

Tali Z. Gal · Elitsur R. Aussenberg · Saul Burdman  
Yoram Kapulnik · Hinanit Koltai

## Expression of a plant expansin is involved in the establishment of root knot nematode parasitism in tomato

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**Abstract** A group of plant proteins, expansins, have been identified as wall-loosening factors and as facilitators of cell expansion in vivo. The root knot nematode *Meloidogyne javanica* establishes a permanent feeding site composed of giant cells surrounded by gall tissue. We used quantitative PCR and in situ localization to demonstrate the induction of a tomato (*Lycopersicon esculentum* cv. VF36) expansin (*LeEXPA5*) expression in gall cells adjacent to the nematode feeding cells. To further characterize the biological role of *LeEXPA5* we have generated *LeEXPA5*-antisense transgenic roots. The ability of the nematode to establish a feeding site and complete its life cycle, the average root cell size and the rate of root elongation were determined for the transgenic roots, as well as the level of *LeEXPA5* expression in non-infected and nematode-infected roots. Our results demonstrated that a decrease of *LeEXPA5* expression reduces the ability of the nematode to complete its life cycle in transgenic roots. We suggest that a plant-originated expansin is necessary for a successful parasitic nematode-plant interaction.

**Keywords** Cell wall · Egg mass · Gall · Giant cell · Parasite · Root growth

**Abbreviations** CBD: Cellulose binding domain · d.p.i: Days post inoculation · E: *LeEXPA5*-antisense transgenic lines · GFP: Green fluorescence protein · GLM: General linear model · PCR: Polymerase chain reaction · qPCR: Quantitative PCR · RT: Reverse transcription

### Introduction

The growth of plant cells is controlled largely by the dramatic changes that take place during cell wall expansion. A group of plant proteins, expansins, have been identified as wall-loosening factors (McQueen-Mason et al. 1992). Expansins were suggested to function by disrupting the hydrogen bonds between cellulose and hemicellulose polymers in the cell wall (McQueen-Mason and Cosgrove 1995), hence facilitating cell expansion in vivo (Cosgrove 1999). Expansins are encoded by a large multigene family, and the expression of expansin mRNAs and proteins is correlated with growth and ripening in many tissues of plants (Brummel et al. 1999a, b; Cosgrove 1999; Chen et al. 2001 and references therein).

The root knot nematode *Meloidogyne javanica* is a plant parasite with agricultural importance. It establishes a permanent feeding site at the differentiation zone of the root by inducing nuclear division without cytokinesis in host cells. This process gives rise to large, multinucleate cells, termed giant cells, which funnel plant resources to the parasitic nematode. Giant cells may be 100 times as large as normal root parenchyma cells; so an extensive, coordinated remodeling of the cell wall must occur to allow giant cell expansion (Williamson and Hussey 1996). Following giant cell formation, and possibly as a secondary response, division and expansion of cortical

T. Z. Gal · E. R. Aussenberg · H. Koltai  
Department of Genomics, ARO, The Volcani Center,  
50250 Bet Dagan, Israel

S. Burdman  
Department of Plant Pathology and Microbiology,  
Faculty of Agricultural,  
Food and Environmental Quality Sciences,  
The Hebrew University of Jerusalem, 76100 Rehovot, Israel

Y. Kapulnik  
Department of Agronomy and Natural Resources,  
ARO, The Volcani Center, 50250 Bet Dagan, Israel

*Present address:* H. Koltai (✉)  
Department of Ornamental Horticulture,  
ARO, The Volcani Center, 50250 Bet Dagan, Israel  
E-mail: hkoltai@volcani.agri.gov.il  
Tel.: +972-3-9683039  
Fax: +972-3-9669583

and pericycle cells around the giant cells occur, causing the formation of galls. Egg production begins 3–6 weeks after the initial infection, depending on the species and environmental conditions (Williamson and Hussey 1996).

