Ultrastructural characterisation of the cell wall of *Arabidopsis thaliana* transgenic plants

1. INTRODUCTION

Cell wall is a fairly rigid layer surrounding the plant cell and located outside of the plasma membrane. Major events in plant evolution are often accompanied by remarkable changes in cell wall chemistry. Accordingly, plant cell wall has a number of several crucial functions: it lends the cell stability and determines its shape, influences its development, protects the cell against pathogens, and counterbalances the osmotic pressure. Its knowledge is valuable not only as a contribution towards our fundamental understanding of how plants 'work', but also as the basis of commercial attempts to predict and manipulate the growth, morphogenesis, disease resistance and digestibility of plants, the abscission of leaves and the ripening of fruit.

The primary cell walls of elongating cells are still elastic, a property that is lost in fully differentiated cells. It means that the growing walls must be loosened in order to expand. The primary wall is laid out during the first division of the cell and develops between the two daughter cells during early telophase. From a structural perspective, the physical properties of the primary wall govern the shape, size and growth rate of the plant cell as well as determine the plant's resistance to microbial digestion.

Primary cell walls are constituted by cellulose microfibrils (25-40% of the wall's dry weight), embedded in a matrix of polysaccharides (i.e pectins and hemicelluloses (such as xyloglucans) (60-75% of the dry weight), and smaller amounts of structural glycoproteins. In addition, there is a pectin-rich intercellular material, the middle lamella that cements the walls of adjacent cells (Fry, 2004).

During the last years, in our group we have been interested in the study of plant cell wall formation using maize as a model plant due to its scientific and economic relevance.

With the aim of identifying new proteins predominantly expressed in cell walls, we have purified a cell wall enriched protein fraction that was used to raise antibodies against it. These antibodies (raised against cell wall proteins) were then used to immuno-screen a maize elongation root region cDNA expression library. In those

conditions, several immuno-detected clones were isolated and fully sequenced. Among other cell wall proteins, one of these maize cDNAs putatively codes for a new Xyloglucan endotransglucosylase/Hydrolase (*ZmXTH1*).

Xyloglucan endotransglucosylase/hydrolases (XTHs) are enzymes involved in the reorganisation of cell walls by catalysing the cleavage and cross-linking of xyloglucan chains. Thus, XTH enzymes participate in cell wall loosening processes by cutting and rejoining the xyloglucan chains through the Xyloglucan endotransglucosylase (XET) enzymatic activity (Fry et al., 1992). XTHs are involved in several physiological processes such as cell elongation and growth, cell expansion, cell division, and also in processes that involve cell wall degradation.

Although several biochemical works have described the existence of maize XET activities *in vitro*, at present only one XTH has been reported in maize as a homolog of a flooding-induced XTH, Zm1005 (Saabs and Sachs, 1996). Therefore, we decided to characterise ZmXTH1 in maize. The presumably full-length cDNA is 1,225 bp long; the predicted protein has 281 amino acids, and possesses the typical domains of this enzyme family, such as a putative N terminal transmembrane domain, the catalytic domain that is homologous to the one corresponding to the *Bacillus* β-glucanase, a putative *N*-glycosylation motif and four cysteine residues in the central and C terminal regions of the ZmXTH1 protein. Phylogenetic analysis of ZmXTH1 reveals that this protein does not belong to the three major subfamilies of this class of enzymes, but rather to the smaller subgroup four in which only monocot species are so far represented. ZmXTH1 has very low XET activity and represents the first enzyme belonging to the subgroup four characterised in maize. Expression data indicate that *ZmXTH1* is expressed in elongating tissues and is modulated by culture conditions. In addition, *ZmXTH1* expression is also induced by gibberellins, thus suggesting a role of ZmXTH1 in maize cell wall modification processes.

To address the cellular function of ZmXTH1, we produced transgenic *Arabidopsis thaliana* plants that over-expressed ZmXTH1 under the control of the CaMV35S promoter.

