BULG. J. PLANT PHYSIOL., SPECIAL ISSUE 2003, 187–206

PLANT RESPONSES TO DROUGHT AND STRESS TOLERANCE

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Summary. In the natural environment plants are well adapted to minimize damages which only occurs under extreme conditions. In the frame of "physiological window" mild drought induces in plants regulation of water loss and uptake allowing maintenance of their leaf relative water content within the limits where the photosynthetic capacity shows no or little changes. But severe drought induces in plants unfavourable changes leading to inhibition of photosynthesis and growth. The most severe drought stress is desiccation. On the basis of presence or absence of bulk water, the mechanisms of protection are different. While the mechanisms conferring drought tolerance are mainly based on structural stabilization by preferential hydration, desiccation tolerance mechanisms are based on the replacement of water by molecules that form hydrogen bonds. The roles of stomatal and non-stomatal limitation, the behaviour of PS2, specific proteins and Rubisco, lipids and sugars, as well as mechanisms of acclimation and stress tolerance in droughted plants are discussed.

Abbreviations: (Ψ_{PS2} – quantum yield of photosynthetic electron transport of PS2; Ψ_w – leaf water potential; ABA – abscisic acid; Ax – antheraxanthin; Chl – chlorophyll; GLs – glycolipids; PL – phospholipids: DGDG – digalactosyldiacylglycerol; PC – phosphatidyl choline: F_0, F_v, F_m – initial, variable amd maximal Chl fluorescence; WS – water stress; WD – water deficit: MGDG – monogalactosyldiacylglycerol: PSA – photosynthetic apparatus; PS2 – photosystem 2; RCs – reaction centers; Rubisco – ribulose-1.5-bisphosphate carboxylase/oxygenase; RuBP -ribulose-l,5-bisphosphate; RWC – relative water content: Vx – violaxanthin; Ax – anteratxanthin; WUE – water use efficiency; Zx – zeaxanthin.; SOD – superoxide dismutase; POX

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– peroxidase; CAT – catalase; HSPs – heat shock proteins; g_s – stomatal conductance.

Introduction

At the whole plant level the effect of stress is usually perceived as a decrease in photosynthesis and growth, and is associated with alteration in carbon and nitrogen metabolism (Cornic and Massacci, 1996; Mwanamwenge et al., 1999). The plant response is complex because it reflects over space and time the integration of stress effects and responses at all underlying levels of organization (Blum, 1996). Under field conditions these responses can be synergistically or antagonistically modified by the superimposition of other stresses. Water deficit can affect plants in different ways. In the frame of "physiological window" mild drought induces in plants regulation of water loss and uptake allowing maintenance of their leaf relative water content (RWC) within the limits where photosynthetic capacity and quantum yield show little or no change. The most severe form of WD is desiccation - when most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell. According to Larcher (1987) stress contains both destructive and constructive elements and is a selection factor as well as a driving force improving resistance and adaptive evolution. Repair processes lead also to hardening of plants by establishing a new physiological standard, which is an optimum stage of physiology under the changed environmental conditions.

Depending on the differences in behavior of the photosynthetic apparatus (PSA) during desiccation two groups of desiccation tolerant (DT) plants were distinguished – homochlorophyllous desiccation tolerant (HDT) and poikilochlorophyllous desiccation tolerant (PDT) (Bewley, 1979; Gaff, 1989). The most essential difference between HDT and PDT plants during desiccation seems to be that the PSA of the HDT plants is retained in a recoverable form, while in PDT plants the chlorophylls and thylakoid systems are degraded and need to be fully reconstituted and revived (Tuba et al., 1996).

Water stress results in stomatal closure and reduced transpiration rates, a decrease in the water potential of plant tissues, decrease in photosynthesis and growth inhibition, accumulation of abscisic acid (ABA), proline, mannitol, sorbitol, formation of radical scavenging compounds (ascorbate, glutathione, α-tocopherol etc.), and synthesis of new proteins and mRJNAs. Besides these physiological responses plants also undergo morphological changes. One of the largest is the adaptation of plants and chloroplasts to high light (sun) and low light (shade exposure). This sun-type or shade-type chloroplast adaptation is also induced by many other stress factors including drought (Lichtenthaler et al., 1981).

Stomatal and non-stomatal limitation on photosynthesis of droughted plants

The rate of CO_2 assimilation in the leaves is depressed at moderate leaf water deficits or even before leaf water status is changed in response to a drop in air humidity (Bunce, 1981) or in soil water potential (Gollan et al., 1986).

