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Low Temperature, High Light Stress and Antioxidant Defence Mechanisms in Higher Plants

By

G. WINGSLE[1)], S. KARPINSKI[1)] & J.-E. HÄLLGREN[1)]

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S u m m a r y

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In different plant species, different strategies have evolved to acclimate to low-temperature and high-light stress. The emphasis of this review will be to discuss the following topics of low-temperature and high-light stress 1) the evidence for involvement of reactive oxygen intermediates (ROI) 2) the roles of enzymatic and non-enzymatic ROI-scavenging and antioxidant systems 3) the avoidance mechanisms of ROI production in chloroplast. To increase the understanding of the oxidative-stress responses induced, for example, by low temperatures in plants, we have to pinpoint the subcellular compartments and processes, which initiate the specific signalling cascades.

I n t r o d u c t i o n

During evolution trees have developed a number of molecular/ anatomical/ morphological and physiological adaptations that enhance the probability of survival in harsh environments. The focus of this presentation will be the role of the active oxygen scavenging systems in trees and the physiological and biochemical processes and mechanisms that govern protective, repair and acclimation processes. It is our belive that an understanding of these strategies will pave way to create improved stress tolerance of trees and enable a better acclimation of trees to harsh environments.

Woody plants exhibit marked seasonal acclimation, a very active process that is triggered by daylength and low temperature. The two step process, where

[1)] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå Sweden, fax +46 90 786 5901; e-mail: Gunnar.Wingsle @genfys.slu.se

low temperature is needed for full hardening is still not fully resolved at the molecular level. Characteristics which enable the plant to endure low temperature tolerance are both constitutive and facultative. Most stresses also have in common their effect on plant water status (WEISER 1970, KACPERSKA 1989). Accumulating evidence implies that the plant hormone abscisic acid (ABA) also plays a central role in cold (PALVA 1994, WELIN & al. 1996). ABA exhibits a transient increase during cold acclimation (DAIE & CAMPBELL 1981, LALK & DORFFLING 1985) and ABA can substitute for the low temperature stimulus (CHEN & GUSTA 1983). WEISER originally 1970 proposed that cold acclimation require transcriptional activity of specific genes and alteration i gene expression ha been earlier discussed in detail (CLOUTIER 1983, GUY 1990, THOMASHOW 1994, PALVA 1994, HUGHES & DUNN 1996). Genes falling into a few categories will produce protective proteins, such as COR-polypeptides (Cold regulated proteins), CAPs (Cold acclimation proteins), AFPs (anti freeze proteins), and proteins involved in lipid or protein protection have been presented.

The major determinant for cells to survive freezing is their ability to tolerate dehydration and withstand repeated dehydration/rehydration cycles. The plasma membrane appears to be the primary site of injury (STEPONKUS 1984, LYNCH & STEPONKUS 1987), and the extent of injury depend on lipid composition of the membranes and the presence of specific cryoprotectants (HINCHA & al. 1990, LIN & THOMASHOW 1992, NISHIDA & MURATA 1996). To compensate for the reduced osmotic potential of the extracellular liquid during ice formation water is diffusing out of the cells leading to dehydration of the cytoplasm. Cryoprotectants and osmolytes stabilise membranes and maintain protein comformation at low water potential.

Accumulation of compatible solutes including sugar alcohols (e.g. pinitol), amino acids such as proline, quarternary ammonium compounds (e.g. glycine betaine,) polyols and polyamines are known to correlate with increased dehydration tolerance in plants (GUY 1990, BOHNERT & al. 1995, INGRAM & BARTELS 1996). Other mechanisms are also involved and have been described (HÄLLGREN & ÖQUIST 1990, HÄLLGREN & al. 1991) and the protective mechanisms are still a matter of debate. Plants increase their capacity for protein synthesis during cold acclimation (CLOUTIER 1983). An example of protective proteins are dehydrins which have been suggested to protect cytoplasmic proteins against denaturation (CLOSE 1996). Proteins of the dehydrin family are also of interest since they exhibit a high affinity for metals (MANTYLA 1997), and it is well known that metals play a major role in the production of reactive oxygen intermediates (ROIs) (HALLIWELL & GUTTERIDGE 1992).