The aim of the work performed during one month in the laboratory directed by Dr. Katia Ruel in the CERMAV-CNRS of Grenoble (France), was the characterisation at ultrastructural level, by transmission electron microscopy (TEM), of transgenic *Arabidopsis* plants overexpressing ZmXTH1, and the immunolocalisation of ZmXTH1 both in maize and in *Arabidopsis* transgenic plants by immunogold.

2. EXPERIMENTAL PROCEDURES FOR TRANSMISSION ELECTRON MICROSCOPY (TEM)

a. Material

The plant material used in this work were *Arabidopsis thaliana* 5-weeks old plants of wild type (Col 0) and transgenic lines overexpressing ZmXTH1 (35S::ZmXTH1), and *Zea mays* (W64) 3 dag seedlings.

b. Sample preparation: fixation and embedding

Small transverse slices both of basal regions of Arabidopsis stems of wild type (Col 0) and *35S::ZmXTH1* Arabidopsis plants, and of elongation region of maize roots, obtained by free-hand sectioning, were fixed in a freshly prepared mixture of 0.2% glutaraldehyde (v/v), 2% *para*-formaldehyde (w/v) in 0.1 M phosphate buffer (pH 7.0–7.2). After rinsing in phosphate buffer, samples were dehydrated in a graded ethanol series up to ethanol 90%, embedded in LR White resin (London Resin Company) and polymerized 24 h at 50°C.

c. Immunocytochemistry.

Immunolabeling was done on ultra-thin transverse sections (50 nm) floating downward in plastic rings passed on 50 µl drops of reactives deposited on parafilm, as described by Chabannes et al. (2001). The primary antibody used was the immunopurified anti ZmXTH1 at different dilutions (1-30/1-300) in the blocking buffer. The secondary marker was a 6 nm colloidal gold Donkey antichicken IgY(Jackson Immunoresearch) diluted 1:30. The diameter of the 6 nm gold particles was further enhanced using a silver enhancing kit from Amersham. Finally, thin sections were transferred on carbon-coated copper grids and poststained in 2.5% aqueous uranyl acetate. Observations were performed at 80 kV with a Philips CM 200 Cryo-electron microscope.

d. Cytochemical staining for polysaccharides.

Polysaccharides of cell walls were detected by PATAg (Periodic acid-thio carbohydrazide silver proteinate) staining, according to the method adjusted by Ruel

et al (1977). Sections floating in plastic rings were incubated during 1h 30 min in 5% periodic acid, briefly washed with distilled water, incubated 48 h in 0.2% thiocarbohydrazide, then in a graded acetic acid series from 20% up to 2.5%, in distilled water during 1 h and in 1% silver proteinate during 30 min. Finally, sections were rinsed in distilled water and transferred to carbon coated copper grids. Observations were performed at 80 kV with a Philips CM 200 Cryo-electron microscope.

3. RESULTS AND DISCUSSION

a. Ultrastructural morphology

We transformed Arabidopsis with the 35S::*ZmXTH1* construct. The expression analysis established that the transgene was successfully expressed in the Arabidopsis transformants; The most visible phenotype of the transgenic plants was a slight increase in the thickness of stem of transgenic plants. In addition, we analysed the ultrastructural morphology of cell walls by periodate thiocarbohydrazide-silver proteinate (PATAg) method. General contrasting of polysaccharides by this method allows the visualisation in transmission electron microscopy (TEM) of all the cell walls. Microscopic analysis showed alterations in cell walls. Stems from the transgenic plant often showed that the parenchyma cells had a thicker primary wall, which in several cases appeared more collapsed with respect to the one of wild type plants (Fig. 1). This alteration might be caused from a pronounced wall-loosening in the transgenic plants, due to the over enzymatic activity of ZmXTH1, driving the cell wall modification. In addition, the fact that this type of modifications were found only in parenchyma cells, that have only a primary cell wall, suggest that this modification is specific of ZmXTH1 acting on primary cells walls, and does not derives from a secondary effect of ZmXTH1 on other proteins. To achieve a better comprehension of the modifications caused by ZmXTH1, we are presently analysing the polysaccharide composition of the altered cell walls.

b. Immunocytochemistry

In experiments with *Arabidopsis* plants there was no labelling at any dilution of the antibody. We carried out several attempts, changing the aliquot of antibody, so this lack of labelling could be due to a problem related with the antigen that could have been damaged during the preparative procedures. In experiments with maize plants, we found some labelling in the cell wall of parenchyma cells, and this agrees with a possible localisation of ZmXTH1 in primary cell wall; however, these results need to be confirmed.