The relative part of stomatal limitation of photosynthesis depends on the severity of water deficit. Under mild stress it is a primary event, which is then followed by adequate changes of photosynthetic reactions (Cornic and Briantais, 1991). Stomatal control of water loss has been identified as an early event in plant response to WD under field conditions leading to limitation of carbon uptake by the leaves (Chaves, 1991; Cornic and Massacci, 1996). Stomata close in response either to a decline in leaf turgor and/or water potential, or to a low-humidity atmosphere (Maroco et al., 1997). As a rule, stomatal responses are more closely linked to soil moisture content than to leaf water status. This suggests that stomata are responding to chemical signals (e.g. ABA) produced by dehydrating roots (Davies and Zang, 1991). A clear time dependency in stomatal responsiveness to air humidity and water status was also found (Franks et al., 1997), suggesting that some of diurnal changes in stomatal function may result from metabolic processes with a circadian rhythm (Chaves et al., 2002). Changes in cell carbon metabolism are also likely to occur early in the dehydration process as shown by Lawlor (2002). The drought-tolerant species control stomatal function to allow some carbon fixation at stress, thus improving water use efficiency, or open stomata rapidly when water deficit is relieved.

Although stomatal closure generally occurs when plants are exposed to drought, in some cases (severe stress) photosynthesis may be more controlled by the chloroplast's capacity to fix CO_2 than by the increased diffusive resistance (Faver et al., 1996, Herppich and Peckmann, 1997). Non-stomatal responses of carbon fixation such as PS2 energy conversion and the dark reaction of Rubisco carbon fixation are resistant to WD (Chaves, 1991; Dickson and Tomlinson, 1996). In addition, stomatal closure occurs before inhibition of photosynthesis and restricts CO_2 availability at the assimilation site in chloroplasts.

When WS is imposed slowly as is generally the case under field conditions a reduction in the biochemical capacity for carbon (C) assimilation and utilization may occur along with restriction in gaseous diffusion. For example, in grapevines grown in the field, CO_2 assimilation was limited to a great extent due to stomatal closure as summer drought progress, but there was also proportional reduction in the activity of various enzymes of the reductive Calvin cycle (Fig. 1, Maroco et al., 2002; Chaves et al., 2002). The tight correlation between mesophyll photosynthesis and stomatal aperture may reflect a down-regulation of photosynthetic apparatus by low C availability (Tourneu and Peltier, 1995). According to Ort et al. (1994) the response of photo-

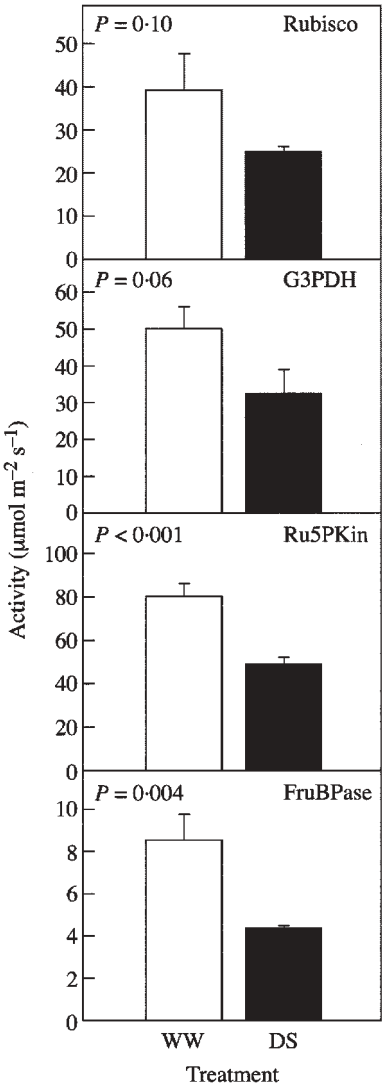

Fig. 1. *In vitro* activity of key enzymes of C metabolism: Rubisco, G3PDH, Ru5Pkin and FruBPase in well watered (open bars) and drought-stressed grapevine (closed bars) in the middle of the summer in Evora, Portugal (From Maroco et al., 2002).

synthesis to internal cell CO_2 (C_i) indicates that the biochemical demand for CO_2 was down-regulated in response to declining CO_2 availability.

