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## Aspects of Glutathione Treatment on the Cytoskeleton in Different Cells of *Picea abies*

By

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With 3 Figures

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**Key words:** Glutathione, root tip cells, callus cells, microtubules, actin filaments, *Picea abies*.

### Summary

URBANEK A., ZECHMANN B., ZELNIG G. & MÜLLER M. 2003. Aspects of glutathione treatment on the cytoskeleton in different cells of *Picea abies*. – *Phyton* (Horn, Austria) 43 (2): 319–333, 3 figures. – English with German summary.

The effects of exogenously applied reduced glutathione (GSH) on the arrangement of the cytoskeleton in different spruce cells were investigated using a monoclonal antibody against  $\alpha$ -tubulin and rhodamin labelled phalloidin for actin staining. Callus cells and root tips of *Picea abies* (L.) KARST. were exposed to two different physiologically relevant concentrations (500  $\mu$ M and 1000  $\mu$ M) of GSH.

Exogenously applied GSH had different effects on the plant tissues. The microtubules and actin filaments of the GSH treated cells showed modifications in the interphase in a similar way. They were shortened and wavy or appeared as bright fluorescent particles in the cortical area of the cell. Furthermore, some alterations in the arrangement of the preprophase band (PPB) were visible in GSH treated cells. Most of the PPBs were rotated, possibly leading to modifications in the symmetry of the cell division site. In dividing cells no significant changes were visible in the form of the cytoskeleton after GSH treatment.

### Zusammenfassung

URBANEK A., ZECHMANN B., ZELNIG G. & MÜLLER M. 2003. Der Einfluss von Glutathion auf das Cytoskelett in verschiedenen Zelltypen von *Picea abies*. – *Phyton*

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(Horn, Austria) 43 (2): 319–333, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die vorliegende Studie untersuchte den Einfluss von exogen angebotenem reduzierten Glutathion (GSH) auf die Anordnung des Cytoskeletts in verschiedenen Zelltypen der Fichte. Unter Verwendung fluoreszenzmikroskopischer Techniken wurde  $\alpha$ -Tubulin mit Hilfe eines Alexa konjugiertem monoklonalen Antikörper gefärbt und zum Nachweis der Aktinfilamente wurde Rhodamin konjugiertes Phalloidin eingesetzt. Kalluszellen und Wurzelspitzen von *Picea abies* (L.) KARST. wurden mit zwei unterschiedlichen, physiologisch relevanten GSH Konzentrationen (500  $\mu$ M und 1000  $\mu$ M) behandelt.

Das exogen angebotene GSH hat verschiedene Auswirkungen auf das pflanzliche Gewebe. Mikrotubuli und Aktinfilamente der GSH behandelten Zellen zeigten ähnlich ausgeprägte Modifikationen in der Interphase. Sie waren verkürzt und gewellt oder erschienen als hell fluoreszierende Partikel im corticalen Bereich der Zelle. Weiters waren Veränderungen in der Anordnung des Preprophasebandes (PPB) in GSH behandelten Zellen zu sehen. Die meisten PPB waren versetzt, was auf eine Änderung der Symmetrieachse während der Teilung schließen lässt. Die GSH Behandlung hatte keinen signifikanten Einfluss auf die Form des Cytoskeletts in den entsprechenden Teilungsstadien.

## Introduction

GSH is a significant member of low molecular weight thiols and is ubiquitous in plants and animals (e.g. FOYER & RENNENBERG 2000). Transformed plants with a lack of  $\gamma$ -glutamylcysteine-synthetase have underdeveloped roots and this symptom could be relieved by GSH supply, which demonstrates its role in postembryonic cell divisions and plant development (VERNOUX & al. 2000). Furthermore, GSH is known to be of great importance in assimilation, storage and transport of reduced sulphur and is also a crucial part of the antioxidative system in plants and animals by scavenging active oxygen species (AOS) and detoxification of xenobiotics. GSH is present in all plant organs, but its contents differ between organs as well as within the cells at different developmental stages (FOYER & RENNENBERG 2000). The GSH levels are not only regulated internally, but are also induced and accumulated as a respond to various stress conditions (HERSCHBACH & al. 1995, MAY & al. 1998, TAUSZ & GRILL 2000, MÜLLER & al. 2002).

