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Desiccation and the Subsequent Recovery of Cryptogamics that are Resistant to Drought

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Summary

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In the plant kingdom, ability to survive desiccation is restricted to a number of poikilohydric plants and, within fairly tight limits, to dormant seeds and spores. The majority of higher plants, in contrast, can not survive desiccation.

Recently, at least three groups of adaptation mechanisms have been outlined for seeds and seedlings that are able to survive cellular desiccation. One of these mechanisms is the capability to scavenge desiccation-induced free radicals and this is afforded by antioxidative and/or enzymic pathways. Indeed, desiccation-induced free radicals were found in numerous tissues, and some authors correlated desiccation tolerance with maintenance or synthesis of antioxidants as glutathione (γ-glutamyl-cysteinyl-glycine, GSH), ascorbic acid (AA) or tocopherols and/or enzymes scavenging cytotoxic oxygen species as superoxide dismutase, catalase or peroxidases.

In this paper, we review the role of some antioxidants and enzymes related to protection from oxidative stress in desiccation tolerant and desiccation sensitive cryptogamics. In detail, we discuss the role of glutathione, that plays an important part for overcoming desiccation. This is demonstrated by studies on three lichen species differing in their tolerance to desiccation. Here, the formation of oxidised glutathione (GSSG) during desiccation and its reduction upon rehydration is interpreted as an adaptation mechanism to drought. Indeed, these results fit well to a thiol-disulphide cycle postulated recently for plants overcoming desiccation. This hypothesis depends upon the idea that protein thiol groups are protected from irreversible auto-oxidation by the reaction of GSSG with protein thiol groups (PSH), thus forming protein-bound glutathione (PSSG) and GSH during desiccation. With further desiccation, the oxidation of GSH continues leading to high final concentrations of PSSG and GSSG. By these oxidation steps, PSH and GSH are protected from desiccation-induced oxidative injury such as irreversible formation of intramolecular cross links in proteins or uncontrolled oxidation of SH groups, e.g. to sulfonic acids. Moreover, these studies on

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lichens indicate that tolerance to desiccation can be correlated with the mobilisation of two enzymes, glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) needed for the reduction of the GSSG formed under desiccating conditions. The latter is the key enzyme of the oxidative pentose shunt that provides the NADPH required for the action of GR. This pathway has special importance for resurrection plants that are not able to provide NADPH from photosynthesis in the first stages of recovery following desiccation.

Abbreviations

AA, ascorbic acid; ACP, acyl carrier protein; ACP-S-S-G, ACP-conjugated glutathione; AP, ascorbate peroxidase; DH, dehydration; G6PDH, glucose-6-phosphate dehydrogenase; GP, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidised glutathione; GST, glutathione-S-transferase; PSH, protein thiol groups; PSSG, protein-bound glutathione; RH, rehydration; SOD, superoxide dismutase.

Desiccation tolerance requires a complex interplay of several adaptation mechanisms

As water leaves a cell that does not tolerate desiccation, the cell undergoes detrimental processes. In general, oxidative stress is reported to increase during drought stress in plants, as in response to a wide range of environmental stresses, such as high light irradiance, soil and air pollution, or nutritional disorder (SMIRNOFF 1993, ELSTNER & OSSWALD 1994). Cellular desiccation, however, additionally causes alterations in ionic strength and pH, crystallisation of solutes and denaturation of proteins. One of the harmful effects associated with desiccation is the formation of disulphide bonds in proteins. These conformational changes in protein structure are probably primary sites of desiccation injury, in particular irreversible formation of intramolecular disulphides (LEVITT 1980). Additionally, water deficit leads to increased lipid peroxidation. As a consequence of these deleterious effects such as lipid peroxidation and denaturation of proteins, membranes become disrupted leading to solute leakage and loss of compartmentation. Moreover, severe water removal may cause cellular collapse.

In the plant kingdom, proficiency to survive desiccation is restricted to a number of poikilohydric plants and, within fairly tight limits, to dormant seeds and spores, respectively. Poikilohydry is a feature of some ferns but of only few phanerogams, however, it is widespread among algae, cyanobacteria, fungi, and particularly among lichens and bryophytes, many of them being desiccation tolerant. The majority of higher plants, in contrast, can not survive desiccation, with the exception of the seeds of many species.