Lawlor and Cornic (2002) and Lawlor (2002) discussed photosynthetic carbon assimilation and associated metabolism in relation to RWC in higher plants and distinguished two general types of relation of photosynthetic potential (A_{pot}) to RWC. In Type 1 response A_{pot}, measured under saturated CO_2 is unaffected by a small loss of RWC (from 100 to 75%) but becomes progressively more inhibited and less stimulated by elevated CO_2. Decreased stomatal conductance (g_s) results in smaller photosynthesis (A) and lower CO_2 concentration inside the leaf and in the chloroplast, the latter falling possibly to the compensation point. In Type 2 A_{pot} and stimulation of A by elevated CO_2 decreases progressively as RWC falls. As RWC declines, the relative limitation of A by g_s decreases and metabolic limitation increases in both types. The authors suggest that decreased A_{pot} under low RWC is caused by impaired metabolism (shortage of ATP) limiting RuBP synthesis without inhibition or loss of photosynthetic carbon reduction cycle enzymes including Rubisco. Decreased ATP content and imbalance with reductant status affect cell metabolism substantially. It was reported (Yordanov et al., 1997a,b, 1998, 1999; Todorov et al., 1998) that application of some cytokinins (kinetin, 4-PU-30, some phenyl amines) alleviated the plant damage provoked by WS. Cytokinins induce the formation of sun type chloroplasts which possess not only different morphology and chemical composition, but are more tolerant against water and temperature stresses (Lichtenthaler, 1981).

Drought stress and PS2 activity

Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sink for PS2 activity during mild drought (Cornic and Fresneau, 2002). It was shown that PS2 functioning and its regulation were not quantitatively changed during desiccation. The CO_2 molar fraction in the chloroplasts declines as stomata close in drying leaves. As a consequence, in C_3 plants RuBP oxygenation increases and becomes the main sink for photosynthetic electrons. Depending on the prevailing photon flux density (PFD), the O_2 through photorespiratory activity can entirely replace CO_2 as an electron acceptor or not.

Havaux (1992) has investigated the impact of various environmental stresses (drought, heat, strong light) applied separately or in combination on the PS2 activity. The existence of a marked antagonism between physicochemical stresses (e.g. between water deficit and HT) was established, with a water deficit enhancing the resistance of PS2 to constraints as heat, strong light (Fig. 2 and 2A). Similar results were obtained on bean plants (Yordanov et al., 1999). The data of Flagella et al. (1998) show that quantum yield of PS2, as related to Calvin cycle metabolism, is reduced only under drastic water deficit.

Long-term drought reduction in water content led to considerable depletion of pea PS2 core. The remaining PS2 complex appeared to be functional and reorganized

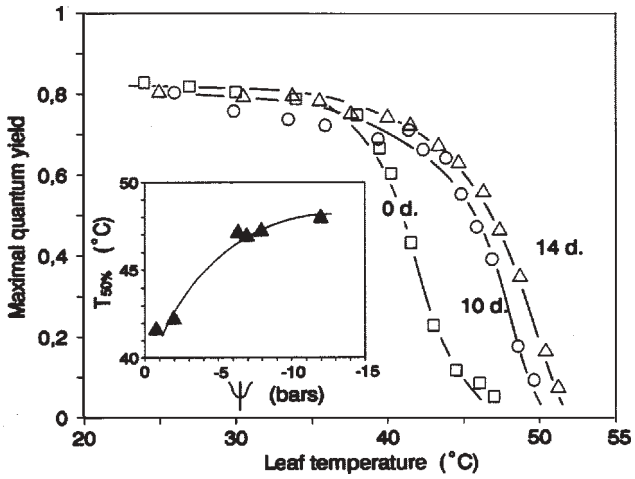

Fig. 2. Temperature dependence of the (ϕ_P^{open} of PSII in potato leaves exposed to slow water stress induced by withholding irrigation to the plants for 0, 10, and 14 d (Ψ = –2, –10, and –12 bars, respectively). Inset, Plot of the temperature ($T_{50\%}$) corresponding to 50% inhibition of ϕ_P^{open} versus leaf Ψ during slow water stress. Heat treatments and fluorescence measurements were done as in Fig. 3 and 4. (according to Havaux et al., 1992)

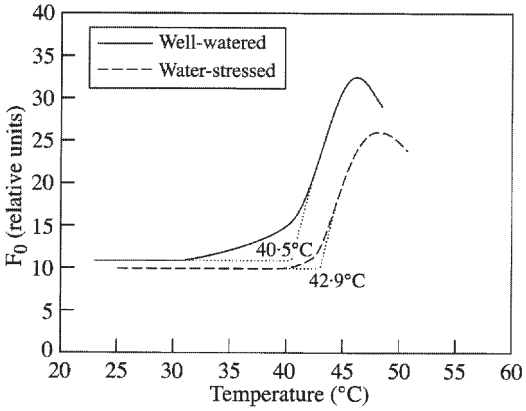

Fig. 2A. Response of basal chlorophyll a fluorescence to leaf temperature in well watered and water-stressed *Lupmiis albus* L. (From Chaves et al., 2002).

with a unit size (LHCP/PS2 core) twofold greater than that of well irrigated plants, and enhanced degradation of CP43 and Dl proteins (Girardi et al., 1996). In addition, PS2 complexes are able to change their location and structure as in PS2-β centers and state-transitions.WS increased PS2-β inactive centers in drought-sensitive more than in drought-tolerant bean cvs. (Yordanov at al., 1997b, Gonzales et al., 2001).

