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Microfibril orientation during cambial xylem derivatives differentiation in stems of Scots pine trees grown under polluted environment

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Abstract: The arrangement of microtubules during differentiation of tracheids in wood of pine trees grown under polluted environment was examined. Using immunofluorescent staining of the microtubules the changes of microtubules arrays were observed. In cambial region and in tracheids at the stage of radial cell enlargement the microtubules were arranged randomly whereas during subsequent stage of differentiation changed rotatively from a flat Z – helix to a steep S –helix. It was observed that in the samples collected from polluted site among the tracheids of early wood the cells with abnormally thin or/and thick lignified secondary wall were observed. It seems that this kind of cell wall structure abnormality could be connected with altered number of microfibril layers deposited during a given orientation of rotating microtubules.

Keywords: air pollution, cambium, microfibrils, Scots pine, tracheids, wood

INTRODUCTION

It is generally assumed that cortical microtubules, one component of cytoskeleton composed of tubulin dimers, control the orientation of new deposited cellulose microfibrils (Ledbetter and Porter, 1963). It is also known that during formation of the secondary cell wall of tracheids or fibers, the arrays of the cortical microtubules are well ordered and their orientation changes progressively as the process of differentiation proceeds (Abe et al. 1995; Funada et al. 1997). The orientation of cellulose microfibrils on the innermost surface of the cell walls changes in a similar way to that of the cortical microtubules, what provide strong evidence for the co-alignment of the cortical microtubules and the cellulose microfibrils during formation of texture of the tracheids cell wall.

In conifers, the process of tracheids differentiation is categorized by following stages: cambial cell division, radial cell enlargement, cell wall thickening, lignification and cell death (Wodzicki, 1971). When the growth of radial dimension is ceased, the secondary cell wall with well-ordered cellulose microfibrils is deposited on the inner surface of the primary wall. With the onset of the secondary wall deposition, the process of lignification starts at the middle lamellae, progressing to the primary and secondary wall. Then, the autolysis of cytoplasmatic contents occurs and the mature tracheids are dead cells with thick, lignified cell wall. Their secondary wall is made up of multiple layers called S1, S2 and S3. The S2 layer is the thickest.

The external factors (air pollutions included) affect the cambial cell derivatives differentiation (Zajączkowski, 1996, Savidge, 1996, Tulik and Rusin, 2005). The aim of our investigations, therefore, was to examine the changes in the array of cortical microtubules during consecutive stages of wood formation in pines growing under environmental stress conditions.

MATERIAL AND METHODS

The pine forest stands were located in Miasteczko Śląskie, in southern part of Poland in neighbourhood of zinc and lead smelting factory. The samples containing phloem, cambium, differentiating tracheids and mature wood were taken at breast height (1.3m) from stems of Scots pine trees (*Pinus sylvestris* L.) during the season of active cambial growth in 2003. The age of pines was 20-28 years. The samples were obtained from two forest stands. The first was located in the most polluted area in Imielów forest district, and the second - in Mikołeska, the district free of air pollutants, which served as a control.

The orientation of microtubules was studied using method of immunolocalization of tubulin.

Small blocks containing cambium, phloem and xylem derivatives were excised from collected samples and treated as described elsewhere (Bohdanowicz et al. 2005). In brief, tissue blocks were fixed with 4% paraformaldehyde, embedded in Steadman's wax and sectioned transversely, radially and tangentially at thickness of approximately 30 μm with a rotary microtome. Sections were stretched on glass slides, dewaxed and rehydrated. Tubulin was stained with mouse anti β-tubulin (SIGMA-Aldrich) and antimouse FITC-conjugated goat antibody. Nuclei were counterstained with 1 μg/ml 4',6'-diamidino–2-phenylindole dihydrochloride (DAPI) in PBS. Specimens were mounted with DAKO fluorescent mounting medium and observed under magnifications of x40 or x100 using Olympus AX80 fluorescent microscope equipped with appropriate filters and CAMEDIA C4040 camera.

RESULTS AND DISCUSSION

The microscopic examination of samples, taken from the trees grown in polluted and control sites showed the successive changes in the orientation of microtubules starting from the cambium to the mature tracheids (Fig. 1).

