Oxylipins are implicated as communication signals in tomato–root-knot nematode (Meloidogyne javanica) interaction

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Throughout infection, plant-parasitic nematodes activate a complex host defense response that will regulate their development and aggressiveness. Oxylipins—lipophilic signaling molecules—are part of this complex, performing a fundamental role in regulating plant development and immunity. At the same time, the sedentary root-knot nematode Meloidogyne spp. secretes numerous effectors that play key roles during invasion and migration, supporting construction and maintenance of nematodes’ feeding sites. Herein, comprehensive oxylipin profiling of tomato roots, performed using LC–MS/MS, indicated strong and early responses of many oxylipins following root-knot nematode infection. To identify genes that might respond to the lipidomic defense pathway mediated through oxylipins, RNA-Seq was performed by exposing Meloidogyne javanica second-stage juveniles to tomato protoplasts and the oxylipin 9-HOT, one of the early-induced oxylipins in tomato roots upon nematode infection. A total of 7512 differentially expressed genes were identified. To target putative effectors, we sought differentially expressed genes carrying a predicted secretion signal peptide. Among these, several were homologous with known effectors in other nematode species; other unknown, potentially secreted proteins may have a role as root-knot nematode effectors that are induced by plant lipid signals. These include effectors associated with distortion of the plant immune response or manipulating signal transduction mediated by lipid signals. Other effectors are implicated in cell wall degradation or ROS detoxification at the plant–nematode interface. Being an integral part of the plant’s defense response, oxylipins might be placed as important signaling molecules underlying nematode parasitism.

Meloidogyne species of root-knot nematodes (RKN) are one of the main devastating plant parasites, infecting an estimated 5000 plant species1–3. The RKN are obligatory sedentary endoparasitic biotrophs, with more than 90 known Meloidogyne species distributed ubiquitously. The most destructive species are M. incognita, M. arenaria, M. hapla and M. javanica, causing crop losses amounting to hundreds of billions of US dollars each year4–6. Successful parasite penetration, migration, establishment and maintenance rely mainly on the secretion of effectors through the stylet that promote and establish an intimate long-term interaction with the host7–9. These effectors are predominantly synthesized by the esophageal glands, two subventral and one dorsal; and other organs, such as amphids and cuticle, also participate in the parasitic secretion10. Multiple effectors of plant-parasitic nematode species have been successfully isolated and characterized using transcriptome, secretome and RNAi approaches11–19. Of the various effector categories, some effectors have been found to act as immunomodulators that manipulate, via mimicry or suppression, the host immune system.

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Although lipids are vital cellular components, increasing evidence suggests their supplementary role in plant immunity. Lipids and their derivative signaling molecules are involved in responses to biotic and abiotic stresses\(^{22-24}\). Several studies have suggested that these compounds are subject to pathogen manipulation and interference. Nematode-derived fatty acid- and retinol-binding (FAR) proteins are predominantly secreted into the host cell for developmental processes, but may facilitate parasitism by interfering with lipid signaling related to plant defense\(^{22-24}\). Similarly, in a recent study, overexpression of the *M. javanica* FAR effector (mj-far-1) rendered plants more susceptible to nematodes infection\(^{25,26}\). One of the universal plant-defense mechanisms upon action of 9-/13-lipoxygenases (9-/13-LOX), α dioxygenase (α-DOX), or a non-enzymatic pathway in the presence of singlet oxygen\(^{30-32}\). The hydroperoxide products serve as substrates for at least six alternative enzymatic functions, classified as hydroperoxides, hydroxides, ketotrienes, ketodienes, epoxides and diols, trials, dicarboxylic acids, and ketols, among others. Among the oxylipins, the jasmonate (JA) group has been well-characterized and is shown to mediate plants’ defense response against necrotrophic pathogens and herbivores\(^{33-34}\). While the functions of the vast majority of oxylipins have yet to be elucidated, their production has been shown to change dramatically upon microbial and fungal pathogen invasion, suggesting an important role in defense\(^{42-43}\). Deficiencies in the LOX pathway lead to alterations in the plant’s response to pathogen attack, supporting the prominent role of oxylipins in the establishment of resistance\(^{35}\). Oxylipins synthesized via the 9-LOX pathway are involved in plant development, including root architecture, senescence and seed germination, and in the stimulation of plant defense upon pathogen attack. The 9-LOX derivatives are among the most active oxylipins in terms of antimicrobial and/or antifungal activity and, in their ability to regulate the programmed cell death responses\(^{40-45}\).

