The symplastic cell interconnection changes during maize and reed root cortex differentiation

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Introduction:

Symplastic connection via plasmodesmata (PDs) takes essential role in transport as well as in plant morphogenesis (for review see Oparka 2005). Electron microscopy based plasmodesmograms generally do not cover root symplast as complex system changing along the axis of differentiation (Ma *et* Peterson 2000, 2001, Zhu *et al.* 1998, for review see Gunning *et* Robards 76). Presented method based on immunolabeling of fixation induced callose synthesis enabled us to investigate active PDs in wider set of samples than electron microscopy routine. Current paper is focused on changes of PDs distribution within differentiating root cortex.

Methods:

Plants of reed (*Phragmites australis*) and maize (*Zea mays*) were grown in hydroponic ¼ Hoagland’s solution (Hoagland and Arnon 1950) under 16/8 hours photoperiod and 25/18°C temperature regime.

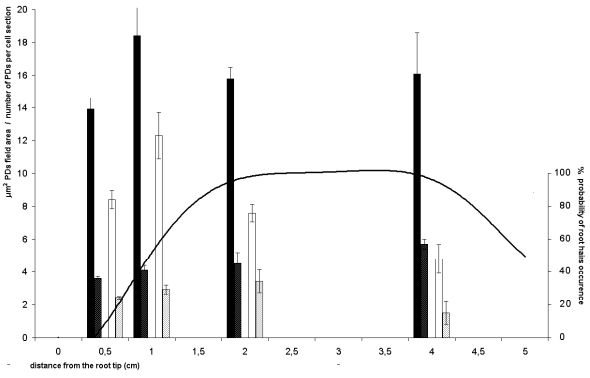
Root segments (~5mm) were sampled from rapidly growing roots at distances of 0.5cm, 1 cm, 2 cm and 4 cm from the root tip (n=15). Samples were fixed in 2% formaldehyde (1x PBS, pH=7.3; 3 hours under vacuum). Fresh cross-sections 200µm thick were prepared with aid of hand microtome. Callose deposition was detected with (1→3)-β-glucan antibody (Biosupplies Australia Pty. Ltd.; see Meikle *et al.* 1991). Spots of callose deposition indicative of plasmodesmata fields were quantified from fluorescence images (callose – FITC, cell wall – UV autofluorescence) with Lucia image analyser (LIM, Prague) with respect to radial and tangential cell walls. Estimated frequencies were related to length of joint cell walls and average length of cortical cells in particular zone.

Controls were fixed with ice cold MetOH or processed without fixation (very low callose signal), primary antibody was omitted (no signal) and inhibitor of callose synthesis 2-deoxy-D-glucose was applied (very low callose signal). Attachment of plasma membrane to PD rich cell walls was tested on thick sections (1 mm) fixed in 2% formaldehyde to lower the membrane dynamics. Cells were plasmolysed in 1,5 M sucrose and membranes were stained with FM4-64 (Biotium Inc., California). Electron microscopy of glutaraldehyde fixed and Spur′s resin embedded samples was managed in standard way (Hayat 2000).

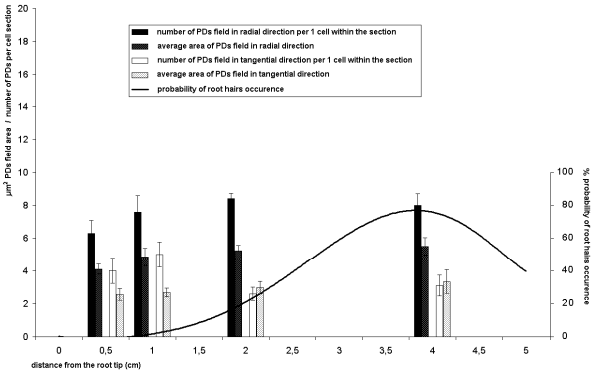
Results:

Fine diffused signal of primary plasmodesmata fields was observed in 0,5 cm segment but disappeared in basipetal direction during differentiation. Concurrently distinct large fields of

Maize (*Zea mays*)



Reed (*Phragmites australis*)



secondary PDs formed within the joint cell walls principally in radial direction. Dynamics of such changes differed between species. Parallel occurrence of small primary PDs together with newly formed fields caused temporary increase of PDs frequency in maize at 1cm behind the tip. The interconnection in tangential direction was significantly reduced within 2 - 4 cm from the apex. More proximal, fully differentiated zones were not systematically sampled as aerenchyma was already well developed and callose signal was poor or undetectable.

Discussion:

Deposition of callose within the neck of plasmodesmata is considered either permanent fyziological modulator of PDs conductivity (Currier 1957, Turner *et al.* 1994) and/or defense mechanism induced after tissue wounding, including sample processing artifacts (Redford *et a.l* 1998. Callose induced by FA treatment was proved to be reliable marker of PD field presence.

PDs related callose signal was never observed within cell walls where primary PDs originating from cell divisions were originaly present (e.g. cell walls facing intercellular spaces) and therefore we assume that material different from callose is responsible in permanent PDs closure.

Used method cannot provide absolute quantification of PDs density such as EM but allows wider screening of zones along the root, analysis of more samples and spatial reconstruction. However, both attitudes estimate only frequencies not directly related to physiological properties of the connections. We also presume, that fixation induced callose deposition indicates only functional "active" PDs.

We have originally hypothesized that formation of symplastically related domains can coordinate aerenchyma channels formation. However we were not able to confirm such supposition and changes in PDs distribution related to later formed aerenchyma distribution were not found. The pattern of symplastic interconnection changes was relatively stable and pattern of PDs reorganization was similar for both species. Observed changes correlate well with the distance from the root tip (R^2= 0,95 in maize and R^2= 0,93 in reed). We assume therefore that observed changes are related to mechanisms of general root differentiation.

Although observed dynamics of PDs change slightly differs for both species, the general impression is similar. The apical-most tissue (0,5 cm) can be characterised by presence of small PDs fields mostly evenly distributed in radial as well as tangential directions. More proximal and differentiated sections (2cm, 4cm) exhibit majority of PDs oriented in radial direction with higher individual area of the spots.

Decrease in tangential and increase in radial connection via PD fields takes place within the root hairs rich zone. We expect therefore, that zone of remarkable radial oriented connection with abundant secondary branched PDs corresponds with the zone of root most active in solutes uptake. However, such interpretation requires further experimental support.

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