Recent investigations made on plants, in which sulphur assimilation and GSH synthesis have been modified by artificial elevation of the cellular GSH content, provided insights into GSH metabolism and its significance for mediating stress tolerance (WINGATE & al. 1988, FOYER & RENNENBERG 2000, ZELLNIG & al. 2000). Positive effects on root growth in form of increased cell division rate in *Arabidopsis* root tissue were reported by exogenously supplied GSH (SÁNCHEZ-FERNÁNDEZ & al. 1997). However, the investigations made on transformed plants, over-producing the enzymes of GSH biosynthesis and regeneration, showed that the ele-

vated GSH levels were not high enough for the increased resistance against AOS production (NOCTOR & al. 1997, STROHM & al. 1999). Moreover, elevated concentrations of GSH in plant tissues were reported to be deleterious. Transformed tobacco plants overexpressing  $\gamma$ -glutamylcysteine-synthetase showed increased oxidative stress related to elevated GSH biosynthesis capacity, stunted phenotypes and leaf necrosis (CREISSEN & al. 1999). Furthermore, chromosomal defects, a decrease in the cell division rate and alterations in the ultrastructure were visible in spruce root meristem and callus cells by applying different concentrations of GSH (ZELLNIG & al. 2000, MÜLLER & al. 2001).

The reported GSH induced chromosomal aberrations and changed cell division rates lead to the assumption, that alterations in the cytoskeleton are responsible for these effects.

The cytoskeleton is a membrane-associated macromolecular network in eukaryotic cells and is crucial for dynamic cell function including cell growth and differentiation, cell division and cytoplasmatic streaming (VOLKMANN & BALUŠKA 1999, DAVIES 2001). Roots and callus tissues are known to provide a convenient source of cells for fundamental studies of cytoskeletal elements and their physiological responses to environmental perturbations (AON & al. 1999, SIVAGURU & al. 1999, BARLOW & BALUŠKA 2000).

In the present investigation a modulation in the thiol pools was caused by applying different GSH concentrations to young spruce seedlings and callus material in order to determine whether physiological concentrations of GSH affect the structure and arrangement of microfilaments (MF) and microtubules (MT) during the cell cycle.

## Material and Methods

### Plant material

#### Spruce seedlings

The seeds of *Picea abies* (L.) KARST. from the Schöckel mountain near Graz (Styria, Austria) were grown on humid perlite and cultivated in a climate chamber (12 hours light/dark, 20 °C/18 °C, 80 % RH, 180  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR) for 10 days prior to the experiments.

#### Cell cultures

Cell cultures of *Picea abies* (L.) KARST. were established from cotyledons by using the solid MS medium (MURASHIGE & SKOOG 1962), containing 3 % sucrose, 1 % agar as gelling agent and growth regulators 1-naphthalenacetic acid (3 mg/l) and 6-benzylaminopurine (1 mg/l) according to MÜLLER & al. 2001. The pH-value was adjusted to 5.8. The cultures were cultivated in petri dishes at 21 °C in a climate chamber and illuminated with two fluorescence tubes (Philips TLD 18W/33 and Sylvania GROLUX F18W/GRO-T8) for 12 hours a day with light intensities between

33 and  $43.7 \mu\text{mol m}^{-2}\text{s}^{-1}$ . The callus tissues were sub-cultured on fresh MS medium every two weeks.

### GSH treatments

#### Spruce seedlings

After ten days the seedlings were transferred to nylon mesh covered 100 ml plastic dishes containing different concentrations of a GSH solution (500  $\mu\text{M}$  and 1000  $\mu\text{M}$ ). The treated plant material was cultivated in a climate chamber (12 hours light/dark, 20 °C/18 °C, 80 % RH,  $180 \mu\text{mol m}^{-2}\text{s}^{-1}$  PAR) for 24 hours. One culture remained untreated as a control. The samples were then harvested for further investigations.