Recent findings have implicated lipid metabolic pathways in the defense responses of plants to *RKN*\(^{46-48}\). For example, microarray-based expression profiling indicated that several fatty acid metabolism genes, including *LOX*, patatin-like protein 1 (PLP1), and 12-oxophytodienoate reductase (OPR), are induced in susceptible soybean roots in response to soybean cyst nematode infection\(^{49}\). Similarly, LOX activity increased in pea root\(^{50}\) and soybean roots\(^{51}\) upon infection with cyst nematodes. Moreover, degradation of trophic cells, indicating a hypersensitive response, is accompanied by increased activity of pea LOX\(^{51}\). Genetic evidence of the involvement of the maize 9-LOX-biosynthesis pathway in resistance mechanisms to *RKN* was further provided by the analysis of the *lox3*-knockout mutant of maize, which displayed increased attraction and susceptibility to *RKN* compared to a near isogenic wild-type line\(^{52}\). Similarly, Ozalvo et al.\(^{53}\) showed that in *Arabidopsis*, the 13-LOX enzyme, LOX4, operates as a root-specific inducer of major defense signaling pathways in response to nematode infection. Recently, a wide screening of oxylipins against *M. javanica* second-stage juveniles (J2) showed that (9S,10E,12Z,15Z)-9-hydroxy-10,12,15-octadecatrienoic acid (9-HOT) application attenuates nematode viability\(^{54}\). 9-HOT is produced by the 9-LOX pathway, controlling root development and inducing cell-wall defense responses such as callose accumulation and reactive oxygen species production\(^{40}\). We hypothesize that the dynamics of host lipid-mediated signaling induces changes in *M. javanica* infective juveniles’ gene-expression profile associated with parasitism. In this study, we report the first comprehensive oxylipin profiling of tomato roots (*Solanum lycopersicum* cv. Avigal 870) challenged by *M. javanica*, using LC–MS/MS, to provide novel insight into the plant response involving fatty acid-derived molecules upon *RKN* attack. By using RNA-Seq transcriptome analysis of *M. javanica* exposed to the 9-HOT and tomato protoplasts, we identified novel potential *M. javanica* transcripts and effectors which might be classified as early mediators regulating nematode parasitism.

**Results and discussion**

**Oxylipin biosynthesis is rapidly induced upon tomato root infection by *M. javanica***. To evaluate alterations in oxylipin-mediated defense pathways during tomato root infection by the *RKN* *M. javanica*, we performed a quantitative analysis of tomato root oxylipins at 5, 15 and 28 days after nematode inoculation (DAI) corresponding to the time of feeding-site formation by infective juveniles, nematode development into J3–J4 stages, and female maturation, respectively as well as of noninoculated roots at the same time points to assess changes in oxylipins accompanying nematode parasitism. A comprehensive oxylipin metabolomic analysis was conducted using the LC–MS/MS platform for quantitative evaluation, and approximately 100 oxylipins were identified (Fig. 1). As shown, inoculated root tissues exhibited an evident alteration in most of the measured oxylipins upon inoculation with *RKN* J2 compared to noninoculated roots, the latter generally exhibiting no significant changes (Table 1). For oxylipins originated from the LOX pathway—the ketones 13-keto-9(Z),11(E)-octadecadienoic acid (13-KOD) and 9-keto-10(E),12(Z),15(Z)-octadecatrienoic acid (9-KOT), as well as from the reductase pathway—the hydroxides 9-HOT and 13-HOT; we observed a sharp accumulation 5 DAI, followed by a lesser accumulation 28 DAI. Oxylipins of the epoxy alcohol synthase pathway, generating the triol group, followed a similar trend, where 9,10,13-trihydroxy-11(E)-octadecenoic acid (9,10,13-THOD), 9(S),12(S),13(S)-trihydroxy-10(E)-octadecenoate (9,12,13-THOD), 9,10,13-trihydroxystearic acid (9,10,13-THOM) and 9,12,13-trihydroxyoctadecenoic acid (9,12,13-THOM) were upregulated at the early time point and gradually decreased as infection proceeded. The presence of oxylipins of the HPL (Hydroperoxide lyase) pathway—azelaic acid and traumatic acid—was measured as well; a significant increase was only observed for azelaic acid 5 DAI.
compared to noninoculated roots, followed by a clear reduction 15 and 28 DAI. A similar pattern of induction 5 DAI was detected with the α-DOX product 2(R)-hydroxy-9(Z),12(Z),15(Z)-octadecatrienoic acid (2-HOT).