The decline in PS2 efficiency is regulatory, serving a photoprotective role. Increased levels of energy dissipation which decrease Ψ_{PS2} may help to protect PS2 from over-excitation and photodamage (Schindler and Lichtenthaler, 1996).

Haupt-Herting et al. (2002) studied the metabolic consumption of photosynthetic electrons and dissipation of excess light energy in tomato plants under WS. They established that O_2 evolution, O_2 uptake, net CO_2 uptake and CO_2 evolution declined. It was concluded that PS2, the Calvin cycle and mitochondrial respiration are down-regulated under WS. The same authors calculated the percentages of photosynthetic electrons dissipated by CO_2 assimilation, photorespiration and the Mehler reaction (Fig. 3): in control leaves more than 50% of the electrons were consumed in CO_2 assimilation, 23% in photorespiration and 13% in Mehler reaction. Under severe stress the % of electrons dissipated by CO_2 assimilation and the Mehler reaction declined while the % of electrons used in photorespiration doubled. The consumption of electrons in photorespiration may reduce the likelihood of damage during WS. Noctor et al. (2002) provided quantitative estimation of the relative contributions of the chloroplast electron transport chain and the glycolate oxidase load placed on the photosynthetic leaf cell. Assuming a 10% allocation of photosynthetic electron flow to the Mehler reaction, photorespiratory H_2O_2 production would account for about 70% of total H_2O_2 formed. When chloroplastic CO_2 concentration rates are decreased photorespiration becomes even more predominant in H_2O_2 generation. At the increased flux through photorespiration observed at lower ambient CO_2 the Mehler reaction would have to account more than 35% of the total photosynthetic electron flow in order to match the rate of peroxisomal H_2O_2 production. According to the authors, the interac-

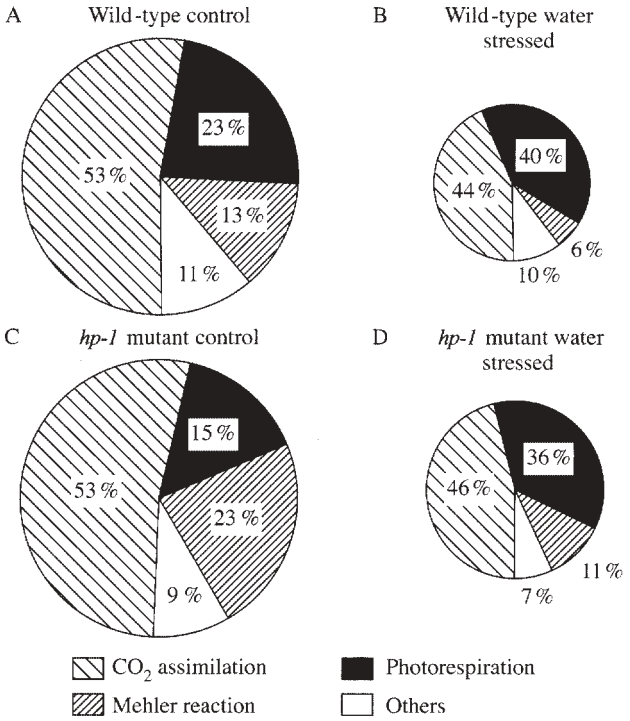

Fig. 3. Photosynthetic electrons (%) consumed/dissipated by CO_2 assimilation, photorespiration, the Mehler reaction and other reactions at 850 µmol photons $m^{-2}.s^{-1}$ in controls (–0.6 M Pa) and water stressed (–1.8M Pa) wild type tomato (A, B) and the hp-1 mutant (C,D). The area of the circles represents 100% of photosynthetic electrons formed; there are 97 (A) and 32 (B) µmol e^{-} $m^{-2}.s^{-1}$ in wild-type leaves, and 112 (C) and 45 (D) µmol e^{-} $m^{-2}.s^{-1}$ in leaves of the hp-1 mutant (According to Haupt-Herting, 2002)

tion between oxidants, antioxidants and redox changes in draughted plants can modify gene expression and photorespiratory H_2O_2 can play role in signaling and acclimation.