During seed maturation, several strategies for coping with the deleterious effects of water removal have been reviewed recently (LEPRINCE & al. 1993). These include, the presence of high amounts of non-reducing sugars, the expression of desiccation- and/or abscisic-acid-regulated genes, and the activity of free radical-scavenging systems. Non-reducing sugars may substitute for water by

forming hydrogen bonds thus maintaining hydrophilic structures in their hydrated orientation (CROWE & al. 1988). In this way, they probably help to stabilise proteins and membranes in dry conditions. Moreover, non-reducing sugars are reported to promote the formation of a "glass-phase" in the cytoplasm, a process that is known as vitrification. In the glassy state, the cytoplasm is characterised as a liquid solution with the viscous properties of a solid. The benefits deriving from vitrification for the quiescent seed are numerous; the high viscosity of the cytoplasm causes chemical reactions to slow down. Consequently, degradative processes such as alterations in ionic strength and pH, and the crystallisation of solutes are prevented. Besides, the glass phase fills space thus preventing cellular collapse following desiccation. Furthermore, water deficit or any treatments affecting the cellular water potential, such as abscisic acid, lead to expression of so called LEA (Late Embryogenesis Abundant) proteins. These are rich in hydrophilic amino acids and have few hydrophobic residues. Therefore, they are largely water-soluble and have high hydration levels and this may additionally contribute to adaptation to desiccation in seeds (citations in LEPRINCE & al 1993). Finally, desiccation tolerance of dormant seeds is based on the ability to scavenge desiccation-induced free radicals (LEPRINCE & al. 1993). Indeed, desiccation-induced free radicals were found in numerous tissues, and in some cases desiccation tolerance was correlated with maintenance/synthesis of antioxidants as glutathione (γ-glutamyl-cysteinyl-glycine, GSH), ascorbic acid (AA) or tocopherols and/or enzymes scavenging cytotoxic oxygen species as superoxide dismutase, catalase or peroxidases (citations in KRANNER & GRILL 1996).

In this paper, we review the role of some antioxidants and enzymes that are involved in free radical scavenging pathways in desiccation tolerant and desiccation sensitive cryptogamics, particularly in mosses and lichens. Finally, we will focus on the role of glutathione in overcoming desiccation.

Drought stress induced formation of free radicals

Free radicals are naturally produced during plant metabolism, particularly in chloroplasts and mitochondria (see HALLIWELL 1987). However, the formation of free radicals occurs with increased frequency during a number of plant stresses (ELSTNER & OSSWALD 1994) including drought. Investigations on drought stress-induced formation of free radicals were completed with seeds and seedlings of higher plants such as *Glycine max*, *Helianthus annuus*, *Zea mays* or *Quercus robur*. Moreover, the formation of free radicals was also reported in grasses and cereals (citations in KRANNER & GRILL 1996)

In cryptogamic plants, there are only few investigations on formation and accumulation of free radicals. A representative study is that of SEEL & al. 1991 who investigated mosses under water stress. By using EPR (electron paramagnetic resonance) and ENDOR (electron nuclear double resonance) studies these authors demonstrated that as well as short-lived peroxy-radicals relatively stable and

carbon centred radicals were formed, and this was proved for both desiccation-tolerant (*Tortula ruraliformis*) and desiccation-sensitive (*Dicranella palustris*) mosses. Such stable free radicals also accumulated in germinating maize (LEPRINCE & al. 1990) and in oak (HENDRY & al. 1992). The authors suggest that these stable free radicals are semi-chinones which result from the final trapping out of radicals involved in a sequence of desiccation-promoted free radical reactions.