The complex changes in the plant gene expression elicited by the root knot nematode infection have been studied for a long time, and many plant development-associated genes have been shown to be involved in the parasitic process (reviewed by Gheysen and Fenoll 2002). Among the genes expressed in the nematode feeding sites are those encoding the plant cell wall-modifying enzymes (Goellner et al. 2000a, 2001; Vercauteren et al. 2002). The tobacco  $\beta$ -1,4-endoglucanase (*Ntcel*) gene was expressed within feeding sites in the early stages of formation of the root knot nematode feeding sites (Goellner et al. 2000a, 2001); upregulation of the promoter of the *Arabidopsis thaliana* endoglucanase 1 gene (*Atcel 1*), which is expressed in a range of plant species (Shani et al. 1997, 2000), was observed in transformed tobacco within giant cells (Goellner et al. 2000b).

Interestingly, a number of nematode genes encode cell wall-modifying enzymes that are secreted by the parasitic nematodes (recently reviewed by Davis et al. 2004). Moreover, Gr-EXP1—a secreted protein from the cyst nematode *Globodera rostochiensis*—shares a homology to plant expansins. Gr-EXP1 is produced and secreted by infective juveniles of the nematode, and its cell wall extension inducing activity was demonstrated in vitro (Qin et al. 2004). Thus, it is plausible that a battery of cell wall-modifying enzymes, from either the plant or nematode source, is temporarily and spatially expressed during nematode parasitism to give rise to the successful parasitic association.

Recently, using microarray technology (Bar Or et al. 2005), we have shown that expression of the tomato expansin 5 gene (*LeEXPA5*; Kende et al. 2004) is induced as part of the plant response to the root knot nematode *M. javanica*. Another study (Brummel et al. 1999a, b) localized *LeEXPA5* mRNA to expanding fruits and full-size maturing green fruits, and demonstrated its decline during the early stages of fruit ripening. Notably, RNA gel blot analysis did not detect *LeEXPA5* expression in healthy, non-infected roots (Brummel et al. 1999a, b).

In the present study, we used quantitative polymerase chain reaction (qPCR) and in situ localization to demonstrate the induction of *LeEXPA5* in gall cells adjacent to the root knot nematode feeding cells. To further investigate the biological role of *LeEXPA5* during root–nematode interaction, *LeEXPA5*-antisense transgenic roots were generated, and the ability of the nematode to establish feeding sites and complete its life cycle within the roots was investigated. Our findings suggest that a plant-originated expansin is required for a successful parasitic nematode–plant interaction.

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## Materials and methods

### Preparation of wild type root cultures and nematode infection

Tomato (*Lycopersicon esculentum* cultivar VF36) seeds (obtained from GeneBank Collection, Volcani Center, Bet Dagan, Israel) were surface sterilized by a 2-min washing with 95% ethanol and a 10-min washing with 3% sodium hypochlorite followed by six 5-min washings with double distilled, sterilized water. Seeds were germinated on the Gamborg medium (Gamborg et al. 1968). Seedling roots were excised and transferred to new Gamborg plates (Gamborg et al. 1968). Root cultures were inoculated with sterile egg masses (clusters) of the root knot nematode *M. javanica* by transferring egg masses laid by nematodes from 42 days post inoculation (d.p.i) root culture, using sterile forcipies. Sterile nematode cultures were prepared initially as described by Zareen et al. (2001).

### RNA purification and quantitative PCR

RNA was purified from infected and non-infected tomato (*L. esculentum* cultivar VF36) wild type and transgenic root cultures (the preparation of the latter is described below), 10 d.p.i using Tri Reagent (Molecular Research Center, Cincinnati, OH, USA). The qPCR was performed for DNase-treated RNA that was reverse transcribed using oligo dT, as described by Gal et al. (2003), using ABGene's Absolute qPCR SYBR Green ROX Mix (ABGene, Espom, UK), and an ABI PRISM 7700 Real-Time PCR machine (Applied Biosystems). For amplification of a 101 bp segment of *L. esculentum* expansin 5 gene (*LeEXPA5*; NCBI accession number: AF059489), forward and reverse primers (5'-CTCAATG GCAACCAATGTCAAG-3' and 5'-ACCATCGCCTG TAGTGACCTTAA-3', respectively) were used and PCR conditions were set according to ABGene instructions. Notably, the sequence amplified by these primers does not overlap the cloned antisense sequence (see below), in order to prevent antisense transcript detection in transgenic root cultures. To minimize mRNA quantification errors, and to correct for inter-sample variations, *L. esculentum* actin (accession no. BT013707) was used as an internal control using specific forward and reverse primers (5'-GAGCAGGAACCTT GAAACCG-3' and 5'-AACGGAACCTCTCAGCACCA-3', respectively). The level of expression of target gene (expansin) was calculated relative to that of the reference (actin) mRNA; the relative efficiency of the target and reference was validated to be approximately equal. (The absolute value of the slope of the log input amount versus the differences in threshold cycles for target and reference was determined according to the Applied Biosystems protocol to be 0.1.) Three replicates were performed for each wild type or transgenic line, for

