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SHORT COMMUNICATION

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Cytomixis in shoot apex of Norway spruce [*Picea abies* (L.) Karst.]

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Abstract Atypical cell walls and nuclei were observed in the apex of Norway spruce shoots from late April to early May on the material collected from a few grafts of a clone of Norway spruce growing on an experimental area. Images of ultrastructure attest to cytomixis. The phenomenon of cytomixis has previously been described in various plant material, both in the meiotic and mitotic cells, but this is the first report of cytomixis in gymnosperms.

Keywords *Picea abies* (L.) Karst · Gymnosperms · Apical meristem · Procambium · TEM

Introduction

As early as in 1885, Tangl found that when the epiderm of onion scales is injured, traumatotropic movements of nuclei towards the wound can be seen in the neighbouring undamaged cells (cited in Tarkowska 1960). Similar migrations of nuclei were also observed in microspore mother cells as described in *Crocus vernus* by Körnicke (1901). He suggested that it was due to injury during the preparation of material. The term cytomixis was introduced by Gates (1911), who made analogous observations in *Oenothera*. He defined it as the process of migration of chromatin from the nucleus of one microspore mother cell to the cytoplasm of the adjacent one. The nuclei migrate through so-called cytoplasmic channels.

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The phenomenon of cytomixis was observed in various plant material: leaf epidermal and subepidermal layers (Tarkowska 1960, 1973), meristematic cells (Tarkowska 1960; Bobak 1976; Kostritsyna and Soldatov 1991), anther culture (Muranty et al. 2002), and pollen mother cells (Datta and Biswas 1984; Sinha 1985; Zheng et al. 1987; Chatha and Bir 1988; Falistocco et al. 1995; Souza and Pagliarini 1997; Zhang 2002; Pan et al. 2002). In the present paper, we report on cytomixis in meristematic cells of Norway spruce shoot apex. This is the first report on cytomixis in gymnosperms.

Materials and methods

Buds were collected from grafts of *Picea abies* (L.) Karst. clone 04-118 (Serwy) in a clonal collection at Zwierzyniec Experimental Forest near Kórnik, Poland (52°15′N, 17°04′ E). The grafts were 20 years old. Material was collected from the middle part of the tree crown every week from January until May.

Embryonic shoots were isolated from the buds and treated with 3% glutaraldehyde and 2% paraformaldehyde with $CaCl_2$ in 0.1 M cacodylic buffer of pH 6.8 for 4 h (all reagents: Poliscience) postfixed in 1% OsO_4 at room temperature for 2 h, in 0.1 M cacodylic buffer; contrasted with uranyl acetate; dehydrated in an ascending series of ethanols, followed by embedding in epoxy resin of low viscosity (Spurr 1969). Ultrathin sections were contrasted with uranyl acetate and lead citrate, and photographed under a transmission electron microscope JEM 1200 EX II (JOEL) at an accelerating voltage of 80 kV.

Results and discussion

Atypical nuclei were noticed in cells of spruce shoots collected and fixed from late April to early May (Fig. 1). The nuclei differed from usual ones both in location and appearance. Cell walls were occasionally atypical, as small fragments of the walls were missing and/or sometimes two

