stress tolerance effectors like protective proteins or low molecular compatible solutes (osmolytes), usually undergo metabolic turnover and therefore, are not present once and for all. Adaptation to a stressor may also lead to a decrease in efficacy of other processes like photosynthesis or growth. Therefore at least in perennial plants stress tolerance must adjust to the respective kind and intensity of a stressor.

6.1 *Frost*

Seasonal changes in hardiness are observed in perennial and in particular in evergreen plants with respect to the prevailing temperatures. In the temperate climate zone evergreen coniferous trees like pine (*Pinus sylvestris*) or spruce (*Picea abies*) undergo frost hardening in autumn and dehardening in spring. During the frost sensitive summer period the leaves of these plants have a high photosynthetic capacity, which decreases to about 40% in the frost-hardy winter state. As frost is an annually recurring stressor that kills sensitive plant organs, the hardening process must start well before the incidence of the first frost event. The day-length, measured via the phytochrome system has been identified as a trigger for the hardening process whereby a short day-length (low level of P_{fr}) promotes hardening while long days (high level of P_{fr}) accelerates dehardening. The highest rate of frost hardening which could be achieved with such a light treatment was –0.7°C per day (Beck *et al* 2004). Upon incidence of the first frost event, the Pine needles had already acquired a moderate frost resistance of about –20°C. The first subfreezing event immediately triggered a second hardening process which proved independent of the day-length and which took place with a daily rate of about –0.9°C. Both processes were additive, resulting in a daily increase of frost hardiness of –1.5°C. After one month of natural frost hardening the needles survived exposure to –70°C. Adaptation to cold involves membrane lipid desaturation (Senser and Beck 1982), attenuation of the photosystems (Vogg *et al* 1998; Ottander *et al* 1995) but increase of the capability of using light energy for cyclic electron transport and photophosphorylation (Senser and Beck 1977), transition from the common starch-dominated carbohydrate metabolism to an oligosaccharide-dominated metabolism, using sucrose and its galactosides as cryoprotectants (Hansen *et al* 1996). Other changes, such as the enhancement of the radical scavenging system may likewise contribute to frost hardening, as in the yew tree (*Taxus x media,* Verhoeven *et al*

2005), but have not yet been investigated with the mentioned model plants. All these processes are reversed in late spring before sprouting of the new shoots.

6.2 *Dehydrins*

Conditions that result in cellular dehydration such as drought and frost induce the expression of dehydrins, which also accumulate in seeds during maturation where they are known as Late Embryogenesis Abundant (LEA) proteins. Dehydrins vary over a wide range of molecular masses from 9 to 200 kD, they are thermostable and contain a high proportion of glycine and lysine residues. Typical structural features of dehydrins are highly conserved sequences, termed K-, S-, and Y-segments (Allagulova *et al* 2003). Although dehydrins are widely distributed and have been found in vascular plants, mosses, ferns, lichens and algae, their molecular functions are not well understood as they do not catalyze any metabolic reaction. A high portion of random coil structures affects their exceptional water binding capacity and the conserved segments give rise to amphipathic α-helices which form lipid binding domains and thus can associate with, and protect lipid aggregates and hydrophobic domains of proteins. The ubiquitous 'K'-segment of dehydrins shows similarity to a class A2 amphipathic α-helix, resembling the lipid-binding domain found in apolipoproteins (Campbell and Close 1997). Because of these physicochemical properties and a clear correlation of the accumulation of dehydrins with dehydration of a plant, a protective function is obvious. The classical view therefore considers dehydrins as membrane stabilizers (Koag *et al* 2003) but other targets, such as proteins and nucleic acids likewise associate with dehydrins.

A comparison of drought resistance of various chickpea species showed enormous differences and from the most