nematode-infected (10 d.p.i) and non-infected (at the same age as infected) root cultures. Means and standard deviations were calculated for all lines in either *LeEX-PA5*-antisense or green fluorescence protein (GFP)-sense groups.

#### In situ localization

In situ reverse transcription-PCR for the localization of *LeEXPA5* transcripts in root tissues excised from *L. esculentum* VF36 wild type root cultures was performed as described (Koltai and Bird 2000, 2002; Pesquet et al. 2004), with a few modifications: reverse transcriptase (Fermentas, Vilnius, Lithuania) was used at a final concentration of 10,000 u ml<sup>-1</sup>. For DNA degradation DNase (Fermentas) at a final concentration of 100 u ml<sup>-1</sup> was used for overnight incubation at 37°C, followed by 30 min incubation at 70°C, for enzyme denaturation. No RNA degradation was performed. *LeEXPA5* cDNA was labeled with the ABGene SYBR Green ROX Fluorescent PCR amplification mix. Forward and reverse primers used for the amplification of a 295 bp fragment of *LeEXPA5* were 5'-CACCTGGAG GACAATTCCGTTAAAAC-3' and 5'-TTATACTATT AAATTGAGCAGTAAAAGCTC-3'. The fragment amplified by these primers does not overlap with the antisense fragment in order to prevent antisense transcript detection in transgenic root cultures. The thermal cycler was set for 41 cycles of 95°C for 15 s, 60°C for 1 min and 72°C for 1 min. For control, the same procedure was applied excluding the primers. Following PCR, the excised tissues were transferred to glass slides containing 10× PBS and were immediately observed using a confocal microscope (Olympus IX81, Tokyo, Japan) to detect the fluorescence signal (excitation and emission wavelengths: 578 and 603 nm, respectively). The BA505I filter and the Argon 488 nm laser beam were modified such that no fluorescence signal was detected in the control. The same microscopic setting was used to detect the signal in the experiment. The liquid left in the PCR tubes following tissue transfer was re-amplified by applying standard PCR with the above primers and gel electrophoresis on ethidium bromide-stained agarose gel to verify the size of the in situ amplified fragment, its presence in the experiment and its absence in the controls. The experiment was repeated twice.

#### Construction of vectors

A 297 bp from the middle region of *LeEXPA5* was amplified by PCR using the following forward and reverse primers: 5'-CTGGA ACTATGGGTGGTGCTT GTGG-3' and 5'-TATAGGCAACAGGA ACTATGCC AGCTCT-3', respectively. The PCR product was excised from the gel, purified, cloned into pstBLUE1 (Novagen) and sequenced for verification. Following

restriction digestion with *SacI* and *BamHI*, the *LeEX-PA5* fragment was cloned into pBIN m-gfp5-ER (Hasseloff and Siemering 1998). The vector was cut beforehand with the same restriction enzymes and the insert was cloned in the antisense orientation, downstream to the 35S promoter, replacing the *m-gfp5-ER* gene.

#### Preparation of transgenic root cultures

The *LeEXPA5*-antisense construct was electroporated into the *Agrobacterium rhizogenes* strain A15834 using Electroporator 2510 (Eppendorf, Hamburg, Germany) according to the manufacturer's instructions. Tomato (*Lycopersicon esculentum* cv VF36) cotyledons were transformed as described by Plovie et al. (2003). Emerging roots were excised and transferred to the Gamborg medium (Gamborg et al. 1968), containing 50 µg ml<sup>-1</sup> kanamycin (Duchefa, Haarlem, The Netherlands), for the selection of emerging roots bearing the transgenic construct. Control root cultures expressing GFP were prepared similarly, using the original pBIN m-gfp5-ER vector, expressing GFP-sense under 35S promoter. The ubiquitous expression of GFP in these controls was verified by microscopic observation (DMLB microscope, Leica, Heidelberg, Germany). Root cultures were inoculated with sterile egg masses as described above.