Measurement of oxylipins of the 9-allene oxide synthase (9-AOS) pathway—10-oxo-11-phytoenoic acid (10-OPEA) produced from C18:2, and 9-hydroxy-10-oxo-octadecaenoic acid (9OH-10KOM) and 9OH-12KOM belonging to the ketol-group products of auto-oxidation of allene oxides, demonstrated increased accumulation 5 DAI which was sustained 15 DAI (Fig. 1). Among the JA, increased accumulation of JA and JA-Ile was observed 5 DAI compared to noninoculated root tissues, along with a gradual decrease as the infection proceeded. Evidence for the functional role of JA in regulating plant defense response to nematode infection is controversial52,54–58. To settle this conflict, a recent study by Gleason et al. 59 has brought new insight to this issue, positioning the JA precursor 12-OPDA, but not JA/JA-Ile, as a key defense signaling molecule involved in regulating plant susceptibility to nematodes59. Our results indicate that while JA and JA-Ile were induced 5 and 15 DAI during compatible interactions with the nematodes, 12-OPDA levels were not affected by nematode infection at the tested time point. In Gleason et al. 59 study, OPDA accumulation was already detected within 2 DAI. In our study, the earliest time point of 5 DAI might therefore have been too late for detecting differences in 12-OPDA levels. Taken together, our results put several oxylipins, among them the hydroxides, triols, ketones, epoxides, ketols and the JA group at the interface during the early time point of the parasitic interaction.

**M. javanica** infection induces expression of tomato oxylipin-biosynthesis genes **LOX1.2, AOS1, OPR2 and α-DOX1.** To correlate oxylipin occurrence with genetic biosynthetic pathways, we used the GUS–promoter bioassay for spatial and temporal expression of oxylipin-biosynthesis genes **LOX1.2,**
AOS1, OPR2 and αDOX1. Transgenic tomato hairy root lines (line 870) carrying pLOX1.2–GUS, pAOS1–GUS, pOPR2–GUS and pα-DOX1-GUS constructs, and a negative control consisting of hairy root lines carrying empty pCAMBIA2300 vector, were tested for GUS activity at 2, 5, 15 and 28 DAI compared to noninoculated transgenic tomato hairy root lines and a negative control (Fig. 2). LOX’s involvement in the plant response to RKN has been previously reported.

LOX1.2 is a 9-LOX gene (solyc01g099210.2.1) that is homologous to LOX1 in Arabidopsis, located in the cytosol and known to catalyze the hydroperoxidation of linoleic acid, and thus be involved in the pathway of 9-oxylipin biosynthesis (UniProtKB—P38416, LOXB_SOLLC). LOX1.2 expression was observed as a mild signal in the vascular system of the forming gall 5 DAI (Fig. 2C1), with only a faint signal observed 15 DAI and 28 DAI (Fig. 2D1,E1). These results are in good agreement with the oxylipin analysis where we observed that the 9-LOX products, including azelaic acid, a mobile product of HPL that primes systemic acquired resistance, were elevated 5 DAI and downregulated 28 DAI. The ketones synthesized by LOX, 9-KOT and 13-KOD, as well as through hydroxide synthesis by reductase, 9-HOT, were induced 5 DAI with a clear reduction 28 DAI. Interestingly, these oxylipins have been shown to exert significant nematicidal properties against J2, as well as antimicrobial activity. Among the products of the epoxy alcohol synthase pathway, the trihydroxy oxylipins 9,10,13-THOD, which have been reported to reduce infection by the fungus Blumeria graminis in barley, were upregulated 5 DAI, and decreased 15 and 28 DAI. Similarly, 9,12,13-THOD and 9,12,13-THOM, which are known to have antimicrobial activity, were upregulated 5 DAI and downregulated 15 and 28 DAI.