#### Cell cultures

Spruce cell suspension cultures were established to improve the homogeneity of the callus cells and for further GSH treatment. Therefore 0.5 g of callus tissue from solid MS medium was transferred in 100 ml Erlenmeyer flasks containing 50 ml liquid sterile MS medium with the same ingredients as the solid growth medium (4.4 g/l MS, 30 g/l sucrose, 3 mg/l of 1-naphthaleneacetic acid and 1 mg/l of 6-benzylaminopurine, pH 5.8). GSH was applied at 500  $\mu\text{M}$  and 1000  $\mu\text{M}$  for 48 hours. The untreated suspension culture was used as a control. After 24 hours of GSH treatment the liquid growth medium of the cultures were exchanged with fresh liquid MS medium containing the adequate GSH concentrations. All cultures remained unshaken to make sure that the GSH was not oxidised. The callus material was harvested and used for further preparations.

### Fluorescence microscopy

#### Indirect immunofluorescence labelling of $\alpha$ -tubulin

Control samples and GSH treated roots and callus cells were prepared for indirect immunofluorescence localisation of  $\alpha$ -tubulin, using a modified method described by WICK & al. 1981 and APOSTOLAKOS & GALATIS 1999. The material was fixed in 4 % paraformaldehyde in a microtubule stabilising buffer (MSB: 50 mM PIPES in 0.1 M KOH, 5 mM EGTA, 2 mM  $\text{MgSO}_4$ , pH 6.8) for 45 minutes at RT, washed in MSB, and digested in 2 % cellulase and 1 % macerozyme in MSB for 45 minutes at RT. Afterwards the samples were washed in MSB for 30 minutes and extracted with 2 % Triton X-100 in phosphate-buffered saline (PBS, pH 7.3) for 120 minutes. After washing in PBS, the specimen were placed on slides in a few drops of PBS, covered with a cover slip and squashed. Slides were then frozen in liquid nitrogen to allow removal of cover slips without disturbing the cells. Squashed cells were incubated with the primary antibody (monoclonal mouse IgG1 anti-  $\alpha$ -tubulin clone B-5-1-2, Sigma) diluted 1:150 in PBS and additionally blocked in 1 % bovine serum albumine for 40 minutes in a moist chamber at 37 °C. After washing with PBS, the samples were incubated with the fluorochrom-labelled secondary antibody (Alexa Fluor 488 goat anti-mouse IgG (H+L) conjugate, Molecular Probes) diluted 1:30 in PBS for 60 minutes at 37 °C in a moist chamber. After extensive washing in PBS, sections were mounted on slides in an antifade agent.

### Staining of actin filaments

Staining of actin filaments was performed using a modified method according to OLYSLAEGERS & VERBELEN 1998 without fixation and detergents and/or organic solvents. The plant material was incubated on slides in actin buffer (100 mM PIPES in 0.2 M KOH, 10 mM EGTA, 5 mM MgSO<sub>4</sub> and 0.2 M mannitol pH 6.9), containing 2 % glycerol and 66 nM rhodamine labelled phalloidin (RLP) for 1 hour.

### Staining of nuclei and chromosomes

Additionally, after the labelling of MT and actin filaments selected material was stained by applying 5 µl of a DAPI solution (4',6'-Diamidino-2-phenyl-indol-dihydrochlorid, 10 µg DAPI/ml distilled water) to 100 µl of corresponding buffer.

### Equipment

Mounted slides were examined with two fluorescence microscopes.

A Zeiss Axioskop equipped with a 100 W mercury arc lamp was used to obtain digital images with a 3-chip-colour video camera (Sony DXC 930 P with Sony-control-system), a frame grabber (ITI MFG-3M-V, Imaging Technology Inc., with variable scan module AM-CLR-VP and colour recording module AM-CLR-VP). Optimas 6.5.1 (BioScan Corp.) was used as image analysis software. Fluorescence images were obtained through a Plan-Neofluoar 40× dry objective (n. a., 0.75), a 63× dry objective (n. a., 0.95) and a Plan-Apochromat 100× oil immersion objective (n. a., 1.4).

MT were investigated with a 450–490 nm excitation and 520 nm emission filter block. Rhodamine labelled actin was visualised with a 546/12 nm excitation and 590 nm emission filter block. Chromosome labelling was obtained at 365/12 nm excitation and the fluorescence was imaged through a 397 long pass filter.

An Olympus microscope (Provis AX 70) with a 100 W mercury arc lamp was used to take analogue images with the standard Olympus PM-DI 35 camera using a Fuji Provia 400 film.