Low-temperature-induced oxidative stress

A few experiments show direct evidence for ROI formation during low temperature stress (KENDALL & MCKERSIE 1989). The evidence for a higher production rate of ROIs during low temperature stress in plants is mostly indirect

and is based on observations of changes in the levels of different ROI-scavengers and antioxidants (BOWLER & al. 1992). The amplitude of an ESR signal, representing unidentified organic free radicals from Scots pine needles, increased with a step-wise decrease in temperature to -40°C (TAO & al. 1992). The best known site for $O_2^{\cdot-}$ production in a plant is by autooxidation of the thylakoid-membrane-bound primary electron acceptor of PSI and of the peripheral reduced ferredoxin (ASADA & al. 1974, FURBANK & BADGER 1983, ASADA 1994). Most of the $O_2^{\cdot-}$ produced in the thylakoid membrane is converted in a $O_2^{\cdot-}$-mediated cyclic electron flow to O_2 and by non-catalytic dismutation to H_2O_2, before it reaches the stroma or lumenal space (ASADA 1994).

For chloroplasts, mitochondria and peroxisomes the electron-transfer chains are well-documented sources of H_2O_2 (CADENAS 1989, ASADA 1994, PASTORI & al 1998). Chloroplasts are thought to be the major H_2O_2 producers (ASADA 1994). It has been shown that H_2O_2 induces membrane energization that leads to the down-regulation of PSII and, in consequence, can provide protection against photoinhibitory damage (SCHREIBER & al. 1991). H_2O_2 is also a strong nucleophilic-oxidizing agent and has been reported to react with SH-groups. Increased levels of H_2O_2 have been shown to be a general response to low temperature stress in chilling-sensitive plants (PRASAD & al. 1994a).

It is a well-known fact that H_2O_2 and $O_2^{\cdot-}$ can react together in biochemical systems to form the hydroxyl radical ($OH^{\cdot}$). In addition, there are other metal-catalysed reactions involving H_2O_2 that produce $OH^{\cdot}$. Thus, the cellular location of metals and reductants such as thiols and AsA, and the site of production of both $O_2^{\cdot-}$ and H_2O_2, will determine the significance of the $OH^{\cdot}$ toxicity.

Additional forms of ROIs are the singlet species. Singlet chlorophyll (^{1}Chl*) is generated by light excitation. The carotenoid pigments appear to have a dual protective role quenching both ^{1}Chl* and singlet oxygen ($^1O_2$). The chloroplast membranes are particularly susceptible to $^1O_2$-induced lipid peroxidation since approximately 90 % of the fatty acid of the thylakoid glycolipids, phospholipids and sulpholipids is the unsaturated fatty acid α-linolenate (KNOX & DODGE 1985). According to our current knowledge there is no direct proof that the singlet species increase during low temperature stress. The mechanism of free-radical-mediated lipid peroxidation involves at least three different phases. There are only a few examples of lipid peroxidation during freezing. Lipid peroxidation was eg. observed in spruce subjected to frost events during the spring (POLLE & al. 1996). On the one hand polyunsaturation of the membrane lipids due to low temperature would increase the potential for oxidative stress damage, but on the other it provides new mechanistic features for membranes (KUSTERS & al. 1991).

Low temperature causes an increase in ROI levels and induces oxidative stress in plants. However, the precise mechanisms remain to be established.

Antioxidants

Protective mechanisms can be divided into two separate categories, those involved in removing ROIs and those involved in reducing ROI production. Generally, the defence system against ROIs in plant cells is a net result of suppression mechanisms, scavenging and repair systems. Higher plants contain numerous enzymatic and non-enzymatic ROI-scavengers and antioxidants, both water- and lipid-soluble, localised in different cellular compartments (LARSSON 1988, DALTON 1995, WISE 1995). Non-enzymatic antioxidants include: pigments, reduced glutathione (GSH), ascorbate (AsA), vitamin E and many others. Interactions between different ROI-scavengers and antioxidants is reviewed by DALTON 1995 and WINKLER & al. 1994.

Non-enzymatic antioxidants

α-Tocopherol is one of the most acknowledged antioxidant (LARSSON 1988, HESS 1993, POLLE & RENNENBERG 1994). α-Tocopherol is the most abundant tocopherol of the four forms found in plants (α–, β–, γ–, δ-tocopherol). Its main location is within the chloroplast. In Scots pine, older needles contain higher α-tocopherol levels than younger and only a small increase in α-tocopherol content can be detected during the autumn in the needles needles (WINGSLE & HÄLLGREN 1993).