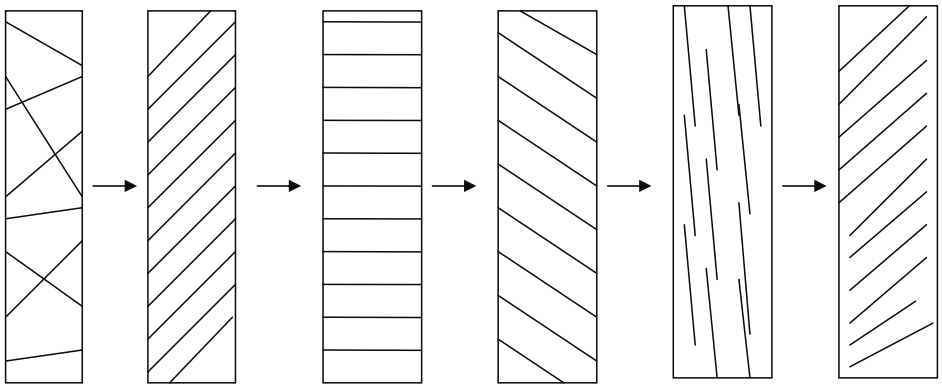

Fig. 1. Scheme of the microtubules orientation during consecutive stages of tracheids differentiation.

The arrays of microtubules in the cambium and in differentiating tracheids, that were at the stage of radial enlargement - when primary cell wall forms, were arranged at random, both in samples from polluted and control sites (Fig. 2A, B).

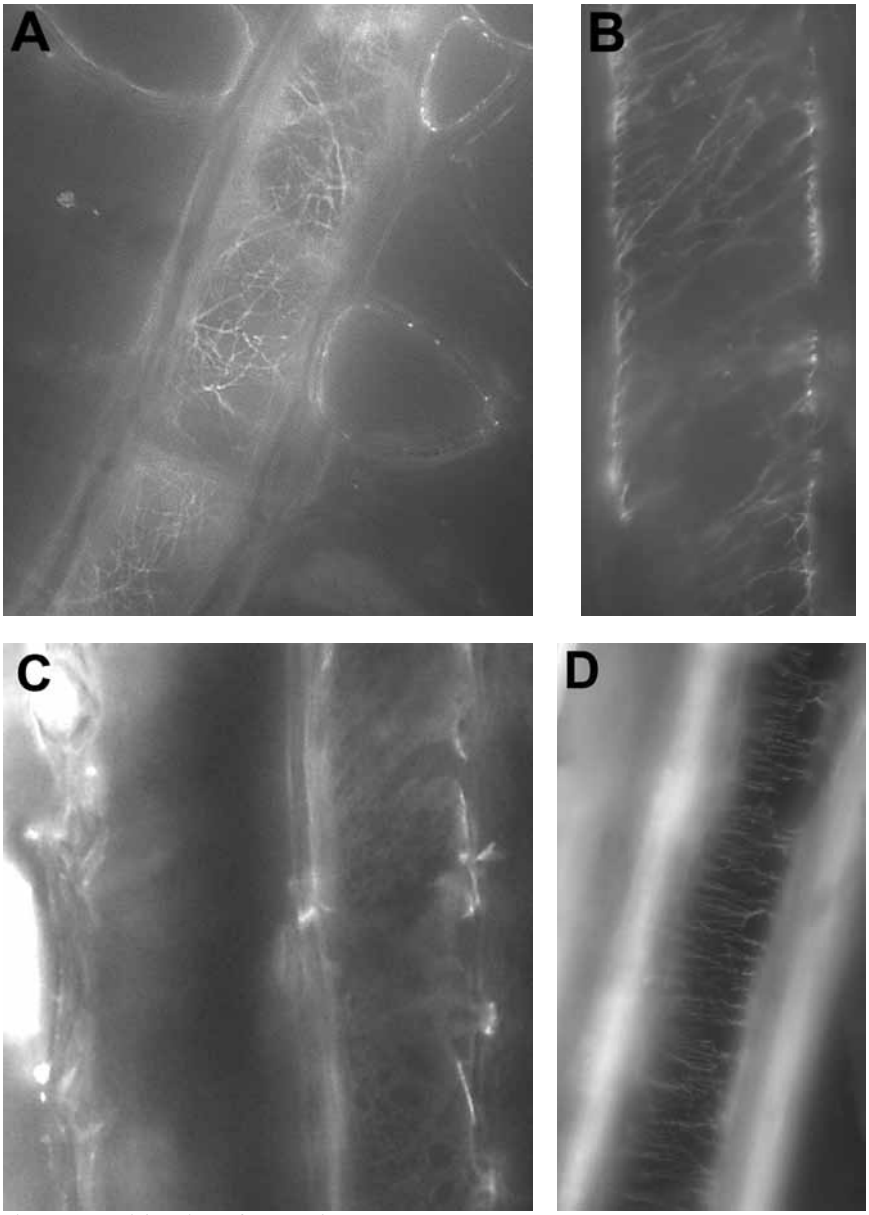

Fig. 2. Tangential sections of *Pinus sylvesris* stem.
A. Randomly oriented microtubules in cambial cell in sample from polluted site.
B. Longitudinal orientation of the microtubules in differentiating tracheid during stage of radial cell enlargement in sample from polluted site.
C. Helically arranged microtubules in differentiating tracheids during the stage of cell wall thickening and lignification in sample from polluted site.
D. Transversely oriented microtubules during the stage of cell wall thickening and lignification in sample from polluted site.