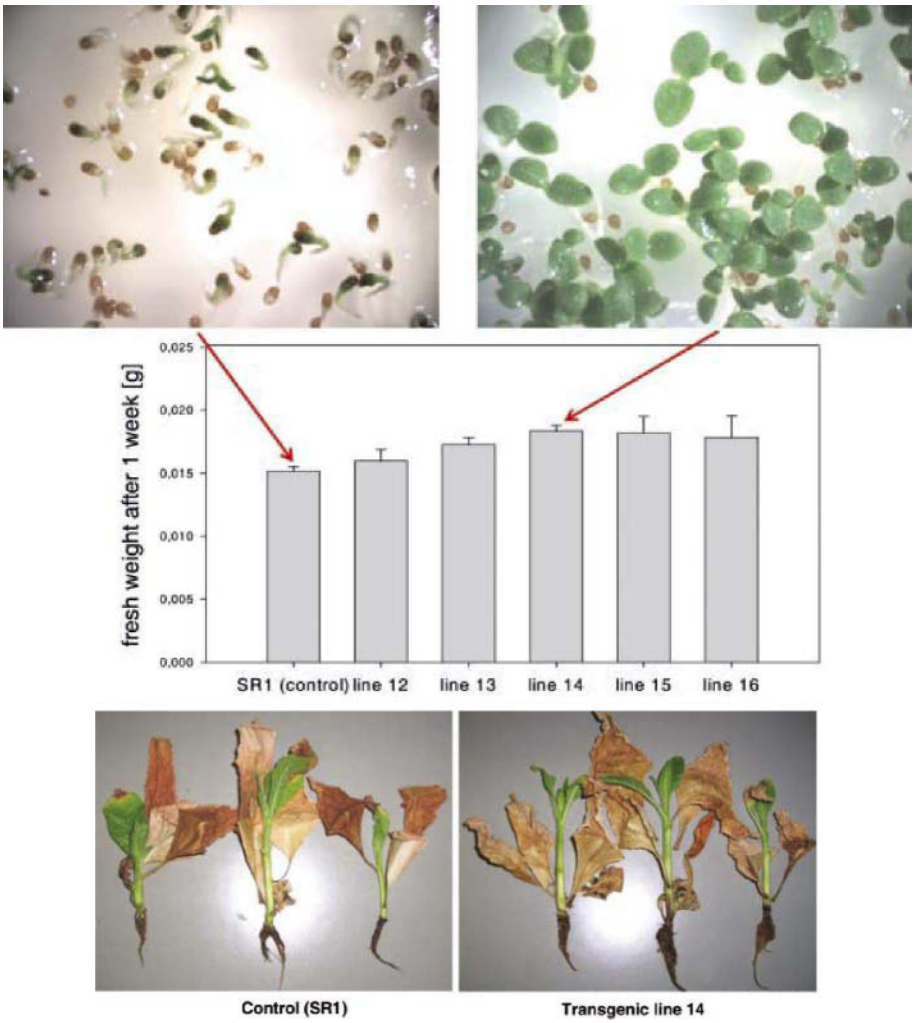

Figure 9. Phenotypes of transgenic tobacco overexpressing *Cicer pinnatifidum* DHN1 under dehydration stress. (**A**) Germination of transgenic tobacco seeds in the presence of 10% (w/v) PEG, 1 week old seedlings. (**B**) Transgenic tobacco plantlets, grown for 5 weeks without irrigation in the greenhouse.

resistant *Cicer pinnatifidum*, one dehydrin gene, DHN1, was isolated (Bhattarai and Fettig 2005). DHN1 can be classified into the class of acidic Y_2K_n dehydrins, but the homology to other dehydrins is very low. Expression of DHN1 in *Cicer* is induced by drought, salt stress and during seed development. ABA is likewise able to sustainably induce DHN1 expression, whereas expression is only transiently enhanced by a low temperature treatment or the application of jasmonic acid. Over-expression of *Cicer pinnatifidum* dehydrin *DHN1* gene in tobacco plants positively affected growth of the seedlings under artificial water stress (figure 9A). The protective effect was less obvious in adult plants from which irrigation was withdrawn for 5 weeks. Although on a first glance both sets of tobacco plants appear very similar, the transformants had a much better developed root system and thus could recover more readily after re-irrigation than the controls (figure 9B, Knake C and Fettig S, unpublished results).