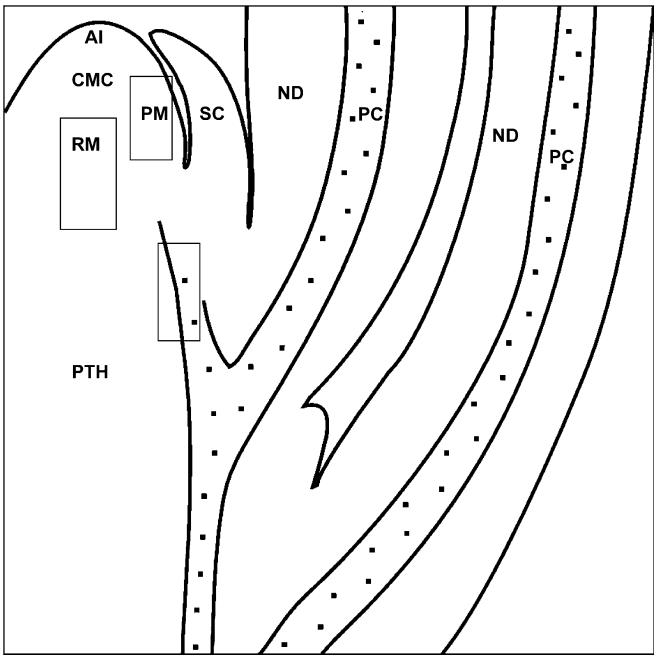

Fig. 1 Part of central longitudinal section of shoot. *AI* apical initials; *CMC* central mother cell; *RM* rib meristem; *PM* peripheral meristem; *PTH* pith; *ND* needle; *SC* scale; *PC* procambium. In the framed regions of the shoot cytomixis was visible in 20% of the shoots that were collected from late April to early May

fragments of walls of the same cell were absent (Figs. 2, 3, 4), but such observations were rare. A nucleus squeezed between cell walls was shared by two different cells and this was recorded mainly in cells of the peripheral meristem (atypical nuclei were visible in 10% of the cells of peripheral meristem). Less frequently, they were also observed in the rib meristem and procambial cells adjoining parenchyma cells (Fig. 4). Such images of ultrastructure attest to cytomixis, which has not been observed in Norway spruce or any other gymnosperm as yet.

The most frequently suggested causes of migration of nuclei include various stimuli, such as mechanical injury, temperature shock and centrifugation. However, not all mechanical injuries cause cytomixis. In the epidermis of onion scales, this phenomenon was observed after a rapid tearing of the epidermis, but not after cutting the scale or piercing it with a needle. Thus, cytomixis was not a direct response to wounding of the tissue (Tarkowska 1960). In spruce shoots, it was not due to any mechanical injury of the tissue during preparation. Observations were carried out with the same methods for more than 10 weeks but cytomixis was observed only in late April and early May, when the apical meristem was particularly active. We observed cytomixis not only in cells of the peripheral meristem but also between a procambial cell (i.e. meristematic) and a parenchyma cell. However, it is not possible to determine the direction of movement of the nucleus. The cause of cytomixis in embryonic shoots of