Rubisco, specific proteins and drought stress

The mechanism by which Rubisco may be downregulated in the light due to tight-binding inhibitors could be pivotal for tolerance and recovery from stress and may be central to integrating the midday depression of photosynthesis (Parry et al., 2002). Additionally, enhanced rates of oxygenase activity and photorespiration maintain the ET rate in response to drought and are quantitatively much more important than the Mehler reaction (Haupt-Herting and Fock, 2002; Noctor et al., 2002). Kanechi et al.

(1995) found a close relationship between Rubisco content and maximal O_2 evolution rate measured at high photosynthetic photon flux density (PPFD) during leaf dehydration. It was established that below –2.0 MPa inhibition of photosynthesis in two maize cvs is in part attributed to stomatal conductance but mostly to the decreased activities of carbonic anhydrase, phosphoenol pyruvate carboxylase and Rubisco (Prakash and Rao, 1996). As mentioned above, the primary site of limitation of maximal O_2 evolution rate, measured at high PPFD, seemed related to significantly reduced RuBP content, not to the amount of Chl or Rubisco. But as mentioned above, Rubisco is not a prime target of water deficit and is not limiting net CO_2 assimilation of leaves submitted to desiccation (Holaday et al., 1992). Decreased supply of CO_2 to Rubisco under both mild and severe water deficit is primarily responsible for the decrease in CO_2 fixation (Lal et al., 1996).

Specific proteins display particular structural features such as the highly conserved domain predicted to be involved in hydrophobic interaction leading to macromolecular stabilization (Close, 1996). The majority of new proteins belong to dehydrin-like proteins, which are abundantly induced during embryo maturation of many higher plants as well as in water stressed seedlings (Pelah et al., 1997). Dehydrins are synthesized by the cell in response to any environmental influence that has a dehydration component, such as drought, salinity or extracellular freezing (Ingram and Bartels, 1996). Dehydrins may stabilize macromolecules through detergent and chaperone like properties and may act synergistically with compatible solutes (Close, 1996; Hoekstra et al., 2002). The steady state levels of major PS2 proteins, including the Dl and D2 proteins in the PS2 reaction center, declined with increasing water deficit possibly as a result of increased degradation. The effects of WD on PS2 protein metabolism, especially on the reaction center proteins may account for the damage to PS2 photochemistry (He et al., 1995).

Drought stress and lipids

Along with proteins, lipids are the most abundant component of membranes and they play a role in the resistance of plant cells to environmental stresses (Kuiper, 1980; Suss and Yordanov, 1986). Strong water deficit leads to a disturbance of the association between membrane lipids and proteins as well as to a decrease in the enzyme activity and transport capacity of the bilayer (Caldwell and Whitman, 1987). Poulson et al. (2002) established that for *Arabidopsis*, polyunsaturated trienoic fatty acids may be an important determinant of responses of photosynthesis and stomatal conductance to environmental stresses such as vapour pressure deficit. When *Vigna unguiculata* plants were submitted to drought the enzymatic degradation of galacto- and phospholipids increased. The stimulation of lipolytic activities was greater in the drought-sensitive than in drought-tolerant cvs. (Sahsah et al., 1998).

Drought stress provoked considerable changes in lipid metabolism in rape *(Brasica napris)* plants (Benhassaine-Kesri, 2002). The decline in leaf polar lipid was mainly due to a decrease in MGDG content. Determination of molecular species in phosphatidylcholine (PC) and MGDG indicated that the procaryotic molecular species of MGDG (C18/C16) decreased after DS while eukaryotic molecular species (C18/C18) remain stable. It was suggested that the prokaryotic pathway leading to MGDG synthesis was strongly affected by DS while the eukaryotic pathway was not. Strong WD results in a profound overall drop in MGDG, the major leaf glycolipid. In drought sensitive seedlings of *Lotus corniculatus* the ratio of MGDG/DGDG declined 3-fold, while the relative part of MGDG was 12-fold lower.

The lipid composition of desiccated *Ramonda* leaves is profoundly modified: the ratio of phospholipids (PLs) to galactolipids (GLs) increased and the relative proportion of MGDG to DGDG drastically decreased. An increase in the PLs relative to GLs in leaves indicate a preferential degradation of the chloroplast membranes (Oquist, 1982).