Fluorescence images were obtained through an UplanFI 40× dry objective (n. a., 0.75), a PlanApo 60× oil immersion objective (n. a., 1.40) and an UplanApo 100× oil immersion objective (n. a., 1.35).

MT were observed using an Olympus filter set (U-MWIBA) with 460–490 nm excitation and 515–550 nm emission. Actin labelling was obtained using an Olympus filter set (U-MWIG) with 520–550 nm excitation and 580 nm emission. DAPI labelled nuclei and chromosomes were visualised using an Olympus filter set (U-MWU) with 330–385 excitation and 420 nm emission.

## Results

### Staining of microtubules

The distribution of MT was investigated using the monospecific anti-body against  $\alpha$ -tubulin.

Control root cells and oblong callus cells from *Picea abies* (L.) KARST. showed cortical MT arrays in interphase, which extended throughout the cell, arranged perpendicular to the cell axis and parallel to each other

(Fig. 1a). In contrast to oblong callus cells, where the axis of division site was indicated, unorganised cortical MT were observed in spherical callus cells of control material (Fig. 1b). Another MT arrangement during the interphase was noticed in association with the nucleus (Fig. 1c). Elongating callus cells and non-dividing root cells showed a longitudinal and parallel extension of MT between the nucleus and the cell membrane, while a MT radiation throughout the cytoplasm was seen in spherical callus cells. Furthermore, MT formed a thick network around plastids, which extended towards other organelles and the cell membrane in all investigated callus cell types (Fig. 1d).

In root and callus cells, exposed to GSH, alterations were observed affecting the form of MT. In cells treated with 500  $\mu\text{M}$  and 1000  $\mu\text{M}$  GSH solution the cortical MT appeared shortened and wavy (Fig. 1e), sometimes the parallel arrangement of MT remained unchanged. Additionally, in root and callus cells increased alterations of cortical MT in form of tubulin-positive dots were observed at both GSH concentrations as well (Fig. 1f).

The observed PPBs were lying perpendicular to the elongation axis in untreated root cells and oblong callus cells with polar cell extension (Fig. 2a). Besides that, only a diffuse PPB was noticed in control callus cells with radial extension. Cells of GSH treated roots occasionally did not form the PPB in the centre of the cell. Furthermore, the PPBs were oriented transverse to the direction of growth. The MT, which were not involved in the PPB, were shortened or they were visible as bright fluorescent dots in the cell cortex (Fig. 2b).

At the beginning of the treatment, most of the cells were at an interphase stage, fewer cells were observed during cell division. Those cells did not show significant modifications in the structure and organisation of MT. Root cells at prophase (Fig. 2d) contained MT arrays forming MT caps at both poles (Fig. 2e). Well abundant kinetochore MT extended from chromosomes to the poles in the meta- and anaphase of the treated cells (Figs. 2f-i). Blind-ended kinetochore MT were not observed. Additionally, no impacts were visible in the organisation of the MT (Fig. 2k) in the telophase of GSH treated cells (Fig. 2j).

However, we observed GSH treated cells in metaphase and anaphase with a transverse position of the equatorial plane and rotated poles. The MT extended within both poles, transverse to the longitudinal axis of the cell growth (Fig. 2c).

### Staining of actin filaments

The distribution of actin filaments in GSH treated root and callus cells was investigated in order to correlate the observed GSH induced modifications of MT with possible alterations of actin filaments. For this research the GSH treated material and control samples were stained with