The central roles of AsA and dehydroascorbate (dAsA) in physiological processes in cells has been thoroughly reviewed (LEWIN 1976, FOYER 1993, ARRIGONI 1994, ASADA 1994, DALTON 1995). Ascorbate, and enzymes that metabolize AsA-related compounds, are involved in the control of several plant growth processes (CORDOBA & GONZALEZ-REYES 1994).

Seasonal changes in AsA have been documented in several investigations of frost-tolerant tree species (POLLE & RENNENBERG 1994). Dormant needles show a significantly higher content of both AsA and dAsA, although the ratio of AsA/dAsA was significantly lower (WINGSLE & MORITZ 1997). These and other findings indicate that AsA metabolism play an important role in low-temperature-induced oxidative stress.

The most abundant thiol in higher plants is glutathione (FOYER & HALLIWELL 1976, FOYER 1997, MULLINEAUX & CREISSEN 1997). The general picture is that the levels of glutathione in its reduced form (GSH) increase several-fold during the winter-time in evergreens and the diurnal and seasonal changes is well documented for tree species (ESTERBAUER & GRILL 1978, SMITH & al. 1990, ANDERSON & al. 1992, WINGSLE & HÄLLGREN 1993, POLLE & RENNENBERG 1994, WILDI & LUTZ 1996). Plants normally have a low GSSG level, for example in Scots pine it is approximately 20-fold lower than the GSH content (WINGSLE & al. 1989). Many factors, including low temperature and other environmental stresses, have been shown to change the ratio or redox status of glutathione [GSH/(GSSG +GSH)] (KARPINSKI & al. 1997) and an accumulation of GSSG can be an indicator of higher oxidative stress (SMITH & al. 1990). The precise roles of glutathione in the oxidative stress response still remain to be established and recent data indicate

that GSH levels play a fundamental role in the regulation of the photosynthetic electron transport (KARPINSKI & al. 1997). The regulatory impact of glutathione and/or the redox status of the glutathione pool on plants' oxidative stress response is discussed below.

The enzymatic ROI-scavenging system

In plant cells the enzymatic ROI-scavenging system consists of such enzymes as: superoxide dismutase (SOD), catalases (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX) and glutathione reductase (GR) (FOYER & HALLIWELL 1976, ASADA 1994, BOWLER & al. 1992, FOYER 1993, INZÉ & MONTAGU 1995, FOYER 1997, MULLINEAUX & CREISSEN 1997).

The enzyme SOD can be taken as an example of the complexity in studying the role of the enzymatic defence system. Different SOD isoforms in plants are differentially expressed and also localised in different compartments within and outside the cell (WINGSLE & al. 1991, PERL-TREVES & GALUN 1991, TSANG & al. 1991, BOWLER & al. 1992, KARPINSKI & al. 1992a,b, KARPINSKI & al. 1993, STRELLER & WINGSLE 1994, BUENO & al 1995, WINGSLE & KARPINSKI 1996, SCHINKEL & al. 1998).

In pine trees there are several isoforms of CuZn-SOD in the chloroplast and in the cytoplasm (WINGSLE & al. 1991, KARPINSKI & al. 1992a,b,1993). There are also extracellular isoforms of SOD (STRELLER & WINGSLE 1994). In addition there exist three Mn-SODs in the needles (STRELLER & al. 1994, SCHINKEL & al. 1998).

SOD mRNA levels have been observed to increase during recovery from naturally-established winter stress, a combination of high light and low temperature stress (KARPINSKI & al. 1993, 1994). In this experiment, in needles protruding above snow, higher mRNA levels were observed for chloroplastic and cytosolic isoforms of CuZn-SOD, in comparison with needles covered by snow. Changes in transcript levels were not reflected in a corresponding increase in protein levels. Moreover, CuZn-SOD activity levels were similar in covered and protruding needles. These results suggest higher turnover rates of CuZn-SOD in needles protruding above the snow. The lack of correlation between mRNA levels and protein activity for CuZn-SODs in response to oxidative stress, has been observed before and was also suggested to be a result of higher turnover rates of CuZn-SODs during oxidative stress (KARPINSKI & al. 1992b).