Oxidative stress and antioxidant defense systems

It was established a link between tolerance to oxidative stress induced by WD and rise in antioxidant concentration in photosynthetic plants (Winston, 1990; Prince and Hendry, 1991). This shows that plants are well endowed with antioxidant molecules and scavenging systems (Larson, 1988). Enzymatic free radical processing systems include SOD, catalysing the dismutation of superoxide (O_2^-) into H_2O_2 and O_2 and those involved in the detoxification of H_2O_2 – catalase, peroxidase, glutathione reductase (GR-ase). In optimal conditions leaves are rich in antioxidant enzymes and metabolites and can cope with activated O_2, thus minimizing oxidative damage. Antioxidant metabolites as ascorbate and glutathione are present in chloropiasts in very high concentrations (Iturbe-Ormaetxe et al., 1998) and apart from their obvious role as enzyme substrates, they can react chemically with almost all forms of activated O_2 (Halliwell and Gutteringe, 1989). The hydrophilic antioxidants ascorbate and glutathione are effective chemical scavengers of oxygen radicals. Enzymatic detoxification systems either quench toxic compounds or regenerate antioxidants with the help of reducing power provided by photosynthesis (Polle and Rennenberg, 1994). Foyer et al. (1997) showed that overexpression of GRase in chloropiasts doubled the concentrations of ascorbate and glutathione (GSH) in leaves and conferred increased resistance to oxidative stress. According to their results drought caused a decrease in the content of reduced glutathione and an increase in that of vitamin E. Carotenoids and vitamin E are the main lipid soluble antioxidants of plant cells. In stressed leaves vitamin E increased significantly.

The photoproduction of monodihydroascorbate (MDA) radical was greatly enhanced by intense light, WS, and leads to suppression of the photosynthetic reactions. Increased MDA levels indicate either increased oxidation of ascorbate or decreased efficiency of ascorbate regeneration, or a combination of both (Heber et al., 1996).

Mechanisms of acclimation to water deficit and stress tolerance *g*

Plants have developed multiple mechanisms in order to protect PSA against different kinds of stresses. At the cellular level, plants attempt to alleviate the damaging effects of stress by altering their metabolism to cope with the stress. Many plant systems can survive dehydration, but to a different extent. According to Hoekstra et al. (2001) on the basis of the critical water level, two type of tolerance are distinguished: Drought tolerance can be considered as the tolerance of moderate dehydration, down to moisture content, below which there is no bulk cytoplasmmic water present – about 0.3 g H_2O g^{-1} DW. Desiccation tolerance refers to the tolerance of further dehydration, when the hydration shell of the molecules is gradually lost. Desiccation tolerance includes also the ability of cells to rehydrate successfully.

Major alterations in patterns of gene expression are known to occur at the early stages of stresses. Some of these changes are thought to provide a long-term protection against stress damage. According to Bohnert and Shen (1999) a nearly universal reaction under stress conditions, including WD, is the accumulation of "compatible solutes", many of which are osmolytes (i.e., metabolites whose high cellular concentration increases the osmotic potential significantly) considered to lead to osmotic adjustment. These observations indicate that "compatible solutes" may have other functions as well, namely in the protection of enzyme and membrane structure and in scavenging of radical oxygen species.

One of the principal mechanisms employed by plants to prevent or to alleviate damage to the PSA is non-photochemical chlorophyll fluorescence quenching (qN) (Ruban and Horton, 1995). In this mechanism excess light energy is dissipated as heat in the light harvesting antenna of PS2. This dissipation is primarily controlled by the trans-thylakoid pH gradient (pH) (Gounaris et al, 1984).

Desiccation induces a Zx- + Ax-mediated photoprotective mechanism in desiccation intolerant *Frullania dilatata* (Deltoro et al., 1998). They propose that when CO_2 fixation and therefore ATP consumption are decreased at low RWC, the functioning electron flow gives rise to an acidification of the thylakoid lumen that induces Zx and Ax synthesis. It has been proposed that the photoprotective process results in the diversion of energy away from the reaction centers (Ruban and Horton, 1995; Medrano et al., 2002). There are, however, experimental data which do not support the statement that the xanthophyll cycle plays a major or specific role in the direct energy dissipation of absorbed light energy (Schindler and Lichtenthaler, 1994). According to Tambussi

et al. (2002) the non-photochemical fluorescence quenching (qN), as well as the content of zeaxanthin and anteraxanthin after moderate WS increased significantly. However, at severe WS a further rise in these xanthophylls was not associated with any increase in qN. In addition, the β-carotene content rose significantly during severe WD, suggesting an increase in antioxidant defense. One tentative scheme of photosynthetic control under drought is proposed by Medrano et al. (2002, Fig. 4).