Next, the expression of 13-AOS1 (solyc04g079730.1.1), located in the thylakoid membrane and involved in JA biosynthesis (UniProtKB—K4BV52, AOS1_SOLLC), was studied. AOS1 reporter lines exhibited GUS staining in the vascular cylinder attached to the developing gall 15 DAI (Fig. 2D2), which strongly increased by 28 DAI in the developed gall (Fig. 2E2).

| Oxylipin (pmol/g FW) | Inoculated | Non inoculated |
|---------------------|------------|---------------|
|                     | 5 15 28   | 5 15 28       |
| 10HOD               | 27.37**   | 9.67**        |
| 10OPEA              | 2364.67** | 3369.57**     |
| 12,12-diHOM         | 188.45     | 93.38         |
| 12,12-EpOD          | 2236.16** | 1105.21**     |
| 12,13-EPOM          | 669.09*** | 204.16**      |
| 12OPDA              | 316.06     | 240.42        |
| 13HOD               | 1937.49** | 1269.64**     |
| 13HOT               | 565.65*   | 205.65*       |
| 13KOD               | 4309.86*  | 1831.64*      |
| 13OH-12KOD          | 1208.85*  | 1013.99*      |
| 2HOT                | 344.74*** | 38.06***      |
| 9,10,13-THOD        | 54.052.1*** | 1677.46***   |
| 9,10,13-THOM        | 10714.0*** | 5715.1***     |
| 9,10-EpOM           | 8339.47*** | 2385.13***    |
| 9,12,13-THOD        | 3272.46*** | 726.85***     |
| 9,12,13-THOM        | 15563.3*** | 4670.7***     |
| 9HOD                | 11,215.8*** | 8343.5***    |
| 9HOT                | 3477.26*** | 1645.98***    |
| 9KOD                | 14491.3*** | 7874.9***     |
| 9-KOT               | 2198.54*** | 624.23***     |
| 9OH-10KOD           | 248.4      | 127.73       |
| 9OH-10KOM           | 8693.3***  | 12345.2***    |
| 9OH-12KOD           | 134.34*    | 110.15*      |
| 9OH-12KOM           | 786.13     | 722.36       |
| Azelaic acid        | 8375.7***  | 3674.29***    |
| Jasmonic acid (JA)  | 79.48***   | 90.88***      |
| JA-Ile              | 419.77*    | 320.28       |
| Traumatic acid      | 109.4***   | 53.39***      |

Table 1. Summary of oxylipin concentrations in tomato roots that were or were not inoculated with M. javanica, at different time points (5, 15 and 28 DAI). Data were analyzed by one-way ANOVA (p < 0.05), and compared by Student t-test to test for significant differences between inoculated and noninoculated values at the same time point, and Tukey HSD test for statistical significance between different time points for the same treatment. Uppercase letters refer to significant differences between means of the different time points in the same treatment; lowercase letters refer to significant differences between means of the same time point in different treatments. *p < 0.0001, **p < 0.001.
Functional analysis of AOS1 in rice indicates that rice plants overexpressing AOS1 are less susceptible to *M. graminicola*. Similarly, *Arabidopsis thaliana* AOS mutant dde2 shows more galling by *M. hapla* than the wild type. However, being a wound-inducible gene, AOS1 expression at this late time point might indicate that a localized wound response is induced by the developing nematode. OPR2 (solyc01g0103390.2.1) is known to be weakly expressed in roots and to be involved in the oxylipin-biosynthesis pathway, but as it belongs to the type 1 subfamily, this isoform is not predicted to be a major contributor to JA biosynthesis (UniProtKB—Q9FEX0, OPRL_SOLLC). The RKN-inoculated OPR2 promoter–GUS fusion construct exhibits no GUS signal related to root tip or elongation zone. LeOPR1 or LeOPR2 expression and that LeOPR2 is expressed at comparatively low levels in tomato roots, leaves, and flowers. However, GUS detection within the mature gall implicated LeOPR2 in a late response, although the physiological function in the mature gall remains obscure. α-DOX1 (solyc02g087070) is a fatty acid-hydroperoxidase located in the endomembrane system, involved in many processes, such as response to oxidative stress, lipid metabolic processes and a salicylic acid (SA) stimulus (UniProtKB—Q9FE5X, OPRL_SOLLC). α-DOX1 has been previously detected in tomato and *Arabidopsis* roots tissues and its generated oxylipins have been suggested to mediate the response of roots to several environmental stresses. Our analysis indicated that α-DOX1 is expressed within the mature gall. 2-HOT, the main product of α-DOX, which has been shown to be upregulated by bacterial inoculation and herbivore infestation, was found to be significantly upregulated by RKN inoculation 5 DAI. The lack of synchronized expression of the biosynthesis genes and their accumulated products at