SOD isoforms are differentially expressed during recovery from winter stress. A comparison of chloroplastic and cytosolic CuZn-SOD mRNA levels showed a 4-fold higher transcript level for the chloroplastic form until mid-May (KARPINSKI & al 1993). This higher transcript level was also associated with a higher chloroplastic CuZn-SOD activity. Transcript levels were reduced for both chloroplastic and cytosolic CuZn-SODs and reached similar low levels after the repair process of the photosynthetic apparatus was completed and photosynthetic capacity had fully recovered from winter stress (KARPINSKI & al. 1993, 1994).

These data indicate that chloroplasts in evergreens play a major role in generation of ROIs during low-temperature-induced oxidative stress.

The total GR activity was also measured in Scots pine needles (WINGSLE & HÄLLGREN 1993), and a well-known pattern with higher activities in winter and lower in summer was observed (ESTERBAUER & GRILL 1978, ANDERSON & al. 1992, POLLE & RENNENBERG 1994). Different responses and regulatory mechanisms from that for SOD genes have been observed for chloroplastic GOR. In the same experiment (KARPINSKI & al. 1993) GR enzyme activity was induced but the transcript levels of chloroplastic GOR gene were not changed. Later it was demonstrated that GR activity in Scots pine needles can be up-regulated by redox intraconvertion of the enzyme without change in its mRNA and protein levels (WINGSLE & KARPINSKI 1996). Additionally, an estimation of mRNA molecule number for chloroplastic CuZn-SOD and chloroplastic GOR showed that transcript levels were at least 20-fold higher for CuZn-SOD than GOR. However, the protein levels for CuZn-SODs were approximately 4-fold higher than for GR. This result strongly suggests higher turnover rates for CuZn-SOD than GR during low-temperature-induced oxidative stress and indicates different regulation of expression of these genes.

The key enzyme involved in H_2O_2 scavenging is APX, which catalyses the reaction: 2 AsA + H_2O_2 → 2 monodehydroascorbate (mdAsA) + $2H_2O$. Chloroplasts photoregenerate AsA from mdAsA or dAsA. mdAsA is converted to AsA either by reduced ferredoxin or NAD(P)H with MDAR. DHAR is thought to regenerate AsA using GSH as an electron donor. GPX has generated much attention as an important enzyme in the scavenging of H_2O_2 or the products of lipid peroxidation. The role and function of the chloroplastic GPX during cold hardening and low-temperature-induced oxidative stress in trees is under investigation (MULLINEAUX & al. 1998).

Expression of genes encoding different isoforms of the same ROI-scavenging enzyme are regulated differently in response to low-temperature-induced oxidative stress (KARPINSKI & al. 1993). Conflicting results have been presented for seasonal changes in total APX activities in spruce (ANDERSON & al. 1992, POLLE & RENNENBERG 1994, POLLE & al. 1996). MDAR showed elevated levels in the needles during autumn and winter. During bud break, both APX and MDAR showed higher activity levels (POLLE & al. 1996). In Scots pine, activities of such enzymes as SOD, MDR, APX and DHAR increased during cold-acclimation (TAO & al. 1998). However, in many other experiments, total SOD activities have not shown any seasonal variation (KRÖNINGER & al. 1993, WINGSLE & HÄLLGREN 1993). Catalases have also received much attention in respect of plants response to chilling and are thought to play a major role in inducing chilling tolerance (PRASAD 1996). Fig. 1 summarizes changes of different non-enzymatic and enzymatic antioxidants during acclimation to low temperature and long nights in Scots pine. In general both non-enzymatic and enzymatic antioxidants increase in Scots pine due to the cold acclimation.

It can be concluded that regulation of expression of the ROI-scavenging enzymes can occur at different levels, e.g. regulation of enzyme activity, regulation

of amounts of isoforms, as well as and posttranslational processes, steady-state levels of enzymes and transcripts, and regulation of the transcription.