#### Determination of the numbers of egg masses, galls and eggs produced

Root cultures were infected with egg masses 10 days post establishment (i.e., after the transfer to a new plate of a new fragment of root from the previous root culture), as described above. Galls and egg masses were counted 42 d.p.i under a dissecting microscope (Zeiss®, Göttingen, Germany). For the determination of the number of eggs per egg mass, egg masses were collected using forceps from nematode-infected root cultures 42 d.p.i and were re-suspended in 1× PBS. Eggs were counted using a dissecting microscope (Zeiss). Experiments were repeated three times. The results were subjected to general linear model (GLM) statistical analysis ( $P \leq 0.05$ ).

#### Root elongation and cell size determination

For determination of root elongation, a 10-mm sterile root section was transferred from a root culture to a Petri dish containing the Gamborg medium. Root elongation was recorded every 24 h. The experiment was performed three times and the results were subjected to GLM statistical analysis ( $P \leq 0.05$ ).

For the determination of cell length, root fragments from the maturation zone of a 7-day root culture were fixed in FAA (63% ethanol, 5% acetic acid, 2%

formalin), embedded in 6% agarose (Euroclone, Milan, Italy) and sectioned using a Vibratome (Vibratome 1000 plus, Vibratome, St. Louis, MO, USA). Cell length was measured from the cortical cells using a light microscope (Leica DMLB) equipped with a Leica DC200 camera. Cell size measurements were performed from at least 25 cells, from three different roots, using the Leica IM1000 software. The GLM statistical analysis was used to examine the significance of differences between the means ( $P \leq 0.05$ ).

For determination of the diameter of giant cells, galls were stained with Acid Fuchsin (Daykin and Hussey 1985), embedded in 6% agarose (Euroclone) and sectioned using a Vibratome. Giant cell diameter was measured using a Leica DMLB light microscope equipped with a Leica DC200 camera. Cell size measurements were performed using the Leica IM1000 software. Importantly, galls were cross-sectioned through all their length, and the biggest diameter value for giant cells was taken for the diameter calculations. Ten repeats were performed on the experiment. GLM statistical analysis was used to examine the significance of differences between the means ( $P \leq 0.05$ ).

## Results

### Expansin induction in nematode feeding sites

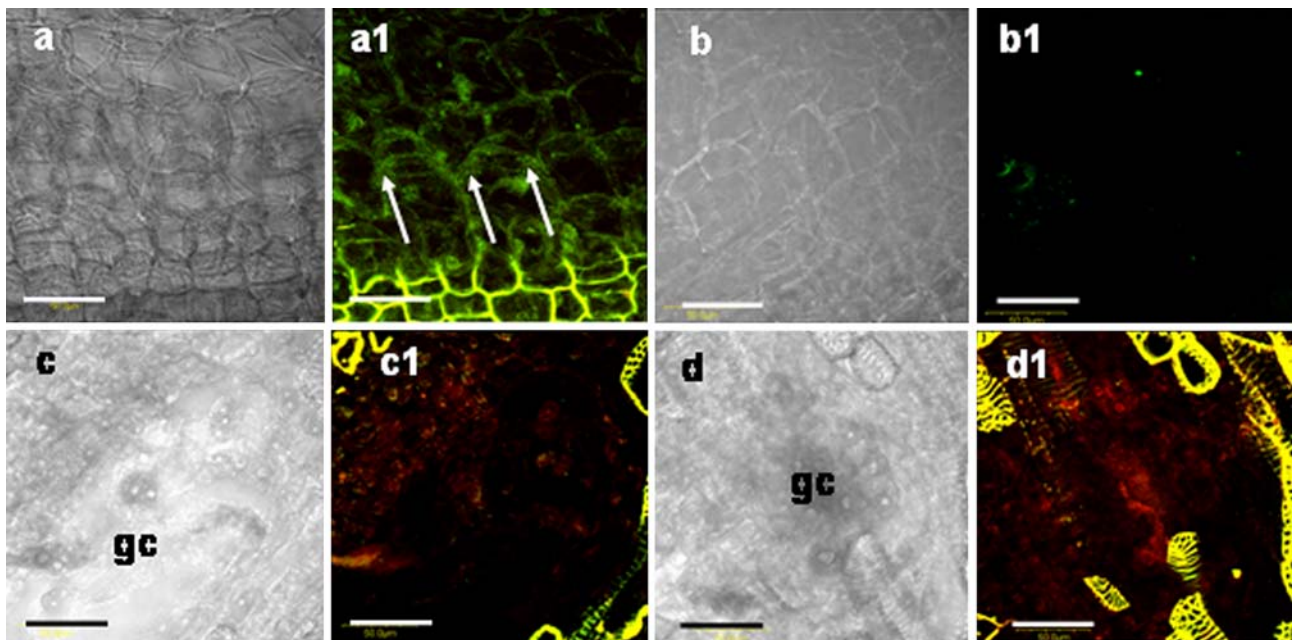
Previous data from microarray experiments suggested that the tomato expansin 5 gene (*LeEXPA5*) is induced

