Research Note

An *Arabidopsis thaliana* Pectin Acetyltransferase Gene Is Upregulated in Nematode Feeding Sites Induced by Root-knot and Cyst Nematodes

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By using differential display, gene expression was investigated in *Arabidopsis thaliana* roots shortly after nematode infection, and a putative pectin acetyltransferase (PAE) homolog (DiDi 9C-12) was found to be upregulated. PAEs catalyze the deacetylation of pectin, a major compound of primary cell walls. mRNA in situ hybridization experiments showed that the expression of DiDi 9C-12 was enhanced very early after infection in initiating giant-cells and in cells surrounding the nematodes. Later on, the level of DiDi 9C-12 mRNA was lower in giant-cells and transcripts were mainly found in parenchyma, endodermis, and pericycle cells of the root gall. Twenty days after infection, DiDi 9C-12 transcripts could no longer be detected. DiDi 9C-12 transcripts were also found in young syncytia and in the cells surrounding the expanding syncytium. Our results suggest that plant parasitic nematodes can modulate the rapid growth of the feeding cells and the expansion of the root gall by triggering the expression of DiDi 9C-12. PAEs, which probably act together with a range of other pectin-degrading enzymes, could be involved in softening and loosening the primary cell wall in nematode-infected plant roots.

Additional keywords: *Heterodera schachtii*, *Meloidogyne incognita*.

Sedentary endoparasitic nematodes are a serious threat to a variety of crop plants. Where in text would you like to cite DeFeyter et al. 1990? and include the most economically important classes, the root-knot (*Meloidogyne*) (Khan and Nasr-Esfahani 1990) and cyst (*Heterodera* and *Globodera*) (Cooke 1990; Sasser and Freckman 1987) nematodes. Endoparasitic nematodes maintain an intimate relationship with their host,

and for the completion of their life cycles, they depend on nutrients withdrawn from feeding cells that are induced inside the roots of a susceptible plant. Although the ultrastructure and function of the feeding cells are similar (giant-cells and syncytia, induced by root-knot and cyst nematodes, respectively), their ontogeny is different. Giant-cells arise from vascular parenchyma cells by repeated mitosis without cytokinesis (Huang 1985; Jones and Payne 1978), whereas syncytia are formed by dissolution of cell walls and fusion of the neighboring protoplasts (Jones and Dropkin 1975).

The plant cell wall is an elaborate extracellular matrix built from cellulose microfibrils interwoven with a network of other polysaccharides, such as hemicelluloses and pectins, and of proteins. Pectins are also the major component of the middle lamella of plant cells and of the intine of pollen grains. Pectins are composed of D-galacturonic acid polymers with interspersed methylgalacturonic acid residues, which in their turn, can be esterified by acetyl groups. The degree of acetylation modulates the properties of pectin molecules and contributes to the structural complexity of the pectin network (Mc Neil et al. 1984). Pectin acetyltransferases (PAEs; E.C. 3.1.1.1) are found in bacteria (Shevchik and Hugouvieux-Cotte-Pattat 1997), pectinolytic fungi (Searle-van Leeuwen et al. 1992), and plants (Breton et al. 1996; Oosterveld et al. 2000; Stratilova et al. 1998) and catalyze the deacetylation of esterified pectin. Deacetylation of pectin, diminishing the pectin backbone hydrophobicity, increases its solubility in water (Dea and Madden 1986). Removal of acetyl esters from the pectin backbone also makes the polysaccharide more accessible to pectin-degrading enzymes, such as pectate lyases. For example, pectate lyase secreted by *Erwinia* sp. cleaves only those galacturonic acid residues that are not acetyl-esterified (Davis et al. 1984).