7. Possibilities to modify frost- and drought-tolerance of plants by genetic approaches

The aim of generating frost- and drought-tolerant crop plants has been tackled since many years using classical breeding and targeted gene transfer. However, the achieved progress is still small. This is due to the fact that the damage and tolerance or hardiness are multifactorial syndromes rather than result of a single reaction or gene (Mittler 2006; Shinozaki and Yamaguchi-Shinozaki 2000; Bohnert *et al* 1995). Nevertheless, tackling of the primary stress reactions (the so-called strains, Levitt 1980) by gene transfer can also alleviate the secondary strains and therefore generate plants with a higher stress tolerance than those which have been genetically tailored against a specific secondary strain, e.g. by the enhanced production of radical scavengers. Over-expression of a transcription factor or transcriptional activator of a multigene-sequence whose products directly or indirectly confer improved stress tolerance turned out as the most promising strategy to cope with the fact of a strain syndrome. This has been demonstrated with the low-temperature transcriptional activator CBF1 (C-repeat binding factor), which binds to the C-repeat/dehydration – responsive element (DRE with the core sequence CCGAC) of the promoter of cold- and drought-regulated genes (Jaglo-Ottosen *et al* 1998*;* Stockinger *et al 1997;* Kasuga *et al* 1999). It has been shown that also cold sensitive cereals (rice and maize) contain such cold-regulated genes in their genomes but that expression of these genes is only in the tolerant species (Sarhan and Danyluk 1998). This is a good example for a kind of a master switch which still may require co-activators for full activity (Yamaguchi-Shinozaki and Shinozaki 2005). Alternative approaches aim at a genetic analysis of the entire strain and whole response complexes *via* quantitative trait loci (QTLs) (Tuberosa and Salvi 2006). Methods for a comprehensive screening have been proposed (Verslues *et al* 2006).

References

Allagulova C R, Gimalov F R, Shakirova F M and Vakhitov V A 2003 The plant dehydrins: Structure and putative functions; *Biochemistry (Moscow)* **68** 945–951

Antikainen M, Griffith M, Zhang J, Hon W C, Yang D S C and Pihakaski-Maunsbach K 1993 Immunolocalization of antifreeze proteins in winter rye leaves, crowns, and roots by tissue printing; *Plant Physiol.* **110** 845–857

Beck E H, Heim R and Hansen J 2004 Plant resistance to cold stress: Mechanisms and environmental signals triggering frost hardening and dehardening; *J. Biosci.* **29** 449–459

Bhattarai T and Fettig S 2005 Isolation and characterization of a dehydrin gene from Cicer pinnatifidum, a drought-resistant wild relative of chickpea; *Physiol. Plant.* **123** 452–458

Bohnert H J, Nelson D E and Jensen R G 1995 Adaptations to environmental stresses; *Plant Cell 7* 1099–1111

Bray E A 1997 Plant responses to water deficit; *TIPS* **2** 48–54

Browse J and Xin Z 2001 Temperature sensing and cold acclimation; *Curr. Opin. Plant Biol.* **4** 241–246

Campbell S A and Close T J 1997 Dehydrins: genes, proteins, and association with phenotypic traits; *New Phytol.* **137** 61–74

Carpita N, Sabularse D, Montezinos D and Delmer D P 1979 Determination of the pore size of cell walls of living plant cells; *Science* **205** 1144–1147

Emsminger I, Busch F and Huner N P A 2006 Photostasis and cold acclimation: sensing low temperature through photosynthesis; *Physiol. Plant.* **126** 28–44

Fowler S and Thomashow M F 2002 *Arabidopsis* Transcriptome Profiling Indicates That Multiple Regulatory Pathways Are Activated during Cold Acclimation in Addition to the CBF Cold Response Pathway*; Plant Cell* **14** 1675–1690

Griffith M and Yaish M W F 2004 Antifreeze proteins in overwintering plants: a tale of two activities; *TIPS* **9** 399–405

Griffith M, Lumb C, Wiseman S B, Johnson R W, Wisniewski M and Loewen M C 2003 Winter rye antifreeze proteins are not cryoprotective, rather they modify the growth of ice in plants; *Acta Hortic.* **618** 31–37

Hansen J and Beck E 1988 Evidence for ideal and non-ideal equilibrium freezing of leaf water in frost hardy ivy *(Hedera helix*) and winter barley (*Hordeum vulgare*); *Bot. Acta* **101** 76–82

Hansen J, Vogg G and Beck E 1996 Assimilation, allocation and utilization of carbon by 3-year-old Scots pine (*Pinus sylvestris* L.) trees during winter and early spring; *Trees* **11** 83–90

Huner N P A, Öquist G and Sarhan F 1998 Energy balance and acclimation to light and cold; *TIPS* **3** 224–230

Jaglo-Ottosen K R, Glimour S J, Zarka D G, Schabenberger O and Thomashow M F 1998 *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance; *Science* **280** 104–106

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K and Shinozaki K 1999 Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor; *Nat. Biotech.* **17** 287–291

Koag M-C, Fenton R D, Wilkens S and Close T J (2003) The binding of maize DHN1 to lipid vesicles. Gain of structure and lipid specificity; *Plant Physiol.* **131** 309–316