in nematode feeding sites (Bar-Or et al. 2005). Among the expansin gene family, *LeEXPA5* is the only one that was detected as induced in our system. qPCR of whole root culture preparations of nematode-infected wild type tomato roots showed a  $4.3 \pm 0.3$ -fold increase in the level of *LeEXPA5* expression 10 d.p.i, as compared with the preparation of non-infected roots, hence confirming the previous microarray results. In situ localization of the *LeEXPA5* transcript demonstrated its presence in the gall tissue of nematode-infected wild type tomato roots 4 and 10 d.p.i. The level of expression was enhanced in the tissue adjacent to developing galls (Fig. 1a1, b1), which may be destined to become part of the still-expanding galls. *LeEXPA5* transcripts were not detected in tissues that are remote from the developing gall (not shown). Notably, *LeEXPA5* transcripts were not detected in giant cells throughout the experiment (Fig. 1c1, d1).

### Determination of the ability of the nematodes to complete their life cycle on transgenic roots

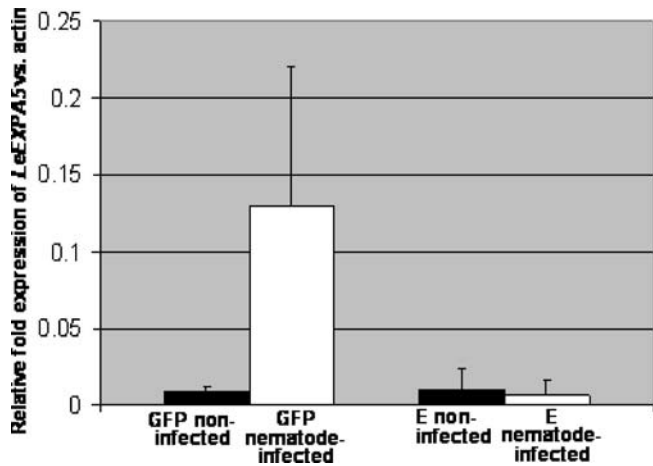
In order to examine the role of *LeEXPA5* on nematode parasitism, we generated transgenic roots expressing a *LeEXPA5* at the antisense orientation, for *LeEXPA5* transcriptional silencing. Ten *LeEXPA5*-transgenic root lines were generated and their phenotype was examined.

Most of the transgenic lines could be infected by nematodes, and galls were produced. To determine the expression of *LeEXPA5* in both nematode-infected and non-infected roots, we determined the level of *LeEXPA5*



**Fig. 1 a–d** In situ localization of *LeEXPA5* transcription products in the root knot nematode *Meloidogyne javanica* feeding site (**a**, **c**) and negative controls (**b**, **d**). Differential interference contrast (**a**, **b**) and fluorescent images (**a1**, **b1**) of a longitudinal section through a gall. Green fluorescence indicates the expression of *LeEXPA5* (arrows, **a1**). No signal was detected in the negative control (**b1**).