Signalling and Regulation

Agents involved in signalling include salicylic acid (SA); H_2O_2 (LEVINE & al. 1994, PRASAD & al. 1994a, PRASAD 1996); $O_2^{\cdot -}$ (TSANG & al. 1991); GSH and GSSG (HÉROUART & al. 1993, WINGSLE & KARPINSKI 1996, KARPINSKI & al. 1997); Calcium (Ca^{2+}; PRICE & al. 1994, KNIGHT & al. 1996); photoreceptors with Ca^{2+} (NEUHAUS & al. 1993); ABA (GIRAUDAT 1995) and recently the redox status of plastoquinone pool (KARPINSKI & al. 1997). However, very little is known about the signalling cascades initiated by these responses. ROIs are known to be involved in the regulation of such diverse processes as the hypersensitive response and systemic acquired resistance (DIXON & LAMB 1990, LEVINE & al. 1994); chilling responses (PRASAD & al. 1994b); cross tolerance to different abiotic stresses (BOWLER & al. 1992) and regulation of photosynthesis (HORMANN & al. 1993).

	Long Night	Low temperature
Glutathione	±	↑
Ascorbate	±	↑
MDAR	±	↑
DHAR	±	↑
GR	↑	±↑
APX	↑	±
SOD	±	±↑

Fig.1. Summary of changes of different non-enzymatic and enzymatic antioxidants during acclimation to long nights and low temperature in Scots pine (WINGSLE & HÄLLGREN 1993, KARPINSKI & al. 1992b, 1993, 1994, KIROSHEEVA & al. 1996, WINGSLE & MORITZ 1997, TAO & al. 1998).

Generally, Ca^{2+} is considered to function as a secondary messenger in plants' oxidative stress response (NEUHAUS & al. 1993, KNIGHT & al. 1996). It was demonstrated that Ca^{2+} can regulate enzymatic ROI-scavengers and the oxidative stress response (PRICE & al. 1994). ABA plays an important role in signalling of drought and low temperature stress. ZHU & SCANDALIOS 1994 demonstrated that different members of the Mn-SOD gene family in maize respond differently to ABA and high osmoticum. ABA has recently been shown to increase both GR and APX activities in *Arabidopsis* (O'KANE & al. 1996).

A regulatory role for H_2O_2 as a signalling molecule in different secondary messenger systems in humans and in animals is well documented (RAMASASARMA 1982, MEYER & al. 1993, GINNPEASE & WHISLER 1996). In plants the ability to control H_2O_2, $O_2^{\cdot -}$ and GSH levels is an important factor in biotic and abiotic stress responses. Recently, it was shown that CuZn-SOD4 and CuZn-SOD4A transcript levels in maize increase in response to H_2O_2 treatment (KERNODLE & SCANDALIOS 1996).

Relevant functions of GSH in the context of oxidative stress, are those where GSH participates in redox reactions and therefore oxidised glutathione (GSSG) is generated (FOYER & HALLIWELL 1976). In plants, high concentrations of GSH, but not GSSG, enhanced the expression of genes encoding enzymes involved in phytoalexin and lignin biosynthesis and suggested a general role for GSH in signalling systems in biological stress (WINGATE & al. 1988). Recently, we reported that changes in the glutathione levels and/or redox status of glutathione pool have a regulatory impact on the expression of genes encoding cytosolic and chloroplastic isoforms of CuZn-SOD in Scots pine (WINGSLE & KARPINSKI 1996) and cytosolic APX in *Arabidopsis* (KARPINSKI & al. 1997). Our results, that GSH reduced the cytosolic CuZn-SOD transcript level, are in agreement with findings for human CuZn-SOD and Mn-SOD genes which were found to be down-regulated by thiols (SUZUKI & al. 1993). It is suggested, that the levels of GSH and GSSG, or the redox state of the glutathione pool, play an important role in the in vivo regulation of the expression of genes encoding the enzymatic ROI-scavenging system in plants. We conclude that the mechanisms regulating the expression of SOD and GOR genes respond differently to altered levels of GSH and GSSG in Scots pine needles (WINGSLE & KARPINSKI 1996). The activity of GR increased per se (but not the GOR transcript level) in response to higher levels of GSSG, suggesting that the enzyme itself undergoes redox intraconversion in vivo. However, the transcript levels of cytosolic and chloroplastic CuZn-SOD were reduced by GSH.