There is a widespread agreement that antioxidants function in scavenging of free radicals and cytotoxic oxygen species, respectively, in particular GSH, ascorbic acid, α-tocopherol, β-carotene and the xanthophyll cycle pigments (SMIRNOFF 1993, ELSTNER & OSSWALD 1994). Furthermore, other compounds such as flavonoids, sugars, polyols, proline and polyamines have antioxidant properties (citations in SMIRNOFF 1993). Moreover, cytotoxic oxygen species are scavenged by enzymic pathways that include reactions catalysed by superoxide dismutase (SOD), ascorbate peroxidase (AP) and other peroxidases, mono- and dehydroascorbate reductases, glutathione reductase (GR) and catalase (for an overview see ELSTNER & OSSWALD 1994). A well-balanced interplay of antioxidants and enzymes in scavenging cytotoxic oxygen species was first suggested by FOYER & HALLIWELL 1976. They postulated an ascorbate-glutathione cycle for the scavenging of the H_2O_2 that derives from superoxide, catalysed by SOD. This cycle is known for eliminating the risk of oxidation of enzymes by H_2O_2. It involves reactions of GSH, AA, GR, AP, mono- and dehydroascorbate reductases. This cycle may also be linked to the lipid-soluble antioxidant α-tocopherol which protects membrane proteins from free radical reactions (FINCKH & KUNERT 1985).

Desiccation and rehydration in mosses is correlated to protection from oxidative stress

As mentioned above, the formation and accumulation of stable free radicals were demonstrated in both desiccation-sensitive (*Dicranella palustris*) and desiccation-tolerant (*Tortula ruraliformis*) mosses (SEEL & al 1991, Table 1). However, their association with molecular damage depended on the adaptation to desiccation. In subsequent investigations SEEL & al. 1992 compared free-radical-scavenging processes in these mosses (Table 1). In the hydrated state, *T. ruraliformis* had a higher SOD activity than *D. palustris* but similar or lower activities of the chloroplastic H_2O_2-processing enzymes AP and peroxidases determined in vitro using guaiacol as a substrate. Desiccation caused a significant increase in SOD activity, but did not affect the activities of peroxidase and AP in *T. ruraliformis*. In contrast, desiccation in combination with irradiance led to a decrease in peroxidase activity in *D. palustris*, but had little effect on the activities of other oxygen-processing enzymes. The extrachloroplastic enzyme catalase was 7-fold more active in hydrated *T. ruraliformis* than in *D. palustris*, but desiccation resulted in significantly decreased enzyme activity in both species. During

desiccation, a depletion of AA occurred in both species. When *T. ruraliformis* was desiccated in light, there was synthesis of γ-tocopherol and maintenance of α-tocopherol and glutathione. Activities of the antioxidant recycling enzymes, dehydroascorbate reductase and GR, were not significantly increased by desiccation in either moss. These authors did not find that desiccation caused cellular damage in the desiccation tolerant moss. However, water removal in the desiccation-sensitive species led to destruction of chlorophyll, loss of carotenoids and increased lipid peroxidation. This cellular damage was correlated with a depletion of the antioxidative system (SEEL & al. 1992).

Table 1. The results of SEEL & al 1991, 1992 indicate that desiccation tolerant and desiccation sensitive mosses are well endowed with an antioxidative system. However, the desiccation-tolerant moss seems to have the capability for activating and maintaining its antioxidative system upon dehydration, the desiccation-sensitive moss may not.

Tortula ruraliformis desiccation tolerant	*Dicranella palustris* desiccation sensitive
controls	
peroxidase and ascorbate peroxidase similar	
catalase 7 fold higher	
SOD activity high	SOD activity low
desiccated plants	
formation of free radicals	
no destruction of chlorophyll	destruction of chlorophyll
no destruction of carotenoids	destruction of carotenoids
no lipid peroxidation	increased lipid peroxidation
depletion of ascorbic acid	
increasing SOD activity	decreasing peroxidase activity
synthesis of γ-tocopherol, maintenance of glutathione and α-tocopherol	γ-tocopherol, α-tocopherol and glutathione not detected

These data from desiccation tolerant and desiccation sensitive mosses fit well to a study on oak seeds. HENDRY & al. 1992 associated desiccation in seeds of the recalcitrant (i.e. desiccation sensitive) species *Quercus robur* with loss of viability, a rise in lipid peroxidation and build-up of free radicals. Here, two different molecular defence mechanisms against oxidative attack were discussed: in the cotyledons a predominantly enzymic protection with high activities of SOD and GR was found, whilst the protection from free radicals in the embryonic axis was afforded by the antioxidants AA and α-tocopherol. These authors suggest that the decay in both defence mechanisms - that occurred during the storage and thus desiccation of the seeds - was directly linked with free radical formation and lipid peroxidation and that these events may contribute to loss of viability in recalcitrant seeds.