Differential interference contrast (**c**, **d**) and fluorescent images (**c1**, **d1**) of a longitudinal section through a gall at the site of giant cells (**gc**). Yellow or red fluorescence indicate auto-fluorescence; no signal was detected for the *LeEXPA5* transcription products either in experiment (**c1**) or in the negative control (**d1**). Bars 50  $\mu$ m



**Fig. 2** The level of *LeEXPA5* transcription products in the GFP-sense (represented as the mean of lines GFP2, GFP3 and GFP4) and *LeEXPA5*-antisense (represented as the mean of lines E6, E8, E9, E11–E13, E15–E18) transgenic root lines. The level of *LeEXPA5* transcripts was measured by qPCR relative to that of *Lycopersicon actin*. *Black columns* represent the mean ratio of the *LeEXPA5* mRNA level in non-infected GFP-sense or *LeEXPA5*-antisense transgenic lines; *white columns* represent the mean ratio of the *LeEXPA5* mRNA level in nematode-infected GFP-sense or *LeEXPA5*-antisense transgenic lines

mRNA in GFP-sense (Fig. 2) and in the *LeEXPA5*-antisense transgenic lines E. The results demonstrated that *LeEXPA5* transcription is relatively low in healthy roots, in both GFP and E lines. Nevertheless, upon nematode infection (10 d.p.i) the level of *LeEXPA5* transcription increased in the GFP lines. No increase in its transcription level was observed in the E lines (Fig. 2). The rather modest increase in *LeEXPA5* expression in galls as compared to controls may also be due to a dilution effect of using excised-whole roots for analyses (rather than the nematode feeding sites only).

A number of galls may be affected by the number of viable eggs in the egg mass that was used to infect the transgenic root culture, and this may vary between egg

masses. Thus, we determined the ratio of egg masses produced as a fraction of the number of galls in a certain root culture plate. This parameter allowed assessing the fraction of nematodes that were able to complete their life cycle (and thus, produce egg mass). In the *LeEXPA5*-antisense transgenic lines, a significant reduction ( $P \leq 0.05$ ) in the ability of the nematodes to produce egg masses was observed 42 d.p.i (Table 1). Nine weeks post infection, similar differences were observed between the lines (not shown), suggesting a reduction rather than a delay in the ability of the nematodes to complete their life cycle. The number of eggs per egg mass produced by the nematodes was determined 42 d.p.i, and the mean was shown to be significantly reduced ( $P \leq 0.05$ ) in *LeEXPA5*-antisense transgenic lines as compared with the GFP-sense control lines (Table 1).

The results suggest that the major difference in *LeEXPA5* transcription between the GFP and E lines relays in the absence of *LeEXPA5* transcription elevation during nematode infection in the *LeEXPA5*-antisense transgenic lines.

#### Determination of giant cell size in transgenic roots

Since giant cells, rather than the gall parenchyma cells, are the primary nutritional source for the parasitic nematode (Williamson and Hussey 1996), we hypothesized that giant cells may be distorted in *LeEXPA5* silenced lines, leading to the dramatic reduction of egg mass production observed in these lines. To examine that, we compared the average size of giant cells in mature galls (42 d.p.i) between *LeEXPA5*-antisense and GFP-sense transgenic lines. A significant reduction ( $P \leq 0.05$ ) in the diameter of giant cells (in cross-sections of galls) was observed in *LeEXPA5*-antisense transgenic lines as compared with the GFP control (Table 1). In addition, in the *LeEXPA5*-antisense transgenic lines, galls were smaller than those of the GFP control, and in most of the former lines the parasitic nematodes did not

**Table 1** Mean ratio of egg mass per gall, mean number of eggs per egg mass, mean diameter of giant cells in nematode feeding sites, mean cell length and mean rate of root elongation of GFP-sense and *LeEXPA5*-antisense transgenic lines (GFP and E lines, respectively)

Transgenic Line	Mean ratio of egg mass per gall	Mean number of eggs per egg mass	Mean giant cell diameter ( $\mu\text{m}$ )	Mean cell length ( $\mu\text{m}$ )	Mean rate of root elongation (mm/day)
GFP3	0.252	350	94.3	82.0	5.7
GFP4	0.117	430	66.5	86.4	3.4
GFP5	0.133	250	115.071	55.5	4.7
Mean GFP Lines	0.17 <sup>a</sup>	322 <sup>a</sup>	101 <sup>a</sup>	74.6	4.6
E6	0.167	50	51.2	60.1	6.1
E8	0.165	172	60.7	37.1 <sup>b</sup>	1.5
E9	0.117	108	38.0	38.5 <sup>b</sup>	5.6
E11	0.093	44	56.7	93.8 <sup>c</sup>	5.5
E12	0.000	0	35.5	51.4	5.0
E13	0.025	90	41.8	45.7	2.7
E15	0.049	36	60.8	44.2	4.0
E16	0.010	70	41.5	135.2 <sup>c</sup>	1.6
E17	0.049	50	31.8	103.6 <sup>c</sup>	6.1
E18	0.029	56	47.9	66.8	1.8
Mean E Lines	0.09 <sup>a</sup>	70 <sup>a</sup>	49 <sup>a</sup>	53.64	3.99