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**Figure 2.** Microscopic analysis of GUS expression patterns in root-knot nematode-inoculated tomato roots harboring LOX1.2, AOS1, OPR2 and, α-DOX1 promoter: GUS fusion constructs. (A1–E1) Micrographs of LOX1.2–GUS reporter line. (A2) Noninoculated root harboring the LOX1.2–GUS fusion construct exhibits no GUS signal related to root tip or elongation zone. (B1) Roots 2 days after inoculation (DAI). (C1) Inoculated roots 5 DAI. (D1) Developing galls 15 DAI. (E1) Mature galls 28 DAI. (A2–E2) Micrographs of AOS1–GUS reporter line. (A2) Noninoculated root harboring the AOS1–GUS fusion construct exhibits no GUS signal related to root tip or elongation zone. (B2) Roots 2 DAI. (C2) Inoculated roots 5 DAI. (D2) Developing galls 15 DAI. (E2) Mature galls 28 DAI. (A3–E3) Micrographs of OPR2–GUS reporter line. (A3) Noninoculated root harboring the OPR2–GUS fusion construct exhibits no GUS signal related to root tip or elongation zone. (B3) Roots 2 DAI. (C3) Inoculated roots 5 DAI. (D3) Developing galls 15 DAI. (E3) Mature galls 28 DAI. (A4–E5) Micrographs of α-DOX1–GUS reporter line. (A4) Noninoculated root harboring the α-DOX1–GUS fusion construct exhibits no GUS signal related to root growth. (B4) Roots 2 DAI. (C4) Inoculated roots 5 DAI. (D4) Developing galls 15 DAI. (E4) Mature galls 28 DAI. (A5–E5) Micrographs of pCAMBIA2300–GUS reporter line; empty pCAMBIA2300 vector fused to GUS reporter served as a control. (A5) Noninoculated root harboring the pCAMBIA2300–GUS fusion construct exhibits no GUS signal related to root tip or elongation zone. (B5) Roots 2 DAI. (C5) Inoculated roots 5 DAI. (D5) Developing galls 15 DAI. (E5) Mature galls 28 DAI. Arrows indicate GUS staining (C1, D2, E2, E3, D4 and E4): Micrographs viewed under a light microscope. Bright-field image of roots and galls photographed through a stereomicroscope. Bars: A1–A5, B1, B4, B5, C1, C4–C5, D3–D5 = 50 μm; B2–B3, C2–C3, D1–D2, E1–E5 = 500 μm.
Table 2. Summary of statistics for individual RNA-Seq paired-end reads and library.

| Biological assay | Library name | No of paired-end reads | No of paired-end reads after quality and trimming | % paired-end reads mapped to *Meloidogyne javanica* transcriptome (%) |
|-----------------|--------------|------------------------|-----------------------------------------------|---------------------------------------------------------------|
| (1)             | Protoplasts  | 21,228,091             | 13,670,602                                     | 73.42                                                          |
| (2)             | Protoplasts  | 21,776,598             | 18,074,731                                     | 60.81                                                          |
| (1)             | 9-HOT        | 20,313,528             | 14,682,317                                     | 73.40                                                          |
| (2)             | 9-HOT        | 21,446,366             | 14,672,689                                     | 72.17                                                          |
| (1)             | MES buffer + Ethanol | 23,253,618            | 16,242,352                                     | 74.07                                                          |
| (2)             | MES buffer + Ethanol | 21,049,270             | 14,001,192                                     | 71.96                                                          |
| (1)             | J2           | 19,814,441             | 14,227,256                                     | 72.44                                                          |
| (2)             | J2           | 21,904,546             | 16,190,321                                     | 71.59                                                          |

early time points, observed for OPR2 and α-DOX1, might be explained by a low level of expression requirement or much earlier expression occurring upon infection. Overall, multiple expression patterns of oxylipins derived from the diverse branched pathways might be the result of plant immunity and/or induced by the nematode for successful establishment. As noted by Prior et al. and Iberkleid et al., nematodes’ FAR protein binds to linolenic and linoleic acids, which are precursors of the oxylipin molecules, and have further been found to inhibit LOX-mediated products. Our results indicate that upregulation of oxylipin biosynthesis is a primary response to nematode inoculation, and as such must be counteracted by an adequate nematode defense response.