At the leaf level, the dissipation of the excitation energy through processes other than photosynthetic carbon metabolism is an important defense mechanism under conditions of WS and is accompanied by down-regulation of photochemistry in the long-term carbon metabolism (Chaves et al., 2002).

Besides the above mentioned mechanisms of energy dissipation, there are also other ways. For example, the energy dissipation in closed stomata can occur via ATP and NADPH, which are used for other metabolic processes, and they are obviously important mechanisms of tolerance and protection against water stress and photooxidative damage (Lichtenthaler, 1996).

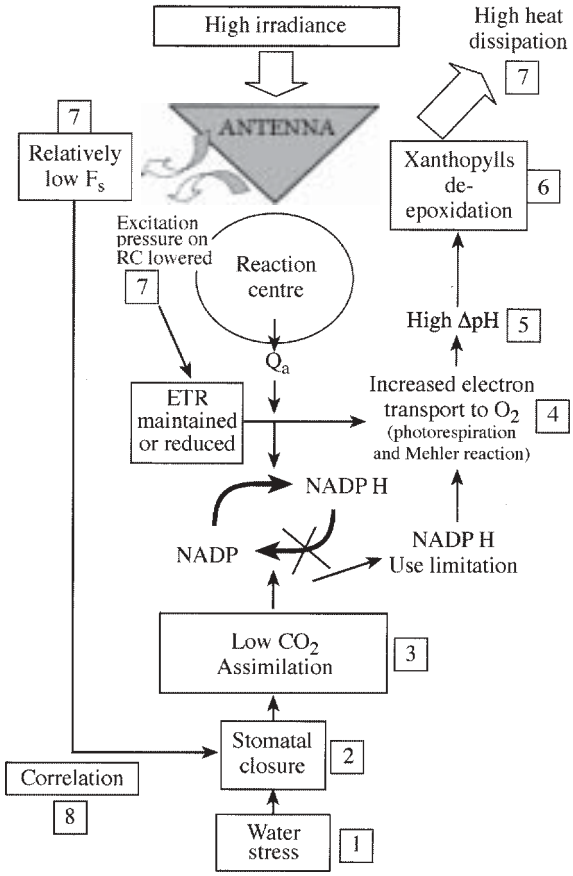

Fig. 4. Tentative scheme of photosynthetic control under drought. Under drought, stomatal closure in proportion to the degree of the stress progressively limitating ΔpH in the chloroplasts. CO_2 assimilation is reduced and CO_2:O_2 ratio drops thereby increasing photorespiration and/or Mehler reaction. Since these processes consume relatively less ATP than does photosynthesis, they should lead to a certain increase of trans-thylakoid ΔpH. Impared ATP-ase and/or ETR may also interfere with the build up of of trans-thylakoid ΔpH. The xanthophyll deepoxidation that follows increased ΔpH should lead to increased NPQ. Thermal dissipation in the antenna becomes progressively more important and Fs is consecutively lowered. The relationship between Fs and g-s provides a method for remote sensing stress. (from Medrano et al., 2002).

The physiological basis of desiccation tolerance in resurrection plants is complex. Some mechanisms may vary between different species (Bartels and Salamini, 2001). As mentioned above, some species retain plastids during dehydration, whereas other not. During a slow desiccation of photosynthetically fully active leaves of the PDT monocotyledon *Xerophyta scabrida* the CO_2 assimilation, thylakoid activity and respiration declined and chlorophylls and carotenoids are successively broken down (Tuba et al., 1996). In contrast, in HDT plants the decline and cessation of net CO_2 assimilation is due to a slow desiccation and results not from a degradation but inactivation of the thylakoid system, which is preserved during desiccation in the non functional but easily recoverable form (Bewley, 1979; Schwab et al., 1989). In these plants stromal enzymes are apparently only inactivated (but not degraded) since they are able to fix CO_2 even at extremely low osmotic potential (Nash et al., 1990).