Other important studies on antioxidants and activated oxygen processing enzymes in desiccated and rehydrated mosses were completed by DHINDSA 1987, 1991 (Table 2). First, he examined the glutathione status and its relationship to

protein synthesis during desiccation and subsequent rehydration in *Tortula ruralis*, a drought tolerant moss (DHINDSA 1987). He demonstrated that the speed of dehydration affects the oxidation/reduction processes of glutathione during rehydration. During slow drying GSSG increased from 2%, expressed as a percentage of total glutathione, in controls to about 22% in desiccated plants. Upon rehydration of slowly dehydrated moss, GSSG declined to the control level within 2h. During rapid drying there was only a small increase in GSSG. However, GSSG increased up to 45% during the subsequent rehydration, started to decrease after 2h of rehydration and reached the control level after 10h.

In the following study on *T. ruralis* (DHINDSA 1991), the activities of GR, GP, and glutathione-S-transferase (GST) were found to increase during slow drying or during rehydration following rapid drying of the moss. An increase in GSSG during rehydration following rapid dehydration was correlated positively with the levels of lipid peroxidation and solute leakage and negatively with the rate of protein synthesis. The inhibition of protein synthesis by high GSSG concentrations was also reported by several other authors (FAHEY & al. 1980, ERNST & al. 1978, 1979, JACKSON & al 1983). It was shown in animal tissues that GSSG inhibits the initiation of protein synthesis by activating a protein kinase which phosphorylates and thus inactivates the α-subunit of initiation factor 2 (ERNST & al. 1978, 1979).

Table 2. Summary of the investigations completed by DHINDSA 1987, 1991. In the drought tolerant moss *Tortula ruralis* oxidation of glutathione and activation of the enzymes glutathione reductase (GR), glutathione peroxidase (GP) and glutathione-S-transferase (GST) were induced only by desiccating the moss slowly. In contrast, rapid dehydration did not lead to either oxidation of glutathione or activation of these enzymes. However, glutathione was oxidised and these enzymes were activated upon rehydration following the rapid dehydration. In our opinion, this leads to the conclusion that the moss has received a drought stress-induced signal for oxidising glutathione and activating GR, GP and GST. However, a response to this signal was not possible in case of dehydrating the moss too rapidly, therefore, the plant could only respond to the signal during the subsequent rehydration.

slow desiccation	**rapid desiccation**
increase in GSSG	no increase in GSSG
increase of GR, GP and GST activities	no increase in the activities of these enzymes
rehydration following slow desiccation	**rehydration following rapid desiccation**
rapid decrease in GSSG to control level	increase in GSSG to a maximum after 2 h, decrease to control level after 6 h
protein synthesis similar control	decreased protein synthesis
decrease of GR, GP and GST activities to the control level	increase of GR, GP and GST activities, started to decrease after 8 h

Desiccation tolerance in lichens may be correlated with the ability to reduce GSSG during rehydration

Recently, changes in the redox status of glutathione and in the activities of GR and glucose-6-phosphate dehydrogenase (G6PDH) were investigated during desiccation and rehydration of three lichens differing in their desiccation tolerance (KRANNER & GRILL, received for publication). In general, all three species are desiccation tolerant; however, within these species there are different grades of desiccation tolerance with *Pseudevernia furfuracea* being the most desiccation tolerant and *Peltigera polydactyla* the most desiccation sensitive of these three species. *Lobaria pulmonaria* has an intermediate position.