<sup>a</sup>Means of groups (GFP and E) are significantly different ( $P \leq 0.05$ )

<sup>b</sup>Mean cell length is significantly shorter than the control ( $P \leq 0.05$ )

<sup>c</sup>Mean cell length is significantly longer than the control ( $P \leq 0.05$ )

develop to obtain their characteristic pear-like shape (not shown).

In conclusion, the results demonstrate that in some of the *LeEXPA5*-antisense transgenic lines a marked reduction was observed in the ability of the nematodes to establish parasitism and complete their life cycle.

#### Determination of root growth and cell elongation in transgenic roots

Expansin is a protein needed for cell elongation (Cosgrove 1999). Thus, we determined the rate of root elongation and cell length for each of the transgenic lines. In two of the lines—E8 and E9—the cells were significantly ( $P \leq 0.05$ ) shorter than the GFP-sense, control lines. In contrast, three lines—E11, E16 and E17—consisted of cells that were significantly ( $P \leq 0.05$ ) longer than those of the GFP control lines (Table 1). There were no significant changes in the rate of root elongation between the *LeEXPA5*-antisense and the GFP-sense transgenic lines, and no clear correlation between cell length and rate of root elongation (Table 1).

#### Correlation between rate of root elongation and nematode infection

The reduction of susceptibility to the nematode (as determined by the reduction of egg production, described above) in transgenic lines may be affected by a decrease of successful root penetration by the nematode, as a result of shortening of the differentiation zone (the site of nematode penetration). Our data suggest that in some of the transgenic lines, such as E11, E12, E15, E17, despite the relatively high rate of root elongation, only few eggs were produced (Table 1). Thus, the reduction in egg production at least in these transgenic lines is not likely to be a result of shortening of the nematode penetration zone, but rather a nematode specific effect.

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## Discussion

In the present work we have examined the functional role of a plant expansin during the parasitic association of root knot nematodes and the host plant. We have demonstrated that a tomato expansin, *LeEXPA5*, is upregulated in gall cells adjacent to the nematode feeding cells, and that reduction in the level of *LeEXPA5* mRNA hinders completion of the root knot nematode life cycle.

Expression of *LeEXPA5* is induced in nematode feeding sites. This was demonstrated first by a microarray analysis (Bar-Or et al. 2005) and validated via the qPCR of nematode-infected root culture and in situ localization studies (this work). The in situ localization studies indicated that *LeEXPA5* is mainly expressed in

the developing gall tissue. During the early stages (first few days) of nematode parasitism these cells expand to form the gall. The signal for *LeEXPA5* expression in developing gall cells might suggest that the *LeEXPA5* expression is required for gall cell expansion and thus gall formation.

Notably, we did not detect any giant cell staining for the *LeEXPA5* transcript, 4 or 10 d.p.i. During these time points the nematode giant cells were already clearly evident (Fig. 1), whereas the galls were still small and expanding. We cannot exclude the possibility that expansin is expressed in giant cells very early during parasitism (i.e., first few hours post infection), once giant cells are induced by the nematode and substantially expand. Nevertheless, these very early time points are difficult for in situ analysis due to the inability to localize the site of the destined giant cells (no giant cells nor galls may be distinguished).

Alterations (both decrease and increase) in cell length were demonstrated during the exogenous application of cellulose binding domain (CBD), a bacterial homolog of a plant expansin (Shpigel et al. 1998). It was suggested that CBD either compete with the cellulose cross-linking component (i.e., xyloglucan; XG) on binding to cellulose, or alter cellulose synthesis, thus changing the plant cell elongation (Shpigel et al. 1998). Similarly, in expansin over expressing tomato, cortical and epidermal cells were shorter and wider than those in wild type plants, and the overall growth was reduced in transgenic lines (Rochange et al. 2001).