RNA-seq analysis of *M. javanica* J2 following exposure to 9-HOT and tomato protoplasts. To identify transcripts and effectors that are subject to plant host lipid-signaling regulation, the oxylipin 9-HOT, which—among other oxylipins—is induced in tomato roots by RKN infection (Fig. 1), was chosen as the inducer. Similarly, in an attempt to mimic exposure to endogenous metabolites secreted within plant tissue during the parasitic stage, J2 were exposed to tomato protoplasts. Freshly hatched J2 incubated in MES buffer or MES + ethanol served as controls for tomato protoplasts and the studied oxylipin, respectively. Altogether, eight cDNA libraries were constructed, generating a total of 170,786,458 reads, 121,161,460 reads after quality and adaptor trimming, and 81,389,649 reads after quality, adaptor, and TRIMMomatic trimming (Table 2). The eight libraries represented: (1) biological duplicates of freshly hatched J2 with 15,208,788 reads; (2) biological duplicates of J2 exposed to protoplasts for 3 h with 15,572,666 reads; (3) biological duplicates of J2 exposed to 9-HOT for 3 h with 14,677,503 reads; (4) biological duplicates of J2 in MES + ethanol with 15,121,772 reads. All RNA-Seq raw data reads were uploaded to NCBI under BioProject Accession PRJNA480605. From the 112,732,357 high-quality paired-end reads, 72.01% of the freshly hatched J2, 67.11% of the J2 exposed to protoplasts, 52.78% of the J2 exposed to 9-HOT and 73.01% of the J2 exposed to MES + ethanol were mapped to the reference genome of *M. javanica*, available on WormBase ParaSite BioProject PRJEB8714 and sequenced by Blanc-Mathieu et al. (Table 2).

Uncovering transcriptomic changes in *M. javanica* J2 upon exposure to oxylipin 9-HOT and other plant signals. To measure transcript regulation of *M. javanica* J2 by 9-HOT and plant signals, we measured changes in gene expression of infective J2 exposed to tomato protoplasts compared to their control (MES), and of infective J2 exposed to 9-HOT compared to their control (MES + ethanol). Statistical analysis of the differentially expressed genes (DEG) was performed using the DESeq2 package. The threshold for DEG was FDR ≤ 0.001 and log2 fold change (FC) smaller than −2 or greater than 2. Overall 7530 DEG were identified among the treatments—J2 exposed to protoplasts and J2 exposed to 9-HOT—compared to their respective controls. Principal component analysis (PCA) was conducted to determine and visualize the significant correlation between the different treatments using R (version 3.0.0) (http://www.R-project.org) and the FactoMineR R package. PCA of the transcriptomic data was performed for all expressed gene profiles (Fig. 3A). The two dimensions made up 98.51% of the total variance, indicating that most of the factors included in the data were responsible for the significant variation in DEG between treatments. Dimension 1 accounted for 97.46%, and dimension 2 for 1.05% of the variation. The first and second PC axis separated the freshly hatched J2 exposed to 9-HOT from all other groups. Taken together, these results suggest that the different treatments can be divided into two major expression profiles of DEG: (1) freshly hatched J2, J2 exposed to protoplasts and MES + ethanol and (2) J2 exposed to 9-HOT, the latter demonstrating the most variation (Fig. 3A).

Next, all 7512 DEG were subjected to Venn diagram analysis illustrating DEG distribution among treatments (Fig. 3B). A total of 6085 DEG (81%) and 1057 DEG (14.1%) were found to be expressed exclusively in the 9-HOT and protoplast treatment, respectively, and 370 DEG (4.9%) were expressed in both treatments (common DEG) (Fig. 3B.1). A total of 4580 DEG were found to be upregulated (83.3%), with 3813 (83.3%) and 751 (16.4%) expressed in the 9-HOT and protoplast treatment, respectively, and 16 (0.3%) expressed in both treatments (Fig. 3B.2). GO enrichment analysis of the annotated DEG revealed several enriched biological processes (Fig. 3C.1) and molecular functions (Fig. 3C.2). Detailed analysis of identified DEG following exposure to protoplast and 9-HOT revealed that the key enriched biological processes were negative regulation of endopeptidase (GO:0061135), peptidyl-proline modification (GO:0031543), glycosylceramide catabolic process (GO:0004348), cellulose metabolic process (GO:0030243), galactosylceramide metabolic process (GO:0006683), and the key enriched molecular functions included polygalacturonase activity (GO:0004650), calcium ion binding...
To further classify the observed changes, all 7512 DEG were analyzed for Pathway enrichment using the web-server of KOBAS 3.078 KEGG, GO and PANTHER database enrichment analysis. When compared to Loa loa for glutathione metabolism (loa00480), which plays important roles in antioxidant defense8, 15 DEG were upregulated and 7 DEG downregulated, for a total of 22 out of 25 known genes involved in this pathway (Fig. S1A).