4. REFERENCES

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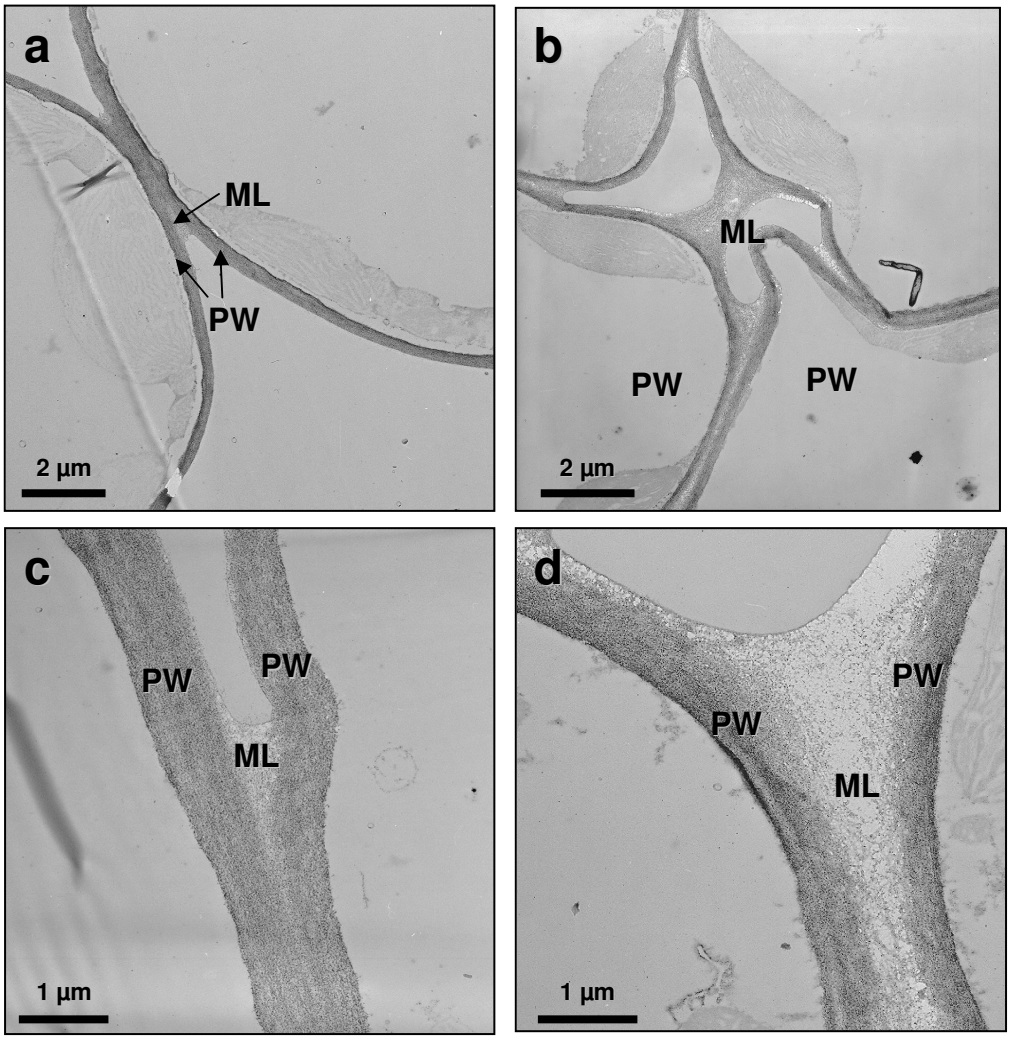

Fig. 1: Ultrastructural organisation of the parenchyma cells in the *Arabidopsis* wild type (a-c) and in the *Arabidopsis* transgenic plants over-expressing ZmXTH1 (b-d). PW: Primary Wall .ML: Middle Lamella