In the present study, some of the *LeEXPA5*-antisense transgenic lines had significantly shorter cells and some had significantly longer cells than the GFP-expressing control. Our qPCR and Brummel et al. (1999a, b) data suggested that *LeEXPA5* transcription in healthy roots is minor. Thus, *LeEXPA5* expression may not be needed for normal root growth. Hence, either the differences seen in cell size and root length in the *LeEXPA5*-antisense lines are a natural variation, and not a result of *LeEXPA5* expression silencing, or suppression of other, root-expressed expansins with homologous sequences may be triggered in the *LeEXPA5*-antisense transgenic lines. Cell size did not correlate in our study with the rate of root elongation. This may be due to the in vitro effect on the propagation of root segments, rather than whole plants (Han et al. 2004).

The root knot nematode establishes its feeding site in the root differentiation zone (above the elongation zone; Williamson and Hussey 1996). Thus, a possible explanation for the reduction of the ability of the nematode to establish in *LeEXPA5*-antisense transgenic roots is the shortening of the differentiation zone, as a result of the variation in root elongation. However, the presence of transgenic lines, which despite their normal rate of elongation (compared to the control) displayed a reduced number of eggs produced, suggested that at least in those lines, the reduction of the ability of the nematodes to successfully complete their life cycle is not merely a result of shortening of the differentiation zone,

but a specific effect of the reduction of *LeEXPA5* transcription on nematode parasitism.

The reduced size of giant cells formed in those normally elongated *LeEXPA5*-antisense transgenic lines further supports this notion. Nevertheless, giant cell size in individual lines was not necessarily correlated to the levels of the success of the parasitic process (Table 1). In addition, the *LeEXPA5* expression was not demonstrated in giant cells. Thus, we cannot exclude the possibility that the recorded effect of *LeEXPA5* silencing on nematode parasitism is other than a direct result of reduced giant cell size. Rather, improper gall formation may be the culprit for the observed effect on nematode infection.

Importantly, since the *LeEXPA5* expression may have only little effect on normal root development, we suggest that the decrease of *LeEXPA5* transcription in the antisense lines following nematode infection, rather than the reduction in the level of its transcripts in non-infected transgenic lines, is the cause of reduced parasitism.

In conclusion, we suggest that *LeEXPA5* transcription is activated by the parasitic nematode, whereas *LeEXPA5* transcription silencing has a specific effect on nematode parasitism, without a clear effect on root development. Thus, the nematode appears to cause the activation of a plant promoter that is not normally active in root tissue, or is active at very low levels. Interestingly, the same *LeEXPA5* gene was expressed in feeding sites induced by the cyst nematode *Globodera rostochiensis* in the roots of susceptible tomato. During early time points (1–3 day old syncytia, i.e., the cyst nematode feeding site) *LeEXPA5* expression was localized to the syncytia cells and to neighboring cells. In 7 day old syncytia, the older parts of the syncytia contained relatively less *LeEXPA5* transcription products than recently formed parts (Sobczak et al. 2002). Other tomato expansin genes were expressed in or close to the syncytium 10 and 14 d.p.i, whereas in *Arabidopsis* expansins were expressed in nematode-induced syncytia (Golecki et al. 2002). Apparently, the root knot and cyst nematodes both induce members of the expansin gene family. Nevertheless, the differences in the site of induction might reflect differences in the processes of feeding site formation (Williamson and Hussey 1996).

Recently, a cyst nematode-originated expansin was discovered (Qin et al. 2004). To the best of our knowledge none have been discovered in *Meloidogyne*. A root knot nematode expansin may not be excluded; nevertheless, our results suggest that if it exists, it may not be sufficient to support a successful plant parasitism.

Future studies involving analysis of transgenic plants (rather than merely roots) may determine the potential of the alteration of expansin expression for the development of new means for nematode pest control. Such a strategy may use, for example, root specific promoters, thus hindering nematode parasitism towards generation of transgenic plants that are partially or fully nematode-resistant, without significant alterations in plant devel-

opment and productivity. This may considerably contribute to develop more efficient management strategies against this important agricultural pest.

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