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The Role of Glutathione in Stress Adaptation of Plants

By

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S u m m a r y

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The tripeptide glutathione is not only essential in the sulfur metabolism of plants, but also an important redox buffer responsible for antioxidative protection and redox regulation of the cell functions. Changes in foliar glutathione concentrations occur upon various stress impacts, such as drought, cold acclimatisation, high altitude stress, and air pollution impacts. In general, results indicate an increase in glutathione concentrations with increasing stress level, which is ascribed a higher capacity for antioxidative protection, but stress impacts may also cause changes in the glutathione redox state which may have further implications on metabolism, such as enzyme regulation and gene expression. The present paper reviews the effects of environmental stress on glutathione metabolism in plants.

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

The tripeptide glutathione (GSH, γ-glutamyl-cysteinyl-glycine) is the most abundant low molecular weight thiol in plant tissues. Due to the particular properties of the molecule it plays multiple roles in the cellular metabolism. It is a central compound in sulfur metabolism and is considered the main transport form of reduced sulfur (RENNENBERG & LAMOUREUX 1990). It links the sulfur reduction pathways to the protein synthesis and functions as a buffer for reduced sulfur. On the other hand, GSH is also a strong reductant which makes it an effective scavenger of toxic active oxygen species (AOS). Oxidative stress is an inescapable feature of life and AOS are involved in nearly all effects of environmental stresses to plants (DE KOK & STULEN 1993). The capacity of the glutathione redox system to detoxify dangerous AOS is dependent on the pool size of total GSH, on the ratio GSH/GSSG (GSSG = oxidized glutathione), and on the activity of the regenerating

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enzyme system, the NADPH dependent glutathione reductase. Elevated levels of GSH appear to be correlated to active plant responses to environmental stresses and responses of GSH synthesis, GSH redox status, and GSH related enzyme activities have been found repeatedly in plants under stress.

Given the extensive literature on the subject the present review is confined to selected points with emphasis on the role of glutathione in forest trees in response to environmental stresses.

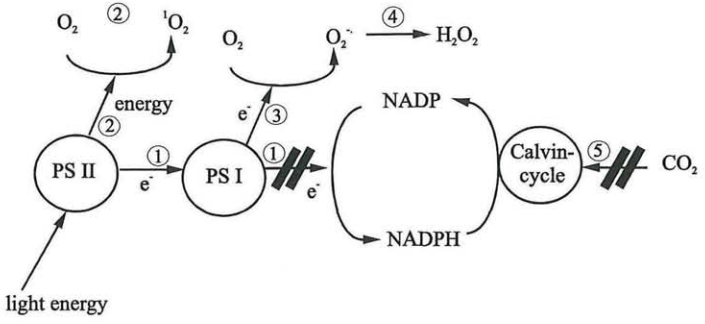

Fig. 1. Possible mechanisms of AOS formation in plant cells under stress: Every stress impact that causes a slower function or malfunction of the Calvin cycle (5) leads to the exhaustion of the primary electron acceptor NADP and to a block in the electron transport to NADP (1). Electrons leak to oxygen (3) yielding superoxide and, via superoxide dismutase reaction (4), H_2O_2. Excess excitation energy may be directly transferred to molecular oxygen yielding singlet oxygen (2).

The common mechanism of stress induced free radical production is based on an imbalance between the consumption of reductant (NADPH) in carbon fixation, and the need of the electron transport chain for regenerated electron acceptor at the PS I site (NADP). Stress impacts such as low temperatures, drought (through stomatal closure), or chemical agents impair the function of the carbon fixation in the Calvin cycle, but they do not slow down light driven electron transport. This leads to an overreduction of the electron transport chains and forces electrons to leak to alternate acceptors, predominantly molecular oxygen yielding superoxide anion $O_2^{\bullet}$ (Fig. 1). Superoxide is detoxified enzymatically forming H_2O_2 which is enzymically detoxified by ascorbate, also the most effective chemical scavenger of AOS. Compared to ascorbate, GSH is less effective in this function, but the regeneration of oxidized ascorbate in the Halliwell-Asada cycle requires GSH (Fig. 2).

Furthermore, the redox pool of GSH/GSSG is able to modify protein structures via interaction with protein–SH groups and disulfide bonds. Hence, this redox state which may change under stress conditions, can regulate enzyme activities or gene expressions (FOYER & al. 1997).