Desiccating these lichens for 2 months resulted in loss of glutathione (15-30% of the control content) that could be explained by GSH consuming processes such as scavenging of desiccation-induced free radicals. Simultaneously, desiccation caused oxidation of GSH in all three lichens. Thalli desiccated for two months contained between 71-93% of the total glutathione in the oxidised form (Table 3). The oxidation of GSH was interpreted as an adaptation to drought, and this fits a thiol-disulphide cycle (Fig. 1) that was postulated recently (KRANNER & GRILL 1996). Oxidation of GSH during desiccation of cryptogamic plants and during maturation of seeds of higher plants has been reported by several authors (Table 4). It seems to be a feature of drought tolerant plants to oxidise GSH during dehydration and to reduce the formed GSSG upon rehydration. However, drought tolerant plants have a strong demand to reduce the accumulated glutathione disulphide upon recovery for at least two reasons: first, to prevent the GSSG-mediated inhibition of protein synthesis and second, to recycle and then maintain their GSH pool.

The reduction of GSSG to GSH during the recovery of desiccated plants that are tolerant to desiccation needs the activities of two enzymes. These are glutathione reductase and glucose-6-phosphate dehydrogenase. The latter is the key enzyme of the oxidative pentose shunt that provides the NADPH required for the action of GR. We believe this pathway is of special importance for resurrection plants that are not able to provide NADPH from photosynthesis in the first stages of recovery following desiccation.

Wetting of thalli desiccated for two months resulted in rapid reduction of GSSG in the most desiccation tolerant lichen, *Pseudevernia furfuracea* and in *Lobaria pulmonaria* whilst its reduction showed a pronounced delay in *Peltigera polydactyla,* the most desiccation sensitive lichen (KRANNER & GRILL, received for publication). The authors suggest that the lichens might have obtained the NADPH required for the action of GR from the oxidative pentose shunt. The activity of G6PDH was decreased dramatically by dehydrating *Peltigera polydactyla* and *Lobaria pulmonaria* for two months, while its activity was not affected in *Pseudevernia furfuracea*. Upon rehydration, the G6PDH-activity significantly increased in *Pseudevernia furfuracea* and in *Lobaria pulmonaria*, but not in *Peltigera polydactyla*. From these results the authors conclude that the reduction of

GSSG during rehydration of *Peltigera polydactyla* might be limited by the availability of NADPH. Furthermore, they assume that desiccation tolerance may be correlated with the capacity to reduce GSSG upon rehydration, and that this reduction of GSSG is depending on the ability to activate or maintain the activities of the enzymes GR and especially G6PDH. Moreover, in these three lichen species the ability to activate G6PDH reflects the different grades of desiccation tolerance.

Table 3. Oxidation of GSH during a desiccation period of two months in three lichen species, and reduction of GSSG during their rehydration as described by KRANNER & GRILL (received for publication). GSSG is given as a percentage of total glutathione. The three investigated lichen species show different grades of desiccation tolerance. *Pseudevernia furfuracea* is the most desiccation tolerant, *Peltigera polydactyla* the most desiccation sensitive of these three species and *Lobaria pulmonaria* has an intermediate position. Detailed information in the text.

species	desiccation	rehydration
Pseudevernia furfuracea	oxidation of GSH (up to 89% GSSG), loss of total glutathione (30% of the content of un-desiccated thalli); GR activated, G6PDH not affected	reduction of GSSG to the control level (18%) within 5 min; GR not affected, G6PDH slightly increased
Lobaria pulmonaria	oxidation of GSH (up to 93% GSSG), loss of total glutathione (23% of the content of un-desiccated thalli); GR not affected, G6PDH dramatically decreased	reduction of GSSG to the control level (18%) within 5 min; GR not affected, G6PDH dramatically increased
Peltigera polydactyla	oxidation of GSH (up to 71% GSSG), loss of total glutathione (15% of the content of un-desiccated thalli); GR slightly, G6PDH dramatically decreased	reduction of GSSG only to 30% of total glutathione within 60 min (not to the control level); GR not affected, G6PDH not significantly increased

A thiol-disulphide cycle postulated for desiccation/rehydration processes of plants whose protoplasm survives desiccation