In the Wnt signaling pathway (loa04310), known to regulate crucial aspects of cell-fate determination during embryonic development80, 24 DEG were upregulated and 5 DEG were downregulated—29 out of the 65 known genes represented in this pathway (Fig. S1B). In the fatty acid biosynthesis pathway (loa00061), which is a precursor for a variety of important building blocks21,81,82, 8 DEG were upregulated, 10 out of the 10 known genes. For retinol metabolism (loa00830), a total of 6 DEG were upregulated out of 6 known genes in this pathway. All of these DEG were enriched following exposure to 9-HOT. Following exposure of J2 to protoplast treatment, the calcium signaling pathway (loa04020) was represented by 27 upregulated DEG out of a total of 57 genes known to be involved in this pathway. In the inositol phosphate metabolism pathway (loa00562), 9 DEG were upregulated out of a total of 38 known genes in this pathway. In the phosphatidylinositol signaling system (loa04070), 9 DEG were upregulated out of 57 known genes in this pathway.

9-HOT application regulates the expression of genes encoding carbohydrate-active enzymes (CAZymes) related to cell wall modification and degradation. To further evaluate the effect of 9-HOT and protoplasts on CAZymes, we investigated families of structurally related catalytic and carbohydrate-binding modules (CBM) of enzymes that degrade, modify or create glycosidic bonds. We focused on the DEG encoding CAZymes related to cell wall biosynthesis, modification and remodeling82 (Fig. S2). Differential expression of four categories of CAZymes were represented following exposure to 9-HOT and protoplasts, i.e., genes encoding carbohydrate esterase (CE), glycoside hydrolase (GH), glycosyl transferase (GT) and polysaccharide lyase (PL) (Figure S2A). Among the CAZyme categories involved in cellulose degradation (Fig. S2B),...
two families of GH were differentially expressed following protoplast and 9-HOT treatments. These included GH5 and GH7 presented by endo-1,4-β-glucanase/cellulase and β-glucosylceramidase and chitosanase. Among the hemicellulose-degrading genes (Fig. S2C), two categories were represented by GH31 and CE1, such as α-galactosidase, α-mannosidase, glucosyltransferase acetyl transferase and carboxylesterase.

9‑HOT induces major differences in nematode’s effector‑encoding gene expression. Given that we were interested in genes involved in governing parasitism, i.e., effectors, our next step was an in-silico analysis to identify differentially expressed transcripts that might encode secreted effectors. DEG that contained a predicted signal peptide according to SignalP5.0 and which do not carry TMHMM (transmembrane alpha helix motifs) were subjected to Venn diagram analysis illustrating DEG distribution among treatments (Fig. 4A). A total of 913 DEG with a signal peptide were identified (12.2% of total DEG). Among these, 707 DEG (77.4%) and 116 DEG (12.7%) were expressed in the 9-HOT and protoplast treatments, respectively, and 90 DEG (10.1%) were expressed in both treatments (Fig. 4A.1). A total of 367 DEG with a signal peptide were found to be upregulated (33.7%) (Fig. 4A.2), and 597 (54.9%) DEGs were downregulated (Fig. 4A.3).

All DEG that were upregulated by 9-HOT treatment were analyzed for GO terms (Fig. 4B) in each of the three main categories (biological process, molecular function and cellular component classification) of the GO classification. The GO cellular component classification of the DEG indicated 12% of extracellular region and 88% of the membrane part. Biological process was represented by 10 categories: 31% by negative regulation of peptidase activity (GO:0010466), 30% by regulation of endopeptidase activity (GO:0052548), about 4–7% each by chitin, ceramide, glucosamine, glycosaminoglycan and glycolshingolipid catalytic process (GO:0006032, GO:0046514, GO:1901072, GO:0006027 and GO:0046479, respectively) (Fig. 4B). Molecular function was represented by 11 categories: 31% by serine-type endopeptidase inhibitor activity (GO:0004867), 18% by metalloendopeptidase activity (GO:0004222), 9% by carboxypeptidase activity (GO:0004180). Next, we validated the expression profile of predicted effectors in protoplast- and 9-HOT-treated M. javanica J2. For that purpose, seven selected DEG from the M. javanica J2 transcriptome were further confirmed and validated by quantitative reverse transcription (qRT)-PCR. Four downregulated genes and three upregulated genes were selected for quantitative analyses, on the basis of being potentially secreted and involved in the pathogenic process and carrying a signal peptide.
peptide. One of the DEG was found in J2 exposed to protoplasts: protoplasts#1 encoding DB domain-containing protein (M.javanica_Scaffold102g012786); six were found in J2 exposed to 9-HOT: 9-HOT#1—unknown gene (M.javanica_Scaffold10526g059067), 9-HOT#2—SCP domain-containing protein (M.javanica_Scaffold139g002482), 9-HOT#3—unknown gene (M.javanica_Scaffold10988g053951), 9-HOT#4—putative esophageal gland cell secretory protein 3 (M.javanica_Scaffold260g024064), 9-HOT#5—calycin-like domain (M.javanica_Scaffold24242g089056), 9-HOT#6—triacylglycerol lipase (M.javanica_Scaffold6853g045742), all of which are shown in Fig. S3. For all qRT-PCR analyses, two housekeeping genes were chosen as reference genes for M. javanica: endogenous reference genes 18S and EF-1α (Table 1S). For all tested transcripts, our analysis remained similar to the transcriptomic trend of the FC data.