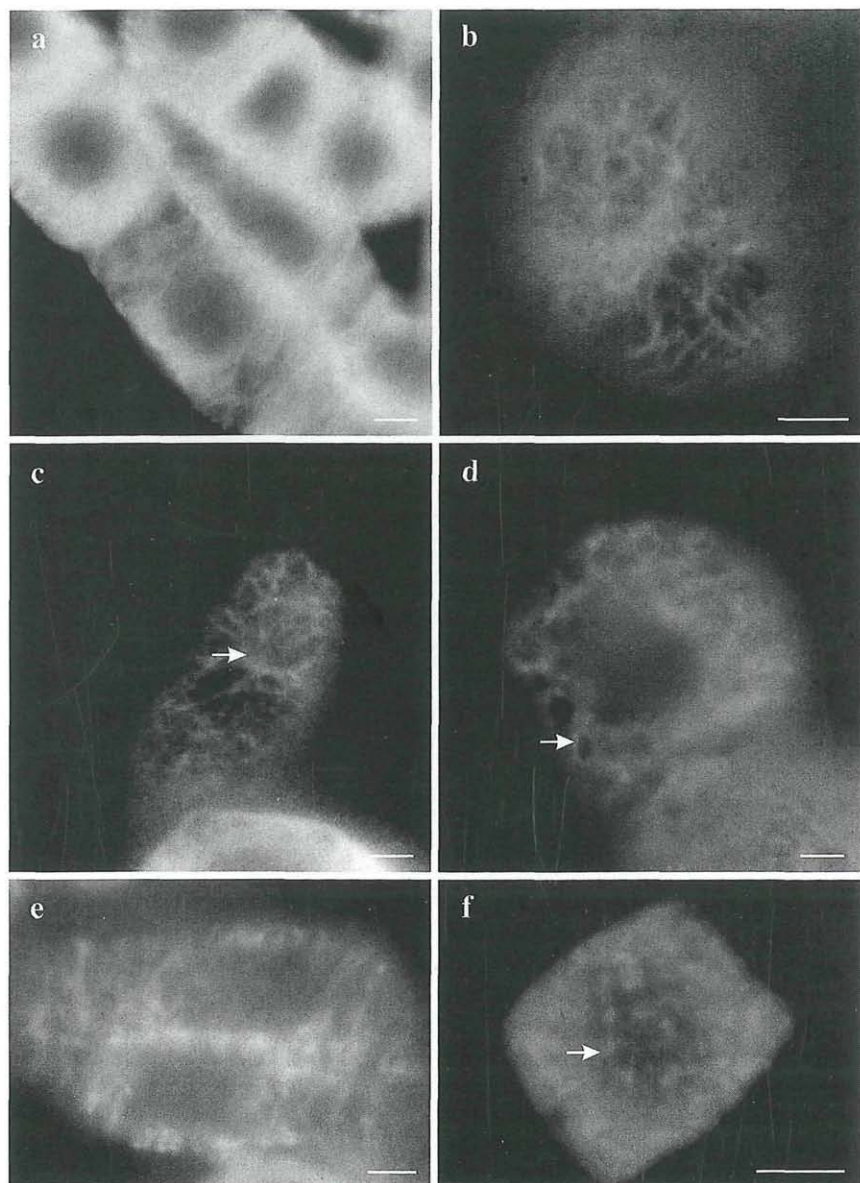


Fig. 1. The MT arrangement in root (a, c, f) and callus cells (b, d, e) of *Picea abies* (L.) KARST. Control cells (a–d), GSH treated cells (e–f). A cortical network with MT arranged parallel to each other and perpendicular to the cell-growing axis (a). In spherical callus cell unorganised cortical MT are observed (b). MT extend from the nucleus (arrow) towards the plasma membrane (c). A thick MT network occurs around the plastids (arrow) (d). After GSH treatment the MT appear shortened and wavy (e) or they are seen as tubulin-positive dots in cortical areas (arrow) (f).

Bars = 10  $\mu$ m.