Glutathione fulfils significant biological functions in catalysis, synthesis and transport (RENNENBERG 1982, MEISTER & ANDERSON 1983, MEISTER 1995), most of them being ascribed to reduced glutathione (GSH). In most tissues glutathione is maintained in the reduced state by the action of GR, and the accumulation of GSSG is therefore commonly correlated with functional disorder. However, a central role for GSSG was suggested recently for overcoming resting stages of plant development including dormancy of seeds and desiccation of resurrection plants. This hypothesis depends upon the idea that protein thiol groups are protected from irreversible autooxidation by the reaction of GSSG with protein thiol groups (PSH) thus forming protein bound glutathione (PSSG) and GSH during desiccation. Upon extreme desiccation, GSH is oxidised further leading to high final concentrations of PSSG and GSSG. In this state, PSH and GSH are protected from desiccation-induced oxidative injury such as irreversible formation

of intramolecular cross links in proteins or uncontrolled oxidation of SH groups, e.g. to sulfonic acids.

As demonstrated in Table 4, a number of cryptogamic plants increase their pools of GSSG and PSSG during desiccation, and they reduce these disulphides upon recovery. The same pattern is demonstrated for seeds during dormancy and the first stages of germination.

Table 4. Glutathione is involved in thiol-disulphide conversions in desiccation / rehydration processes in cryptogamics and in seeds and spores during dormancy and the first stages of germination. From KRANNER & GRILL 1996, modified.

Species / organ	thiol-disulphide exchange during dehydration	thiol-disulphide exchange during rehydration	references
Neurospora crassa spores	GSSG and PSSG increasing under aging / drying conditions	reduction of both PSSG and GSSG during imbibition	FAHEY & al. 1975
Triticum aestivum and *Hordeum sativum* embryos	high PSSG and GSSG concentrations in dormant seeds	reduction of both PSSG and GSSG during imbibition	FAHEY & al. 1980
Tortula ruralis leaves	oxidation of GSH to GSSG during slow dehydration	reduction of GSSG to GSH	DHINDSA 1987, 1991
Spinacia oleracea seeds	ACP-SSG accumulation during seed maturation	reduction of ASP-SSG to reduced and acylated ACP	BUTT & OHLROGGE 1991
Pisum sativum seeds	high GSSG concentrations in dormant seeds	reduction of GSSG to GSH during imbibition	KRANNER & GRILL 1993
Pseudevernia furfuracea thalli	high GSSG concentrations in dry lichens	reduction of GSSG to GSH during rehydration	KRANNER & GRILL 1994
Lycopersicum esculentum seeds	high GSSG concentrations in dormant seeds, increasing during aging	reduction of GSSG during imbibition	DEVOS & al. 1994

Although mechanism and significance of the formation and accumulation of GSSG in dehydrated tissues are not clearly understood yet, we can consider some possible reactions of GSH that would lead to formation of GSSG during desiccation. First, the oxidation of GSH by oxygen may occur spontaneously as a metal-catalysed reaction. Furthermore, a high concentration of thiyl radicals will lead to dimerisation of thiyl radicals leading to autooxidation of GSH or PSH, respectively. However, to the best of our knowledge, the formation and the reactions of thiyl radicals have not been studied in plants under drought.

Another possibility for the oxidation of GSH is an enzyme-catalysed reaction that leads to formation of GSSG. As outlined by PICHORNER & al. 1992 plant peroxidases have a thiol - oxidase function. Here, the oxidation of cysteine by peroxidases extracted from horseradish and wood of several trees was discussed with respect to lignification. An increase in the activity of glutathione peroxidase has been reported by DHINDSA 1991 for mosses under drought stress. Such an enzyme catalysed oxidation of GSH to GSSG during drought could be interpreted as an adaptation mechanism that serves to provide the high GSSG levels required

for the further reaction of GSSG with PSH (Fig. 1, DH 2). After a further oxidation of the GSH derived from the last reaction, PSSG and GSSG represent stable states that allow a protection from further oxidation of both.