Since the early 1990s, numerous efforts have been made to unravel the molecular details of plant-nematode relationships. Differential screening of cDNA libraries (Gurr et al. 1991; Lambert and Williamson 1993; Van der Eycken et al. 1996), subtractive hybridization (Bird 1996; Bird and Wilson 1994), and β -glucuronidase tagging (Barthels et al. 1997) have been used to identify plant genes upregulated in nematode-infected root tissue. Differential display (Liang and Pardee 1992) was applied to investigate the interaction between soybean and the soybean cyst nematode (Hermsmeier et al. 1998; Mahalingam et al. 1999) and to identify alterations in *Arabidopsis thaliana* (L.) Heynh. gene expression in the early compatible interaction with cyst (Hermsmeier et al. 2000) or root-knot nematodes (Vercauteren et al. 2001).

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Nucleotide sequence data reported are available in the EMBL database under the accession numbers AJ286349, AY050847, and X99348.

Through differential display, a 270-bp-long differential-display (DiDi) fragment was amplified with decamer 9 (5'-CGTGGCAATA-3') and anchor primer T₁₂MC (Genhunter Corporation, Brookline, MA, U.S.A.) from cDNA generated from *A. thaliana* (ecotype Columbia) roots infected with the root-knot nematode *Meloidogyne incognita* 2, 3, 4, 5, and 7 days after inoculation and not from control roots. This fragment was cloned and named DiDi 9C-12. After the sequence of DiDi 9C-12 (accession number AJ286349) had been analyzed, a 102-bp open reading frame was identified that coded for a 34-amino-acid-long peptide (Fig. 1). The deduced protein sequence of the full-length cDNA sequence (accession number AY050847) was similar to PAEs from different plant species, for instance, from *Vigna radiata* (Breton et al. 1996) (accession number X99348; 51% amino acid identity, BLAST score 1091, $P = 1.8 \times 10^{-109}$) (Fig. 1).

The spatial distribution of DiDi 9C-12 transcripts in infected and control roots was analyzed by mRNA in situ hybridization. Root galls and syncytia of *A. thaliana*, together with noninfected control roots, were dissected and fixed overnight at 4°C in 3% glutaraldehyde. Paraffin sections (10 µm) mounted on glass slides were hybridized to a ³⁵S-labeled sense and antisense transcript of DiDi 9C-12. Autoradiography was done by dipping the slides in NBT-2 Kodak emulsion (Sigma-Aldrich,

St. Louis) and by exposing them at 4°C until the signal was detected (de Almeida Engler et al. 2001; Vercouteren et al. 2001). Slides of root galls and of syncytia were developed after exposure times of 4 and 19 weeks, respectively. Very early after infection a clear DiDi 9C-12 hybridization signal was observed in initiating giant-cells and in cells surrounding the nematode (1 to 2 days postinfection [dpi]) (Fig. 2A and B). After infection (2 to 3 days), DiDi 9C-12 messenger RNA was detected both in giant-cells and in the surrounding cells (Fig. 2C). Five days after infection the signal was much lower inside the giant-cells but still strong in the surrounding parenchyma, endodermis, and pericycle cells (Fig. 2D). Seven days after infection, DiDi 9C-12 transcripts were mainly visible in the endodermis and pericycle cells (Fig. 2E), and after 20 days, no clear hybridization signal was detected (Fig. 2F). DiDi 9C-12 transcripts were also found to accumulate in syncytia, although more weakly than in root galls (considering the long exposure time). In young syncytia (3 dpi), the signal was spread throughout the syncytium (Fig. 2G), whereas in expanding syncytia (7 dpi) DiDi 9C-12 transcripts were localized in surrounding cells (Fig. 2H). In sections of healthy uninfected roots, a weak signal of DiDi 9C-12 was detected in the vascular cylinder (Fig. 2I) and a much stronger hybridization signal was seen in the root tips (Fig. 2J). Root sections that were hybridized to sense