At the early stage of radial cell enlargement the predominant orientation of the microtubules was longitudinal and it changes to transverse as the cell expansion proceeded. It was also observed that the microtubules in the differentiating tracheids disappeared locally in circular regions. It means that this is the region where intertracheal bordered pits could be formed, eventually. Similar regions were observed in differentiating tracheids of *Abies sachalinensis* (Abe et al. 1995) and *Taxus cuspidata* (Funada et al. 1997).
When the stage of tracheids enlargement ceased, the microtubules orientation changed progressively from a flat helix to a steep Z-helix and then clockwise rotation was also noted both in samples from polluted and control sites (Fig. 2C, D). This observation is agreed with results published in papers concerning plant microtubules (see Nick, 2000). However, we observed that in trees grown in environment polluted with heavy metals, among the tracheids of early wood the cells with abnormally thin or/and thick lignified secondary wall were observed. This kind of cell wall structure abnormality could be connected with altered number of microfibril layers deposited during a given orientation of rotating microtubules. This example indicates that the dynamics of the cortical microtubules reorientation could be altered by unfavorable environmental factors, such as heavy metals pollution.

CONCLUSION

1. The successive changes in the orientation of microtubules during the primary and secondary wall deposition are observed both in differentiating tracheids from control and polluted environment.
2. The rotational changes of the microtubules orientation during secondary cell wall deposition might have been modified by air pollutions.

It seems that studies concerning arrangement of microtubules during formation of secondary cell wall of tracheids should be continued. The microtubules play an important role during plant morphogenesis. Therefore, the control of the process of wood formation by manipulation of microtubules might provide new tools to improve wood quality.

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Streszczenie: *Orientacja mikrotubul podczas różnicowania pochodnych kambialnych na stornę drewna w pniach sosen (Pinus sylvestris L.) wyrosłych w zanieczyszczonym środowisku.* Orientacja mikrotubul, elementu cytoszkieletu, warunkuje orientację mikrofibryl celulozowych w ścianie komórkowej cewek drewna sosny. Przeprowadzono badania na próbkach obejmujących kambium i różnicujące się drewno, pobranych z pnia głównego sosen (*Pinus sylvestris* L.) rosnących w środowisku zanieczyszczonym metalami ciężkimi (w bliskim sąsiedztwie huty cynku i ołowiu, Miasteczko Śląskie) oraz w środowisku czystym. Obserwacje mikroskopowe przy użyciu techniki imunofluorescencyjnego wybarwiania mikrotubul wykazały, że ich układ zmieniał się dynamicznie, zarówno u drzew rosnących w środowisku czystym, jak i zanieczyszczonym. W komórkach strefy kambialnej obserwowano nieuporządkowany układ mikrotubul, który podczas postępujących faz różnicowania się drewna ulegał stopniowym zmianom. I tak, na początku fazy wzrostu promieniowego różnicujących się cewek przyjmował układ podłużny, natomiast w końcowym etapie tej fazy mikrotubule przyjmowały położenie poprzeczne. Podczas kolejnej fazy różnicowania się drewna, czyli odkładania wtórnej ściany komórkowej, lignifikacji oraz autolizy protoplastu, mikrotubule we wszystkich analizowanych próbkach tworzyły uporządkowane szeregi, a ich układ zmieniał się z płaskiej Z helisy do stromej S helisy i znowu płaskiej helisy Z. Zaobserwowano jednakże, że pomiędzy cewkami drewna wczesnego, w próbkach sosen wyrosłych w zanieczyszczonym środowisku, występują cewki z cienką i/lub grubą ścianą komórkową. Tę anomalię dotyczącą grubości ściany komórkowej cewek wiążemy z zaburzeniami dynamiki zmian orientacji mikrotubul, a mianowicie z możliwością tworzenia odmiennej liczby warstw mikrofibryl celulozowych podczas rotacyjnych zmian układu mikrotubul.

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