Recently, we have demonstrated that exogenous GSH and GSSG can inhibit APX1 and APX2 gene expression in *Arabidopsis* during excess-light stress. Regulation of these genes in *Arabidopsis* is partly controlled by the redox status of the plastoquinone pool (KARPINSKI & al. 1997). To our knowledge there is no data indicating that the changes in the levels of AsA and dAsA and/or the redox status of the ascorbate pool have a regulatory impact on the expression of genes encoding the enzymatic ROI-scavenging system in plants.

The network of signalling pathways regulating expression of genes encoding the enzymatic ROI-scavenging system in plant cells is complex. One gene can be regulated by more than one signalling pathway. Interactions between different signalling pathways are not understood. Fig. 2 shows a schematic outline for the regulation of the ROI-scavenging system.

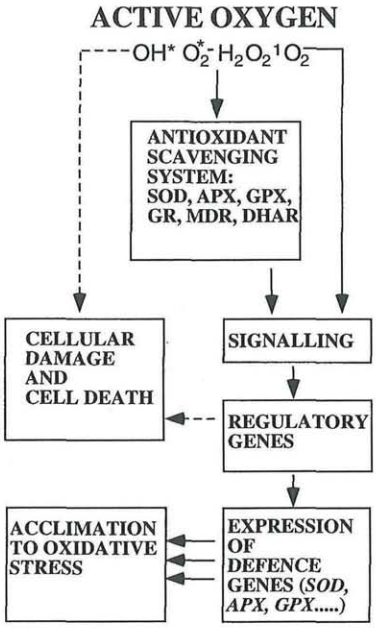

Fig. 2. A schematic outline for the regulation and acclimation to oxidative stress of the ROI-scavenging system in plants.

ROIs in Photosynthesis and Low Temperature

Photosynthesis is generating ROIs, but it can also be involved in the removal of and protection against ROIs. Several excellent reviews exist on photosynthesis, covering the role of oxygen in photoinhibition (KRAUSE 1994), oxygen metabolism (FOYER & HARBINSON 1994) and chilling stress (BAKER 1994, WISE 1995, HUNER & al. 1998).

Photooxidation of needles of conifers in hars environments is manifested as a light and O_2-dependent bleaching of photosynthetic pigments. Chlorophyll bleaching in conifers is much greater in sun-exposed than shaded habitats (KARPINSKI & al. 1994). In Scots pine, during the winter-time, the chlorophyll concentration is lower and the carotenoid levels remain equal, or even increase. At the end of the winter, when the quantum flux density is relatively high, the pigment levels are lowest (KARPINSKI & al. 1994). This coincides with a very low PSII efficiency and reorganisation of the photosynthetic apparatus allow rapid recovery of photosynthesis in the spring (LUNDMARK & al. 1988, ÖQUIST & al. 1992, KARPINSKI & al. 1993, 1994, OTTANDER & al. 1995).

The D1 protein, and the reaction centre in PSII, is generally described as the most sensitive part of the photosynthetic apparatus when plants are subjected to high light and low temperature stress (ARO & al. 1993, BARBER 1995, RUSSELL &

al. 1995, KARPINSKI & al. 1997). In the chloroplast, light reactions will continue while the energy consuming biochemical reactions are more limited in low temperature. Chloroplasts subjected to low temperature may reduce the generation of ROIs by dissipating energy through a number of mechanisms (WISE 1995). Increased energy dissipation can be achieved through decreasing the photochemical PSII activity; by increasing the photorespiratory activity; by the Mehler-peroxidase reaction (SCHREIBER & NEUBAUER 1990); and by an increased conversion of absorbed light into heat (HORTON & al. 1996). The thermal dissipation process occurs within the antenna and the [(violaxanthin (V) + antheraxantin (A) + zeaxanthin (Z); VAZ] cycle is suggested to play a major role (DEMMIG-ADAMS & ADAMS III 1994, HORTON & al. 1996). Between October and January, the VAZ cycle pigments in Scots pine changed their epoxidation state from 0.9 to 0.1 and the D1 proteins content decreases (OTTANDER & al. 1995)

Adaptation of photosynthesis to low temperature is expressed by at least two different strategies in overwintering plants. One is to maintain photosynthetic capacity throughout the winter by different adjustments in the photosynthetic apparatus, and the other is to photosynthesise during warm periods and down-regulate photosynthesis during winter. A correlation between photosynthetic capacity at low temperature and freezing tolerance in winter cereals results from photosynthesis providing energy for cellular metabolism (ÖQUIST & al. 1993).