<i>A. thaliana</i>	VGLTLIRGADS-GAVCLDGTLPGYHLHRGHGSGANSWLIQ	531
<i>V. radiata</i>	VG+T + A + GAVCLDG+ P YH H+G G+G N+W++	66
<i>A. thaliana</i>	LEGGGWCCNNIRTCVYRKTTTRRGSSNYMEKQLQFTGILSDK	650
<i>V. radiata</i>	EGGGWCNN+ +C+ R+ TR GSS M+ QL F+G S+	106
<i>A. thaliana</i>	AQENPDFFNWNRVKLRYCDGASFSGDGQ--NQAAQLQFRG	769
<i>V. radiata</i>	+ NPDF++WNR+K+RYCDG+SF+GD + + A L FRG	146
<i>A. thaliana</i>	ERIWRAAIDDLKANGMRYANQALLSGCSAGGLAAILRCDE	888
<i>V. radiata</i>	SRVFAAVVDDLLAKGMKNAQNAIISGCSAGGLAAILNCDR	186
<i>A. thaliana</i>	FRNLFPGSTKVKCLSDAGLFLDTADVSGGRTIRNLYNGVV	1007
<i>V. radiata</i>	F++L P +TKVKCL+DAG F++ DVSG + I Y+ VV	226
<i>A. thaliana</i>	ELQSVKNNLPRICTNHLDPSTCFPPQNLIQMKTPLFIVN	1126
<i>V. radiata</i>	+ NLP CT+ L P CFFPQN++SQ+ TP+F VN	266
<i>A. thaliana</i>	AAYDTWQIQSSIAPTSADPSGFWHDCRLNHGKCTPAQLRF	1245
<i>V. radiata</i>	AAYD+WQI++ +AP +ADP G W +C+L+ KC+ QL	306
<i>A. thaliana</i>	AAYDSWQIKNILAPGAADPHGTWRECKLDIKKCSSNQLSA	
<i>A. thaliana</i>	LQGFREQMLRVVKGFSMSRQNGLFINSFCAHCQTERQDTW	1364
<i>V. radiata</i>	+Q FR LR S G FI+ C+AHQQT Q+TW	346
<i>A. thaliana</i>	MQVFRTDFLRAFGAVGNPSKGFHIDGCAHCQTGTQETW	
<i>A. thaliana</i>	FADDSPVIRKKAV <u>AI</u> <u>AVGDWYFDRAEVKLVD</u> <u>CPYP</u> <u>CDKSC</u>	1483
<i>V. radiata</i>	+DSPV+ +A AVGDWY+DR K +DC YPC+ +C	386
<i>A. thaliana</i>	LRNDSPVLGSTTI <u>AK</u> <u>AVGDWY</u> <u>DR</u> <u>KPF</u> <u>KQID</u> <u>CAYPC</u> <u>NPTC</u>	
<i>A. thaliana</i>	<u>HNLVF</u>	1498
<i>V. radiata</i>	HN +F	
<i>V. radiata</i>	<u>HNRI</u> <u>F</u>	391

Fig. 1. Alignment of the deduced amino acid sequence (DiDi 9C-12 bold and underlined) of the putative pectin acylesterase (PAE) of *Arabidopsis thaliana* (accession number AY050847) and the PAE of *Vigna radiata* (accession number X99348). + indicates the similar amino acids (one-letter abbreviation).

probes of DiDi 9C-12 never showed any hybridization signal at the different time points tested (data not shown).

As the function of PAEs in vivo remains to be examined in more detail, we can only hypothesize on the role of the putative PAE encoded by DiDi 9C-12 in the host upon nematode infection. In *A. thaliana* roots infected with *M. incognita*, DiDi 9C-12 transcripts were found to accumulate already at 1 dpi, mainly in initiating giant-cells and in cells surrounding the nematodes. In mature giant-cells no signal could be observed. The transcript level of DiDi 9C-12 inside giant-cells could play a role in the ontogeny of the feeding cells (for instance in acytokinetic mitosis). In normally dividing cells, the cell plate, which finally separates the daughter cells, is formed by fusion of vesicles lining up at anaphase. The cell plate, when mature, becomes the middle lamella, which is rich in pectins. In giant-cells however, vesicles accumulate but then disperse, and the cell plate is prematurely aborted (Jones and Payne 1978). Once the feeding nematode has become sedentary, its increasing size results in an expansion of the root that can be tolerated only by

