Cell wall remodelling and biosynthesis during pathogenesis of cyst nematodes

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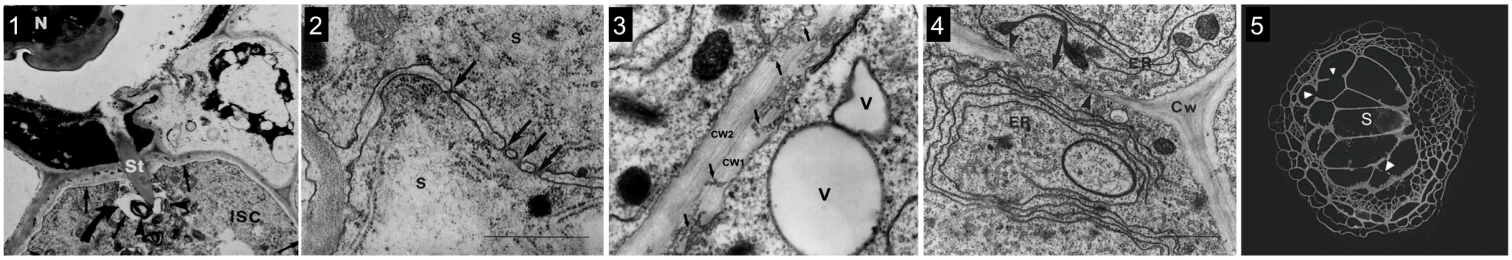
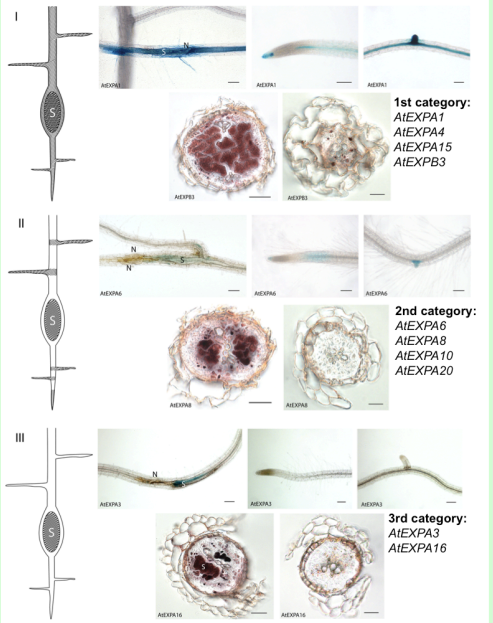
Cyst nematodes are severe root pathogens that cause big losses in the crop production. They induce specific feeding structures, so called syncytia, in the host roots by reprogramming of plant morphogenetic pathways. One of the remarkable events occurring in feeding sites is the extensive remodelling of the cell wall. During induction and development of the syncytium, opposed processes such as cell wall biosynthesis, dissolution and modification occur. This is related to several different tasks that syncytial cell walls have to fulfil. To counteract increased turgor they dramatically thicken what increases their mechanical strength. At the same time, however, they remain flexible to allow the cell expansion and permeable to enable the nutrient transport. At their surface ingrowths are deposited, while the abutting parts of the cell wall get dissolved and cell wall openings are formed. These dramatic changes in cell wall architecture are based on specific regulation of plant's cell wall biosynthetic and modifying enzymes by nematodes. In the focus of our work are expansins, glucanases, pectin lyases and cellulose synthases. We could show that certain members of these families play a crucial role in the induction and further development of syncytia. Moreover, by use of mutants or knock-out lines we observed effects that could potentially be used in the practical breeding for the nematode resistance.

Plant parasitic nematodes, especially beet root nematode *Heterodera schachtii*, cause large crop losses in the agriculture worldwide. Their host plants are sugar beet, potato, tomato, soybean and other plants. The infective juveniles hatch from the eggs in the soil and attracted by root exudates migrate to the roots and invade them. In the central cylinder, starting from the single initial cell (ISC, Fig.1), they induce a feeding site, called syncytium (S). From this structure the nematode withdraws nutrients during its entire life. After becoming sedentary, the second stage juveniles molt three times until they reach adultness. The females swell embedded in the root tissue while the males become mobile again and fertilize the females. After mating, females die as cyst containing eggs. First modifications of the syncytial cell wall are visible already at the onset of feeding site induction. In the initial syncytial cell the callose-like material is deposited around the stylet tip (St) and at the cell wall (Fig.1). Later, between syncytial and neighboring cells cell wall thickening and first cell wall dissolutions can be observed (Fig.2). At later stages, the plasmodesmata are widened and middle lamella dissolve and in this way cell wall openings are formed (Fig.3 and 4). Mature feeding sites are characterised by cell wall dissolutions (arrow heads) between big hypertrophied syncytial elements surrounded by strongly thickened cell walls (Fig.5).