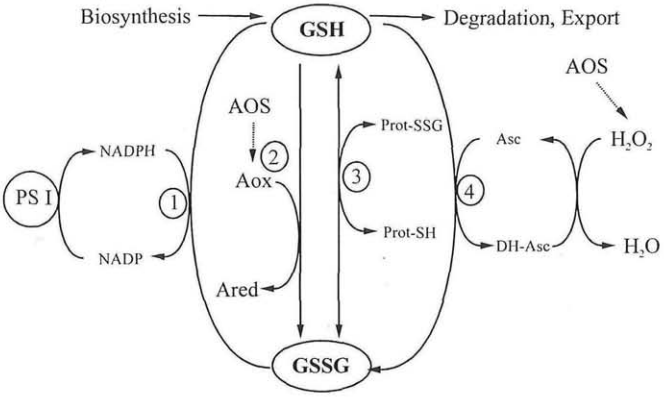

Fig. 2. Some possible roles of glutathione in protection against stress. Composed after DE KOK & STULEN 1993 and FOYER & al. 1997. PS I = Photosystem I, Aox = oxidized substrate, Ared = reduced substrate, Prot = proteins, Asc = reduced ascorbate, DHAsc = dehydroascorbate, AOS = active oxygen species, GSH = reduced glutathione, GSSG = oxidized glutathione. 1 = glutathione reductase activity, 2 = chemical antioxidant property of glutathione, 3 = interaction with proteins and formation of mixed disulfides, 4 = enzymic activities of the Halliwell-Asada cycle.

Air Pollution

In its role as a central compound in sulfur metabolism glutathione concentrations in plant tissues are affected by exposure to sulfurous air pollutants. Early studies on spruce trees revealed significantly higher GSH concentrations in needles harvested in SO_2 polluted areas. In fumigation experiments, exposure to SO_2 and H_2S both significantly increased GSH concentrations in leaf tissues of different plants (TAUSZ & al. 1998a). Although SO_2 is known to induce oxidative stress in chloroplasts, the role of enhanced glutathione concentrations for the stress protection is questionable in this case. Glutathione rather functions as a buffer for reduced organic sulfur.

Oxidative air pollutants, such as ozone, induce oxidative stress in plant tissues. Increases of the GSH pool were regarded a protective response of plant metabolism against this stress (MEHLHORN & al. 1986). However, results in literature are highly inconsistent, since under many experimental conditions GSH pools showed no responses to ozone fumigations, although other effects, e.g. chromosomal anormalities in meristems, were found (WONISCH & al. 1999). A field study on the ozone sensitive species *Pinus ponderosa* showed changes in the GSH/GSSG ratios as an early symptom of ozone injury rather than changes in the total glutathione pool (Fig. 2).

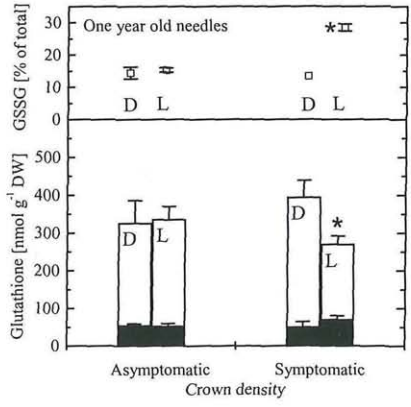

Fig. 3. The glutathione system in needles of *Pinus ponderosa* trees growing at an ozone impacted field plot in Southern California with (Sympt.) and without (Asymptom.) severe crown thinning. The total pool of glutathione (columns) consists of GSH (open part of the column) and GSSG (closed part of the column). Columns show medians of 5 individual trees, error bars show median deviations (omitted when within the symbol). Sunlight adapted needles (L) and needles after overnight darkening of detached branches (D). Asterisks indicate significant differences between light and dark adapted stage. Modified from TAUSZ & al. 1999.

In a comparison between sensitive and tolerant cultivars of different species, a protective effect of enhanced GSH level against ozone was reported in some (e.g. in tobacco), but not in all species (WELLBURN & WELLBURN 1996). Modification of GSH levels in genetically transformed tobacco (about 5-fold the wildtype concentrations) coincided with a slight increase in ozone tolerance (WELLBURN & al. 1998), but studies on similarily transformed poplar did not confirm this result (NOCTOR & al. 1998, STROHM & al. 1999). Ozone sensitive tobacco cultivar BelW3 showed oxidation of the glutathione pool together with a decrease in glutathione reductase (GR) activity upon ozone fumigation, whereas the resistant BelB maintained a high GSH/GSSG ratio and a high GR activity (PASQUALINI & al. 1999).

Light Stress

Glutathione levels are strongly affected by the light environment. Sun needles of *Picea abies* contain more GSH than shade needles (GRILL & al. 1987). A study by SCHUPP & RENNENBERG 1989 revealed a light dependent daily course of GSH concentrations in spruce needles with a midday maximum. This might be due to the biochemistry of GSH synthesis which is clearly light dependent (NOCTOR & al. 1997), or due to transportation processes exporting GSH from the needles, or a combination of both. In pine trees even a small increase of GSH levels was observed upon darkening. In this study, branches were detached after sampling in the light, i.e. GSH export was impaired (TAUSZ & al. 1999, Fig. 3).