softening and stretching of the plant cell walls that surround the nematode. The enhancement of DiDi 9C-12 could thus allow the expansion of the feeding cells and growth of the nematode. DiDi 9C-12 transcript accumulation was also observed inside the syncytia induced by the cyst nematode *H. schachtii*. Whereas galls originate from proliferation and hypertrophy of the root cells, syncytia are formed by cell wall breakdown of neighboring cells and fusion of the protoplasts (Jones and Dropkin 1975) and develop mainly longitudinally along the vascular parenchyma.

Both types of feeding cells (giant-cells and syncytia) are formed next to vascular elements and are typical transfer cells with thickened cell walls and elaborate membrane ingrowths (Jones and Northcote 1972; Pate and Gunning 1972). Wall ingrowths, varying from simple rods to extensive fingerlike structures, greatly increase the surface area of the plasmalemma in the feeding cells, allowing a rapid transport and uptake of solutes. The origin of the enzymes modulating the cell wall architecture of the feeding cells (expansion, invagina-

Fig. 2. In situ localization of DiDi 9C-12 transcripts in *Arabidopsis thaliana* roots infected with *Meloidogyne incognita* and *Heterodera schachtii* and in noninfected control roots. Longitudinal and cross sections were hybridized with 35 S-labeled DiDi 9C-12 sense (data not shown) and antisense RNA probes (A through J). Root tissue was stained with a 0.05% toluidine blue solution. Bright-field digital images were overlaid with dark-field digital images in which only the red channel was retained (signal seen as red dots). **A**, DiDi 9C-12 hybridization signal in cells surrounding the nematode (1 day postinfection [dpi]). **B**, Initiating giant-cells at the binucleate stage (1 to 2 dpi). **C**, Giant-cells and surrounding cells (2 to 3 dpi). **D**, Cells mainly in the neighborhood of giant-cells (5 dpi). **E**, Cells mainly in the endodermis (7 dpi). **F**, Mature root galls (20 dpi). **G**, Young syncytia (3 dpi). **H**, Cells surrounding the syncytium (7 dpi). **I**, Tips of control roots. **J**, Mature root galls (20 dpi) and noninfected control roots, respectively, in which no DiDi 9C-12 hybridization signal could be detected. bc = binucleate cells, e = endodermis, gc = giant-cell, n = nematode, and p = pericycle. Bar = 50 μ m.

tions, and degradation) is still not elucidated. In soybean roots infected with cyst nematodes, two polygalacturonase sequences have been identified (Mahalingam et al. 1999) that code for enzymes that cleave the $\alpha 1$ to $\alpha 4$ linkages of D-galacturonic acid, which forms the backbone of pectins. Recently, five tobacco endo- β -1,4-glucanase genes have been described as being upregulated in giant-cells and syncytia (Goellner et al. 2001). Together with our report, evidence is thus accumulating that cell wall modifications in nematode feeding cells are the result of plant enzymes induced by the nematodes. Hydrolytic enzymes secreted by the subventral glands of the nematodes would then be involved in the disassemblage of plant cell walls during penetration and migration into the plant root (De Meutter et al. 1998; Popeijus et al. 2000; Smant et al. 1998; Wang et al. 1999).

In conclusion, our results suggest that DiDi 9C-12, probably in concert with other pectin-degrading enzymes induced by the nematode, could be involved in the modulation of cell growth by loosening the primary cell wall and middle lamella in plant roots infected with sedentary parasitic nematodes. This mechanism could help the feeding site to develop in plant roots, which is indispensable for completion of the nematode's life cycle.

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