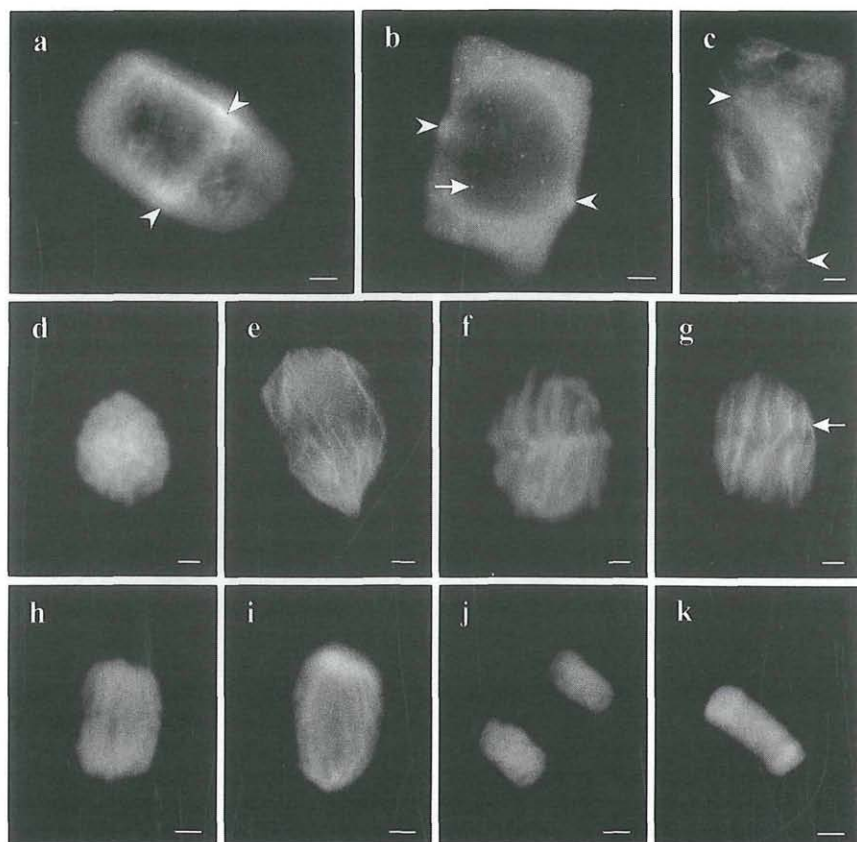


Fig. 2. Chromosomes (d, f, h, j) and division specific arrays of microtubules (a, b, c, e, g, i, k) observed in root meristem cells of *Picea abies* (L.) KARST. Control (a), GSH treated cells (b-k). PPB lying perpendicular to the cell growing axis (arrowheads) (a). The PPB of GSH treated cell are oriented transverse to the direction of growth (arrowheads); the remained cortical MT are visualised as bright fluorescent dots (arrow) (b). Rotated anaphase spindles with transferred poles (arrowheads) (c). A cell in prophase with condensed chromosomes (d) and spindle caps focused at both poles (e). A metaphase cell with chromosomes organised at the equatorial plane (f). Metaphase spindles forming "microtubule fir trees" (arrow) (g). An anaphase cell with chromosomes (h) and kinetochore MT focused at the poles (i). Telophase showing chromosomes at the poles (j) and phragmoplast microtubules (k). Bars = 10  $\mu$ m.

RLP using the glycerol permeation method, which allowed the labelling of actin filaments in cells without fixation. We achieved a long lasting actin staining with well-preserved actin filaments in the epidermal cell layer, in the cortical cells underneath and in the columella of the spruce root tips. In these cells actin cables lying in the cytoplasm were the most prominent