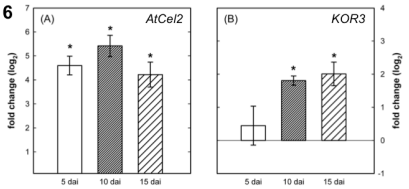
Expansins are cell wall loosening proteins that induce stress relaxation and extension of the plant cell wall without hydrolytic breakdown of its major component.
With different techniques such as syncytium-specific cDNA library, sqRT-PCR, prom::GUS lines, *in situ* RT-PCR and syncytium-specific Affymetrix GenChip we analysed the expression of the entire expansin family in syncytia induced by *H. schachtii*. Figures below show the summary of the results. Grey shading indicates gene expression; S – syncytium.

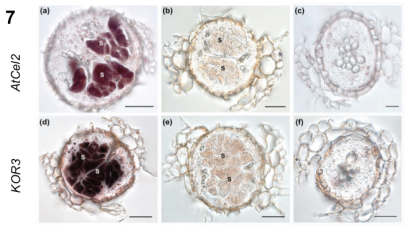


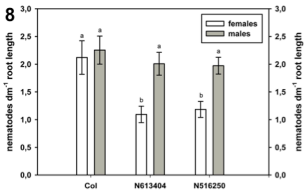
The fourth category comprises of two expansins, *AtEXPA7* and *AtEXPA18*, that are downregulated in syncytia.

Wieczorek *et al.* (2006) Expansins are involved in the formation of nematode-induced syncytia in roots of *Arabidopsis thaliana*. *Plant J.* **48**, 98-112.

Cellulases (EGases, endo-1,4-glucanases) are cell wall enzymes involved in hydrolysis of β-1,4-glucosidic linkages. There are 25 members of this family in Arabidopsis. They are divided into an α- and a β-subfamily which are secreted and act in the outermost cell wall and the γ-subfamily which are membrane-bound and function in the innermost cell wall. According to the Affymetrix GeneChip two genes, a secreted AtCel2 and a membrane-bound KOR3, are strongly upregulated in syncytia. We confirmed their syncytium-specific upregulation with the aid of qRT-PCR (Fig.6) and *in situ* RT-PCR (Fig. 7).





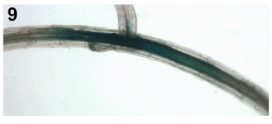


T-DNA lines of both, *cel2* (N613404) and *kor3* (N516250), showed a significant decrease in the number of females per dm root length. Number of males remained unchanged (Fig.8).

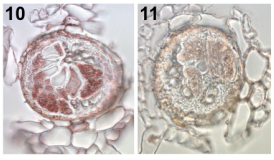
Wieczorek *et al.* (2008) Expression of endo-1,4-β-glucanase gene family in syncytia induced by *Heterodera schachtii*. *Plant J.*, **53**, 336-351

Pectate lyases belong to the systematic class of (1->4)-alpha-D-galacturonan lyases (EC 4.2.2.2). They catalyse the eliminative cleavage of deesterified pectin, which is a major component of the primary cell walls of many higher plants. Pectate lyases are mainly secreted by plant pathogens, they play a crucial role in the maceration of plant tissues.

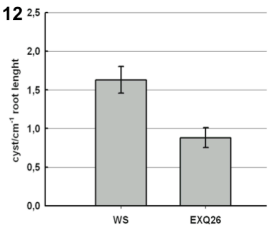
GUS activity of the pectate lyase At4g24780 promoter in syncytia induced by *H. schachtii* was visible at 3, 5 (Fig.9), 7, 10 and 15 dai. The upregulation of At4g24780 could be confirmed by the Affymetrix GeneChip approach and with qPCR (Tab.1). Localisation of the At4g24780 transcripts in syncytia was carried out with the aid of *in situ* RT-PCR. Staining is visible in a 5 dai old syncytium section (Fig.10). Lack of staining in the negative control (Fig.11). T-DNA line of At4g24780 (EXQ26) showed a significant reduction in the number of *H. schachtii* females (Fig.12).



5&15dai vs root	ddct 5dai	ddct 15dai
6,8	2,52	4,71



Additionally, differences in the size of the cyst between the wild type (WS) and the T-DNA line EXQ26 could be observed (Tab.2). The results show that five times more undeveloped cysts are present on the EXQ26 line. Further, less small and more middle cysts were observed on the wild type plants. The number of large cysts on both lines remains similar.



	total number of cysts	Cysts undeveloped	small	middle	large
WS	225	2,7%	16,0%	44,9%	36,4%
EXQ26	80	15,0%	20,0%	26,2%	38,7%

Cell wall modifications in the nematode feeding sites are very complex and include precisely spatially and temporally synchronised processes of cell wall extension, synthesis and in case of syncytia also degradation. Global analysis using GeneChip technology as well as detailed studies employing methods such as qRT-PCR, *in situ* hybridization and promoter::GUS fusions revealed an extended list of plant genes encoding cell wall-modifying enzymes and proteins that are expressed in nematode infected roots. Our studies indicate that expansins as primary factors might be involved in the syncytium-specific wall relaxation thus facilitating easily access to the cell wall for biosynthetic (KOR3), degrading (AtCel2) and modifying enzymes (pectate lyases). Moreover, significant reduction in the number of females on the mutant plants demonstrate how important cell wall modifications are for feeding site induction and expansion as well as nematode development and that such approaches could find successful application in the practical crop plant breeding.