Kreps J A, Wu Y, Chang H-S, Zhu T, Wang X and Harper J F 2002 Transcriptome Changes for *Arabidopsis* in Response to Salt, Osmotic, and Cold Stress; *Plant Physiol.* **130** 2129–2141

Larcher W 2001 *Ökophysiologie der Pflanzen* 6th edition (Stuttgart: Eugen Ulmer) p. 204

Levitt J 1980 *Responses of plants to environmental stresses*. Vol. I *Chilling*, *freezing, and high temperature stress* (London, New York, Toronto: Academic Press)

Mahajan S and Tuteja N 2006 Cold, salinity and drought stresses: An overview; *Arch. Biochem. Biophys.* **444** 139–158

Mittler R 2006 Abiotic stress, the field environment and stress combination; *TIPS* **11** 15–19

Monroy A F and Dhindsa R S 1995 Low temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25°C; *Plant Cell* **7** 321–331

Nakashima K and Yamaguchi-Shinozaki K 2006 Regulons involved in osmotic stress-responsive and cold-responsive gene expression in plants; *Physiol. Plant.* **126** 62–71

Örvar B L, Sangwan V, Omann F and Dhindsa R S 2000 Early steps in cold sensing by plants: the role of actin cytoskeleton and membran fluidity; *Plant J.* **23** 785–794

Ottander C, Campbell D and Öquist G 1995 Seasonal changes in photosystem II organisation and pigment composition in *Pinus sylvestris*; *Planta* **197** 176–183

Pearce R S 2001 Plant freezing and damage; *Ann. Bot.* **87** 417–424

Plieth C, Hansen U-P, Knight H and Knight M R 1999 Temperature sensing by plants: the primary characteristics of signal perception and calcium response; *Plant J.* **18** 491–497

Quatrano R S, Bartels D, Ho T H D, and Pages M 1997 New insights into ABA-mediated processes; *Plant Cell* **9** 470–475

Randy R D 1995 Dissection of oxidative stress tolerance using transgenic plants; *Plant Physiol.* **107** 1049–1054

Sakai A and Larcher W 1987 Frost survival of plant responses and adaptation to freezing stress; *Ecol. Stud.* **62** (Berlin: Springer)

Sarhan F and Danyluk J 1998 Engineering cold-tolerant crops – throwing the master switch; *TIPS* **3** 289–290

Senser M and Beck E (1977) On the mechanisms of frost injury and frost hardening of spruce chloroplasts; *Planta* **137** 195–201

Senser M and Beck E 1982 Frost resistance in spruce (*Piceaabies* (L.) Karst): V. Influence of photoperiod and temperature on the membrane lipids of the needles; *Z. Pflanzenphysiol.* **108** 71–85

Shinozaki K and Yamaguchi-Shinozaki K 2000 Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signalling pathways; *Curr. Opinion Plant Biol.* **3** 217–223

Stockinger E J, Gilmour S J and Thomashow M F 1997 *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit; *Proc. Natl. Acad. Sci. USA* **94** 1035–1040

Tuberosa R and Salvi S 2006 Genomic-based approaches to improve drought tolerance of crops; *TIPS* **11** 405–412

Verhoeven A S, Swanberg A, Thao M and Whiteman J 2005 Seasonal changes in leaf antioxidant systems and xanthophyll cycle characteristics in *Taxus x media* growing in sun and shade environments; *Physiol. Plant.* **123** 428–434

Verlues P E, Agarwal M, Katiyar-Agarwal S, Zhu J and Zhu J-K 2005 Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status; *Plant J.* **45** 523–539

Viswanathan C and Zhu J-K 2002 Molecular genetic analysis of cold-regulated gene transcription; *Phil. Trans. R. Soc. London B* **357** 877–886

Viswanathan C, Zhu J and Zhu J-K 2006 Gene regulation during cold acclimation in plants; *Physiol. Plant.* **126** 52–61

Vogg G, Heim R, Hansen J, Schaefer C and Beck E 1998 Frost hardening and photosynthetic performance of Scots pine *(Pinus sylvestris L.)* needles. I. Seasonal changes in the photosynthetic apparatus and its function*; Planta* **204** 193–200

Yamaguchi-Shinozaki K and Shinozaki K 2005 Organization of *cis*-acting regulatory elements in osmotic- and cold-stress-responsive promoters; *TIPS* **10** 88–94

Zhu J-J and Beck E 1991 Water relations of Pachysandra leaves during freezing and thawing;*, Plant Physiol.* **97** 1146–1153

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