Drought Stress

Higher plants respond to water deficiency with stomatal closure which causes a lack of CO_2 in the chloroplasts. This results in increased oxidative stress in illuminated chloroplast, possibly affecting the glutathione system. Results on non-dessication tolerant plants are contradictory. Some studies reported oxidation of the glutathione pool only at very low relative water content, probably reflecting severe (and probably irreversible) biochemical damages to the tissues at that stage of desiccation (SMIRNOFF 1993). Other studies showed an increase in the GSSG/GSH ratio as the first symptom in the antioxidative system in conifers (S. MONSCHEIN, M. TAUSZ & D. GRILL, unpublished results on spruce, and M. TAUSZ, A. WONISCH, J. PETERS, D. MORALES, M. S. JIMÉNEZ & D. GRILL, unpublished results on pine). These changes in glutathione redox state underwent lightmodulated variations, sometimes showing recovery of the oxidized state in the dark (compare results in Fig. 3).

The situation is clearly different in desiccation tolerant (poikilohydric) plants or in desiccation tolerant resting stages of plants, e.g. seeds. The dehydrated tissues contained a high proportion of GSSG which is clearly not a sign for damage in this case. Upon rehydration, the glutathione pool was quickly conversed to a high GSH/GSSG ratio (KRANNER & GRILL 1993).

Cold Acclimatisation

GSH levels in conifers undergo annual concentration changes with maxima in winter and minima in summer (ESTERBAUER & GRILL 1978). A role in winter hardening is ascribed to increasing GSH levels in fall, but the mechanisms are still unclear (WINGSLE & al. 1999). However, results are not always that clear and in some studies no pronounced seasonal change of GSH content was found in field grown conifer needles (STECHER & al. 1999).

Experiments in climate chambers showed that the increase in foliar GSH concentrations in spruce needles can be triggered by low temperatures (4 °C), but not by a shortening of the day length (HERBINGER & al. 1999). In this experiment, like observations in other field studies (STECHER & al. 1999), changes in the redox state of the GSH pool were not found in winter conditions (HERBINGER & al. 1999). The substantial increase of GR activity which is associated with the increase of GSH levels during winter adaptation (ESTERBAUER & GRILL 1978) might account for the stable redox state of the GSH pool.

Stress Combinations

The complex impacts of environmental stress combinations on the glutathione system of field grown plants were mainly studied in forest research projects. Altitude profiles provided a gradient of natural and man-made stresses. Irradiance, climatic stress, and atmospheric ozone concentrations increase with in-

creasing altitude causing potentially elevated oxidative stress at higher altitudes. Gradient studies on forest trees (TAUSZ & al. 1997) and several herbaceous high alpine plants (WILDI & LÜTZ 1996) revealed increasing concentrations of glutathione with increasing altitude (i.e. increasingly stressful conditions). Fig. 4 shows this observation on spruce trees, but only without the impact of local air pollution above local inversion layers.

Discussion and Prospectives

The mechanisms through which GSH is involved in stress protection and/or stress responses remain largely unclear. Some results contradict each other with respect to an increased protective capacity due to increased GSH levels. GSH is only one component of the cellular antioxidant defence, and plant response is a concerted action of the whole metabolism. WILDI & LÜTZ 1996 showed that different antioxidants may respond differently to stress (e.g. high altitude), and that these responses depend on the species. The study of antioxidative response patterns instead of single responses (such as GSH alone) seems to be more promising (TAUSZ & al. 1998).

Another important aspect is the potential of the glutathione redox pool to participate in enzyme regulation (MAY & al. 1998). Recent results indicate not only a direct regulation of the expression of antioxidant enzymes via glutathione or a redox regulation of enzyme molecules on the protein level in vivo (e.g. for glutathione reductase, WINGSLE & al. 1999). The regulatory aspects of GSH metabolism have also been demonstrated by the capacity of glutathione to induce damages on chromosomes in meristematic tissues observable on the structural level (ZELLNIG & al. 2000).

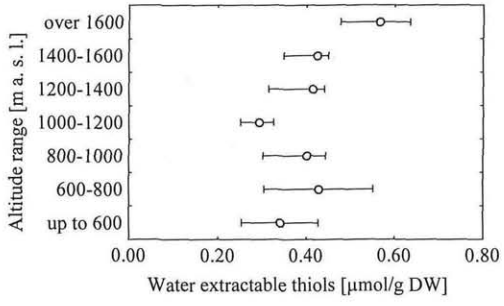

Fig. 4. Altitudinal pattern of water extractable thiols (which is by about 95% glutathione) in previous year's spruce needles of field plots in Austria (modified from TAUSZ & al. 1997). Sites below 1000 m are impacted by local pollution (SO_2), above 1000 m altitudinal increase of thiol contents are observed.

Further research on the initial stages of changes in the redox systems including enzyme activations and metabolic adaptations upon stress is needed.

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