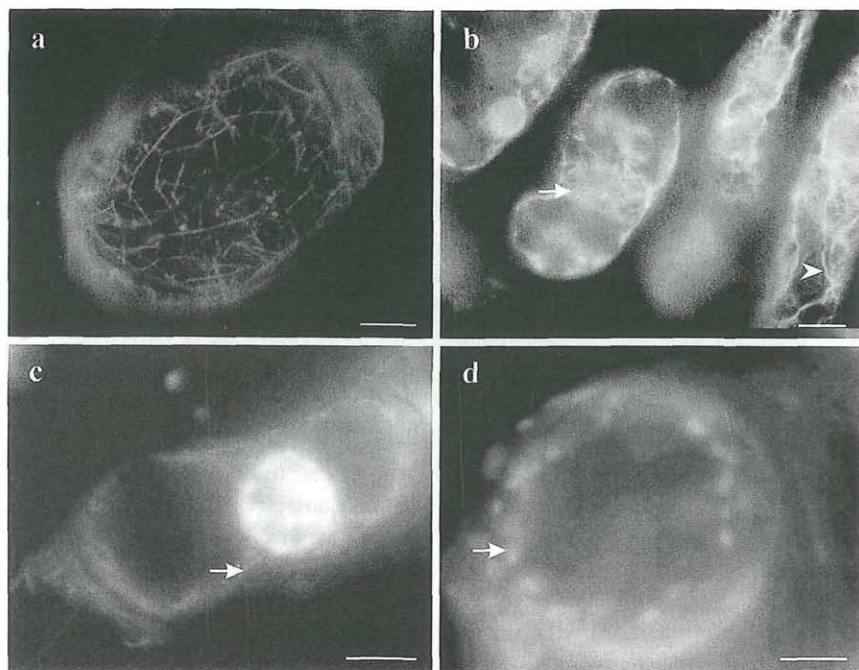


Fig. 3. Distribution of actin filaments in root (b) and callus cells (a, c, d) of *Picea abies* (L.) KARST. Controls (a, b), GSH treated material (c, d). DAPI staining of nucleus (c). Spherical callus cell with cortical MF network and actin filaments, which extended throughout the cytoplasm (a). Actin network in association with the nucleus (arrow), transvacuolar actin bundles (arrowhead) and actin layers along the plasma membrane were visible in root cells (b). Amorphous actin-positive fluorescence is noticed in association with the nucleus in GSH treated callus cell. Strands emerging from the nucleus are shortened and end blind in the cytoplasm (arrow) (c). Diffuse fluorescence was seen at the plastids (d). Bars = 10  $\mu\text{m}$ .

feature. Furthermore, actin layers were distributed along the plasma membrane (Fig. 3b). Actin bundles surrounding the plastids were evident within the columella cells, while cortical arrays were observed seldom.

The callus cells could also be successfully labelled with the applied method. Thick actin cables as well as thin cortical actin filaments were present in most of the cells. Elongated callus cells exhibited parallel arranged actin filaments, an actin extension throughout the cytoplasm was characterised in spherical callus cells (Fig. 3a). Furthermore, a typical actin network around plastids was visible in callus cells, connecting the plastids and binding to the plasma membrane. A dense actin network was visible around the nucleus in form of a red fluorescent envelope in cells of both plant materials (Fig. 3b).

In GSH treated root tip cells and callus cells similar impacts were observed on actin filaments as already described on MT, independent of the GSH concentration. The cortical actin filaments remained as small, tortuous fragments or bright fluorescent dots at interphase. Mostly only a diffuse fluorescence was noticed. The transvacuolar actin cables appearing deeper in the cytoplasm could not be recognised. The actin baskets around the nuclei persisted, however they were amorphous in appearance and only seldom observed as arrays that emerged from the nucleus to the plasma membrane. They ended blind in the cytoplasm (Fig. 3c). Only a diffuse fluorescence was seen around the plastids; an anchoring of plastids at the plasma membrane was lacking (Fig. 3d).

### Discussion

The data presented in this study revealed that the exogenously applied GSH caused serious modifications in the form and arrangement of the cytoskeleton in root and callus cells of *Picea abies* (L.) KARST. Both plant materials were used, as it is known that epidermal MT respond differently from other tissues to certain growth-altering stimuli (BLANCAFLOR & al. 1998).

After the treatment with both GSH concentrations shortened and tortuous MT were observed in all cell types during the interphase. Moreover, in GSH treated cells tubulin-positive dots in the cortical cell area were noticed. A similar phenomenon was described in colchicin treated cells of *Vigna sinensis* (APOSTOLAKOS & al. 1990) and was also observed in cells of *Nicotiana tabacum* after treatment with the plant defense elicitor cryptogein (BINET & al. 2001). In comparison, the interphase cells of control spruce material, showed an intact cortical MT network delineating the plasma membrane. In root and callus cells, which undergo a cell differentiation in a polar manner, parallel arrays arranged perpendicular to the cell-growing axis were evident. Whereas, in spherical callus cells the cortical MT were lying unorganized, as previously described in suspension culture cells of *Solanum tuberosum* (COLLINGS & EMONS 1999).

In GSH treated spruce material similar impacts were observed on cortical actin filaments as described on cortical MT network of the interphase cells. Alterations were also visible on transvacuolar actin cables, nucleus- and plastid-associated filaments. They were observed in form of short stretches or were noticed as diffuse fluorescence structures. Arrays connecting the nucleus and the plasma membrane were observed seldom. Otherwise, MF arrangements were very well preserved within control cells according to other authors (JUNG & WERNICKE 1991, CLEARY 1995, LAZZARO 1996, De RUIJTER & EMONS 1999, KANDASAMY & MEAGHER 1999).

The determination of the main direction of cell expansion, mediated by cytoskeletal elements, has a key role in organogenesis (KOST & al. 1999).