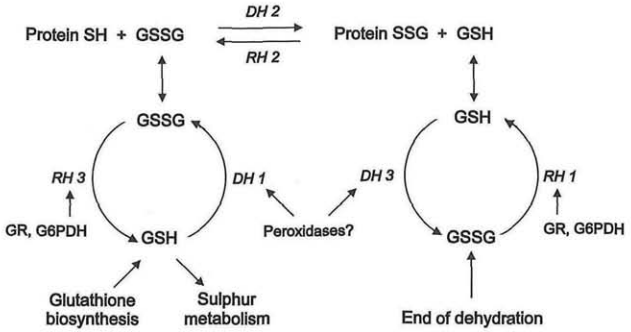

Fig. 1. Glutathione disulphide dependent protection of protein thiol groups during desiccation of plants whose protoplasm survives desiccation. This cycle is organised in dehydration (DH) and rehydration (RH) steps. The cycle starts in the fully hydrated state (left part of the cycle) where glutathione is mainly present as GSH. Dehydration step 1 starts with the oxidation of GSH to GSSG. We propose an enzymatic reaction, maybe catalysed by peroxidases that use GSH as a substrate. In DH 2 the formation of mixed disulphides proceeds yielding PSSG and GSH. The participation of enzymes remains unclear. In DH 3 the GSH formed in DH 2 is oxidised to GSSG, possibly by peroxidases as described for DH 1. On this point, the tissue is in the dehydrated state. On RH 1 GSSG is reduced to GSH by the activity of GR. Rehydration step 2 implicates the reaction of GSH and PSSG yielding reduced protein thiol groups and GSSG. After RH 3 the fully hydrated state is reached leading to reduction of GSSG to GSH by GR. If no further dehydration follows, the normal metabolic processes are started. From KRANNER & GRILL 1996, modified.

Conclusions

Desiccation-tolerance is one of the most fundamental principles for resting stages of plant development. These include the dormancy of seeds and spores and the survival of resurrection plants under drought. In spite of the widespread differences in anatomy and physiology of these organisms such as higher plants, mosses or lichens, they may have developed similar molecular mechanisms that allow protection from the injurious effects of cellular desiccation.

In seeds, several strategies for coping with the deleterious effects of desiccation have been investigated, one of them being the ability to scavenge desiccation-induced free radicals. This is afforded by antioxidative and / or enzymic pathways. The best known antioxidants that play a part in overcoming desiccation are glutathione, ascorbic acid, α-tocopherol and β-carotene. Furthermore, activated oxygen processing enzymes have been shown to function in protection from desiccation-induced free radicals: superoxide dismutase, catalase, peroxidases, glutathione reductase and (mono) dehydroascorbate reductase. Most

of the research on this topic has concentrated on seeds and seedlings of higher plants; cryptogamic plants are by far less frequently investigated. However, there are some representative studies on mosses that indicate that desiccation-tolerant species seem to be able to activate their antioxidative system in the dehydration / rehydration process; desiccation-intolerant may not.

Furthermore, it was demonstrated on lichens, that glutathione undergoes oxidation processes during desiccation that are interpreted as adaptation to desiccation. Glutathione probably plays an important part for overcoming periods of drought, first as an antioxidant, and second by protecting thiol-groups of proteins from formation of intramolecular disulphides by forming protein-bound glutathione. Moreover, it was suggested that desiccation tolerance in lichens can be correlated with the ability to reduce GSSG, that is formed upon desiccation, to GSH in the first stages of rehydration. The reduction of GSSG is catalysed by the NADPH dependent glutathione reductase. This enzyme was shown to have high activities in desiccated and rehydrated lichens. However, a lichen species that is not well adapted to desiccation, did not reduce GSSG to the same extent that desiccation tolerant species do. It was demonstrated, that in this case the reduction of GSSG was limited by the availability of NADPH that was not provided by activating the enzymes of the oxidative pentose shunt as glucose-6-phosphate dehydrogenase. This pathway has special importance for desiccation tolerant plants, because it serves to provide NADPH in the first stages of rehydration when photosynthesis is not possible yet.

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