Cold acclimation does not affect the susceptibility of photosynthesis to photoinhibition in Scots pine. However, there is a distinct increase in resistance to photoinhibition at the level of PSII reaction centres, limiting photoinhibition despite suppression of the capacity for photosynthesis (KRIVOSHEEVA & al. 1996). Clearly, under the similar excitation pressures of PSII as defined by q_p, needles of cold-acclimated Scots pine were much more resistant to photoinhibition than needles of non-hardened pine. Unlike winter varieties of rye and wheat, which respond to cold acclimation by increased capacities for photosynthesis, seedlings of Scots pine respond to cold acclimation by a 25% inhibition of photosynthesis over the studied range of absorbed photon flux density. This is accompanied by increased activities and levels of several enzymes and metabolites of the enzymatic ROI-scavenging system (KRIVOSHEEVA & al. 1996).

The oxygenase reaction leading to photorespiration and the donation of electrons to oxygen to form superoxide in a pseudocyclic electron flow seem to be the major oxygen-consuming reactions, (OSMOND & GRACE 1995). BIEHLER & FOCK 1996, concluded that the Mehler-peroxidase reaction increased in wheat during drought stress when the availability of CO_2 was limited. The reaction of AsA with H_2O_2 is efficient (ASADA 1994) and there is accumulating evidence that the Mehler-peroxidase reaction serves as an important sink for excess electrons (KRIVOSHEEVA & al. 1996), although the role is not well understood and the significance under debate. KRIVOSHEEVA and co-workers, hypothesize that the H_2O_2-scavenging system has two roles in protection of cold acclimated needles from photoinhibition: i) protection from ROIs formed upon excessive excitation in general ii) allows O_2 to function as an electron acceptor, thus opening a fraction of

photosystem II reaction centres and consuming electrons in excess of the requirements of CO_2-fixation.

The photorespiratory pathway is theoretically a possible protection mechanism, however, to our knowledge, no data can be found in the literature to support the hypothesis that increased photorespiration during low temperature stress would protect the photosynthetic machinery in plant leaves. The protective role of photorespiration cannot be explained simply in quantitative terms by energy dissipation (KRAUSE 1994). Heber and co-workers (WU & al. 1991) argued that the limited rate of coupled electron flow facilitated by photorespiration protects the photosynthetic machinery in two ways: i) by maintaining the primary electron acceptor, plastoquinone, in a partly oxidised state and ii) by building up a high proton gradient over the thylakoid membrane. This highly-energized state of the thylakoid membrane may be dependent on cyclic electron flow in the proximity of PSI (WU & al. 1991). Acidification of the thylakoid interior will lead to an increased dissipation of excitation energy via chlorophyll-fluorescence and this energy-dependent quenching mechanism is known to protect against photoinhibition (KRAUSE & WEIS 1991, HORTON & al. 1996).

The above data indicate that the ability of trees to adjust the defence systems against low-temperature-induced oxidative stress depends on a number of factors. The relative role of antioxidants should be considered in further studies on improvement of plants' oxidative stress tolerance.

Recently HUNER & al. 1998 reviewed the question of energy balance and acclimation to light and cold and concluded that changes in environmental conditions result in an imbalance between the light energy absorbed by PSII and the energy utlilized by metabolism. The energy imbalance is sensed by alterations in PSII exitation pressure, and thus the reduction state. This is suggested to give rice to a chloroplastic reduction signal and to initiate a signal transduction pathway. This signal reduction pathway apparently coordinate photosynthesis-related gene expression and influence nuclear expression of genes. Hence, HUNER & al. 1998 suggest that the photosynthetic apparatus might be an environmental sensor.

Finally we would like to add that our experiments showed that there exist a systemic acquired acclimation to excess light in Arabidopsis (KARPINSKI & al. 1998). In essence: a leaf treated with high (excess) light render other leaves on the same plant more resistant to subsequent high light treatment. The signal might well be associated with the redox state of plastoquine pool in the chloroplast (KARPINSKI & al. 1997) but the nature of the signal is not known. Further experiments in this area are underway.

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