Based on this fact, it may be suggested, that through the disruption of the cortical MT and MF network in GSH treated cells the cell elongation could be limited or is not able to take place in a polar way (e.g. KOST & al. 1999, HASEZAWA & KUMAGAI 2002). Furthermore, the alterations on nucleus-associated actin network may also indicate possible abnormalities during the cell division, since actin filaments are involved in the transport of the nucleus to the cell centre prior to the mitotic cycle (NICK 1999).

Another effect of the GSH treatment is the low number of cell divisions, which was observed in both cell types and was previously reported on the same plant material upon all GSH concentrations (ZELLNIG & al. 2000, MÜLLER & al. 2001). The depressed division rate may indicate that the destabilization of cytoskeletal elements in interphase disabled the cell to enter the mitotic cycle. It may be suggested, that GSH induced alterations in the cytoskeleton inhibited the cell elongation and division and limited the plant growth. This hypothesis is supported by investigations showing, that GSH treated spruce seedlings had less developed roots and shoots in comparison to non-treated material (MÜLLER, unpublished data). Moreover, stunted phenotypes were also reported in the transformed tobacco lines overexpressing  $\gamma$ -glutamylcysteine-synthetase, resulting in increased GSH levels in these cells (CREISSEN & al. 1999).

In this study, GSH induced alterations in the arrangement of MT were also found in cells entering the mitotic cycle. The PPBs, which are a characteristic feature of the  $G_2$ -phase cells having an important role by marking out the site of cell division (FOWKE & al. 1990, CLEARY & al. 1992, MARC 1997, NICK 1999), were arranged perpendicular to the elongation axis in control cells, as also reported recently in other papers (TAUTORUS & al. 1992, NICK 1999). In contrast, PPBs of the GSH treated root cells were lying transverse to the direction of cell growth. The alterations of the cytoskeleton observed in the GSH treated cells during the interphase could be one of the reasons for this phenomenon. Since it is known that the MF are involved in the development of the PPB and the position of the division site (MINEYUKI & PALEVITZ 1990, LLOYD 1999), the question arised, whether altered actin filaments were indirectly involved in the changed position of the PPB. Rotated spindles observed after cytochalasin treatment (LLOYD 1999) support this hypothesis. One may speculate, that GSH might also have an indirect influence on the plasma-membrane-associated sites of the cytoskeleton, which determine the position of the PPBs, mitotic spindles and the phragmoplast (BALUŠKA & al. 1998). Disruption of those proteins may change the position of the PPB. No detailed statements about the position of PPBs in GSH treated spherical callus cells in comparison to the control material could be made, since they are less organised and less defined as in root meristem cells (e.g. TAAUTORUS & al. 1992, COLLINGS & EMONS 1999).

Since the PPB determines the position of the future division plane and the division spindle (NICK 1999), alterations in the symmetry of the division site were expected after GSH treatment. Most of the dividing cells of the GSH treated material showed spindle poles lying in the direction of the growing axis. But also asymmetric divisions with rotated spindles in metaphase and anaphase were observed, similar to the results reported by GIMÉNEZ-ABIÁN & al. 1998 in root meristem cells of *Allium cepa* after caffeine treatment.

During the cell division the MT arrays appeared unaffected short and wavy kinetochore MT were not evident. The organisation of the MT during the cell division correlated with the MT arrangement described in protoplasts of *Picea glauca* and *Picea mariana* (FOWKE & al. 1990, WANG & al. 1991).

Taken together, the investigations showed that exogenously applied GSH affected the structure and arrangement of MT and actin filaments and supported recent reports (CREISSEN & al. 1999, ZELLNIG & al. 2000, MÜLLER & al. 2001) indicating that elevated concentrations of GSH in different plant tissues might be deleterious. How GSH was able to cause such impacts on the cytoskeleton is not clear yet. Especially because the externally applied GSH concentrations in the present experiments were within the range of physiological concentrations of GSH in plant cells of up to 5 mM (NOCTOR & FOYER 1998). One explanation could be that the exogenously applied GSH changed the GSH status inside the cells, which caused malfunction of nuclear proteins and changes in the thiol metabolism (ZELLNIG & al. 2000) since GSH is involved in the conformation and the redox status of protein thiol groups (KUNERT & FOYER 1993).

The current opinion, which contributes GSH an intrinsic value as a flavour precursor in food and as a medicinal product (NOCTOR & al. 1998), should be seriously questioned and further investigated in more detail.

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