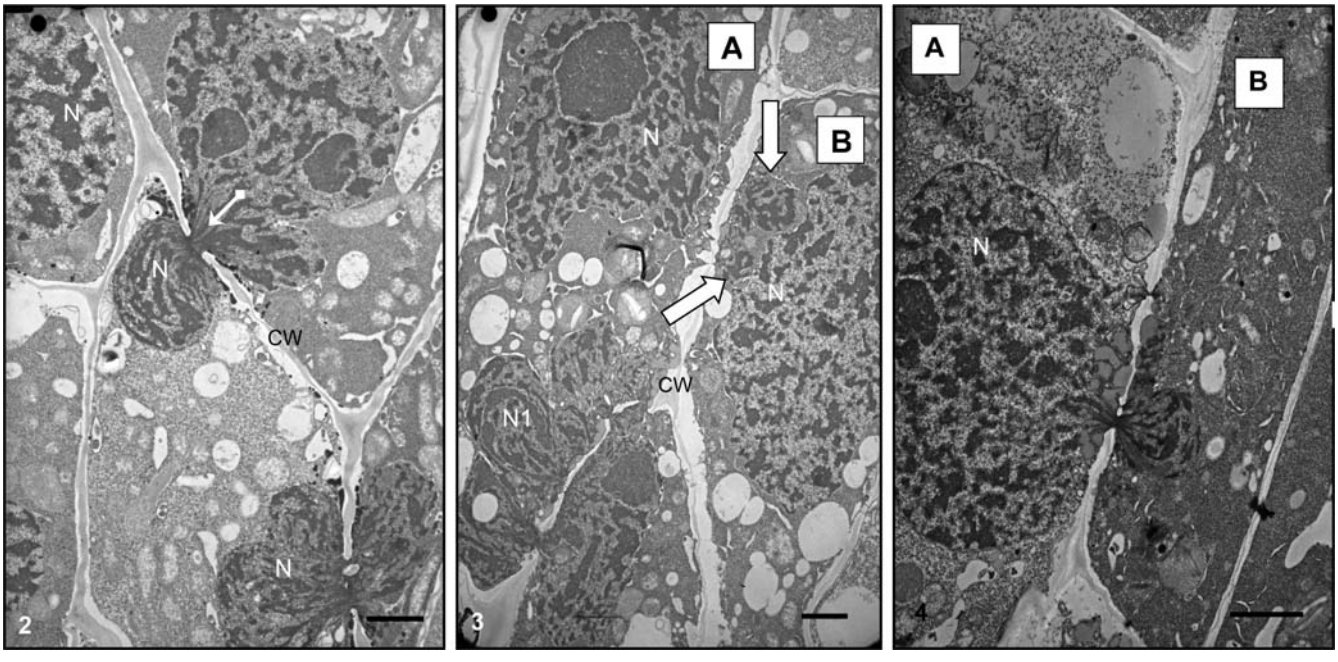

Fig. 2 In cells of the peripheral meristem, cytomictic channels can be seen (*arrow*), with the nucleus located in a gap in the cell wall. Two fragments of walls of the same cell of the peripheral meristem are missing. Aggregations of osmiophilic material are visible near the cytomictic channel. *N* nucleus; *CW* cell wall; *scale bar* 2 μm

Fig. 3 Fragment of cells of the peripheral meristem. In cell *A*, two nuclei are visible: one of them (*N1*) shared by two cells. In the neighbouring cell (*B*), close to the nucleus, circular fragments of nucleus can be seen (*arrows*) and the structure of its chromatin resembles that of the nucleus of cell A. *N* nucleus; *CW* cell wall; *scale bar* 2 μm

Fig. 4 Nucleus shared by a parenchyma cell (*A*) and a procambial cell (*B*). Aggregations of osmiophilic material are visible near the cytomictic channel. *N* nucleus; *CW* cell wall; *scale bar* 2 μm

spruce is still unknown. It could be associated with some differences in osmotic pressure, or result from disturbance to mitotic divisions (which seems probable). Maybe it can be a case of multiple nuclei, but on the other hand in gymnosperms endopolyploidy was not detectable (Nagl 1978; Barow and Meister 2003). It cannot be excluded however that gaps in cell wall, observable on micrographs, are the effect of lysis of a fully developed cell wall. Near these gaps concentrations of some kind of osmophilic material were observed. It is also possible that this phenomenon is related to transformation of the vegetative shoot apex into a generative one.

The importance of cytomixis lies in the formation of aneuploid and polyploid cells (Datta and Biswas 1984; Kostritsyna and Soldatov 1991); cytomixis causes an increase or decrease in chromosome number (Zheng et al. 1987). However, it is often regarded as a phenomenon related to cell degeneration. It has been observed, for example, in root cells of oilseed rape exposed to a cold stress (Egierszdorf, personal communication). This does not seem to be the case in spruce shoot apices. The buds were collected from a few grafts of the same clone of Norway spruce, growing on the same experimental area. Late frosts did not occur during the time of material collection (we have the meteorological data from the weather station nearest to the site, about 2 km away). We did not notice any macroscopic changes of shoots and buds, nor any needle discoloration nor any other damage caused by frost. No degenerative changes in cell ultrastructure were observed in this study, so cytomixis appears to be an element of normal development of the spruce shoots.

Further research is planned in order to explain the role of cytomixis in development of spruce shoot apices and to investigate the fate of the cytomictic cells.

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