On the basis of presence or absence of bulk water, the mechanisms of protection are different. While the mechanisms conferring drought tolerance are mainly based on structural stabilization by preferential hydration, desiccation tolerance mechanisms are based on the replacement of water by molecules that form hydrogen bonds. During dehydration, anhydrobiotes pass through hydration ranges that also necessitate protection against drought. The desiccation tolerance program can be switched on by dehydration and the plant hormone ABA (Ingram and Bartels, 1996). Upon water loss the cellular volume decreases and cell content becomes increasingly viscous and the chance for molecular interactions rises. The danger of protein denaturating and membrane fusion increases. But a range of compatible solutes which do not interfere with cellular structure and function hinder this process. It is considered that at lower water contents molecular oxidants (glutathione, ascorbate, tocopherol) play a preponderant role in elevating oxidative stress. Hoekstra et al. (1997) showed that desiccation may increase the transfer of these amphiphiles from the polar cytoplasm into the lipid phase of membranes. They thought that this partitioning into membrane might be extremely effective in automatically inserting amphiphilic antioxidant into membranes upon dehydration. Reduction of metabolism coincides with survival of desiccation (Leprince et al., 1999). In vegetative tissues genes encoding enzymatic antioxidants such as APX, SOD, GR-ase) are upregulated during drying or rehydration. When the bulk water is removed (below 0.3 g H_2O g^{-1} DW) the mechanism keeping the macromolecules preferentially hydrated through amphiphiles fail to work, because there is no water left for preferential hydrations (Crowe et al., 1990). It has been established that during desiccation, soluble sugars interact with the polar head groups and replace the water molecules. Phospholipid molecules largely retain the original spacing between one other. When water dissipates from the water shell of macromolecules at moisture contents lower 0.3 g H_2O g^{-1} DW the hydrophobic effect responsible for structure and function is lost.

After bulk water is lost the hydrogen bonding and glass formation are the mechanisms by which membranes and proteins are structurally and functionally preserved.

Sugars are special in that they allow the removal of the closely associated water from protein without this leading to conformational changes and loss of enzymatic function. According to the water replacement hypothesis, sugars act as a water substitute by satisfying the hydrogen-bonding requirement of polar groups of the dried protein surface (Carpenter and Gowe, 1983; Wolkers et al., 1998). At around 0.1 g H_2O g^{-1} DW the cytoplasm vitrifies and exists in a so called glassy state - an amorphous metastable state, retaining the disorder and physical properties of the liquid state (Franks et al, 1991). This state decreases the probability of chemical reactions and is indispensable for surviving the dry state. A very important role in this process is played by late embryogenesis abundant proteins (LEAPs), especially their Group 1 – dehydrins, in stabilization and protecting during desiccation. It was observed that their accumulation coincides with the acquisition of desiccation tolerance (Bartels et al., 1988). Group 1 proteins have very high potential for hydration - several times greater than that for "normal" cellular proteins (McCubbin et al., 1985). Because of these special features LEAPs potentially bind to intracellular macromolecules coating them with a cohesive water layer and preventing their coagulation during desiccation (Close, 1996). Upon removal of their own hydration shell these proteins would still be capable of playing a role in stabilizing macromolecular structures. They could provide a layer of their own hydroxylated residues to interact with surface groups of other proteins, acting as "replacement water" (Cuming, 1999; Buitink et al., 2002). Wolkers et al. (1999) suggested that LEAPs embedded in the glassy matrix might confer stability on slowly dried carrot somatic embryos.

Another class of proteins associated with desiccation tolerance are low molecular weight HSPs. Coordinated expression of LEAPs and sHSPs transcripts were observed during embryo development in response to ABA, indicating the existence of common regulatory elements of both LEAPs, sHSPs and desiccation tolerance (Wehmeyer et al., 1996). But so far, there is no direct experimental evidence for a specific role of sHSPs in desiccation tolerance.

Satoh et al., (2002) followed recovery of the photosynthetic system during rewatering in a terrestrial, highly drought-tolerant cyanobacterium *Nostoc comunne.* With absorption of water, the weight of the Nostoc colony increased in three phases with half-increase times of about 1 min, 2 h and 9 h. Fluorescence intensities of phycobiliproteins and PS1 complexes recovered almost completely within 1 min, suggesting that their functional forms were restored very quickly. Energy transfer from allophycocyanin to the core-membrane linker peptide, L-CM, recovered within 1min, but not that from L-CM to PS2. PS1 activity and cyclic ET flow around PS1 recovered within 2 min, while the PS2 activity recovered in two phases after a time lag of 5 min, with half times of about 20 min and 2 h. Photosynthetic CO_2 fixation was restored almost in parallel with the first recovery phase of PS2 reaction center activity. It is interesting that only 10% from water needed for full hydration of Nostoc colony was enough for recovery and maintenance of the PS2 activity.

In conclusion, we would like to repeat the call of Ingram and Bartels (1996) to search for valuable approaches in order to identify those metabolic steps that are most sensitive to drought, and to elucidate which metabolites and gene products are of primary importance for increasing drought tolerance of plants.

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