Differentially regulated effector-encoding genes are implicated in cell wall modifications, stress response, plant immune suppression and nematode development, enabling parasitism. Among the 346 upregulated DEG in the 9-HOT treatment were genes implicated in nematode growth and development, such as MLT-10, the cuticlin-1, epicuticulin gene family and collagen, all of which participate in various cellular and developmental processes required for nematode molting and fecundity. Within the root tissues, RKN undergo three molting stages; in each molt, the makeup of the cuticle surface coat’s compounds changes, one among many strategies acquired by plant-parasitic nematodes to avoid plant immunity. Similarly, previous studies have shown that hormones and different compounds secreted by the roots trigger changes in the surface cuticle of sedentary plant-parasitic nematodes.

In addition, a group of genes implicated in oxidation–reduction activity required for coping with oxidative stress response were induced by 9-HOT (i.e., glutaredoxin, thioredoxin-like domain). Similarly, effectors containing a C-type lectin, which was found to delay the oxidative burst in tobacco leaves following infection by M. graminicola, were upregulated following exposure to 9-HOT (M. javanica_Scaffold6180g042809) and protoplasts (M. javanica_Scaffold16387g074472) (Fig. 4A). In addition, genes involved in lipid modification (several genes of triacylglycerol lipase, calycin domain) were also differentially expressed following 9-HOT treatment. Differential regulation of several proteases was observed: a carboxypeptidase, serine carboxypeptidase (SCP), was studied in depth and was shown to play a critical role in the development, invasion, and pathogenesis of certain parasitic nematodes and other animal pathogens. Another protease, a serine proteinase, that was induced has also been shown to be involved in mediation of host invasion by the parasitic nematode Steinernema carpocapsae. Similarly, alteration in the expression of chorismate mutase (CM) and venom allergen-like protein (Vap2), well-studied effectors that are involved in suppression of defense reactions of the host cell during the infection stages, were detected. Papain inhibitor, which might be related to pathogen effectors that inhibit apoplastic papain-like cysteine protease (PLCP) which are strongly associated with the effector triggered immunity (ETI) response, were strongly upregulated upon 9-HOT treatment. An extensive representation of genes involved in cell wall modification and remodeling, carrying a signal peptide, were altered upon 9-HOT and protoplast exposure, e.g., effectors carrying a Rare lipoprotein A domain found in several effectors, such as Mc-EXP1, GrEXPB1 and GrEXPB2, associated with cell wall extension in M. chitwoodi and G. rostochiensis, respectively, were upregulated upon 9-HOT application. Additional observed differentially regulated genes were involved in cell wall degradation and modification (Fig. S2), including cellulose binding protein (CBP)—a nematode excretion protein that appears to be associated with the breakdown of cellulose present in the plant cell wall—and pectate lyases, known to play a key role in pectin degradation by catalyzing the random cleavage of internal polymer linkages (endopeptinases). Similarly, pectate lyases have been isolated from several sedentary plant-parasitic nematodes, such as species of Heterodera, Globodera, and Meloidogyne, and have been shown to be released into the plant tissue through the stylet of the nematode. GH family 38 (GH38) α-mannosidase was upregulated by 9-HOT; this protein is involved in α-mannose cleavage, carrying hemicellulose activity, and has been identified in several phytopathogenic nematode species. In addition, several endoglucanases belonging to the GH5 family were differentially expressed upon 9-HOT treatment (Fig. S2); these have been shown to facilitate penetration and migration into root tissue and were localized to the esophageal glands of infective juveniles. All of these genes are part of a cocktail of cell wall-degrading and modifying enzymes that are thought to soften and degrade the structure of plant cell walls during nematode migration and to facilitate infection. Their fluctuation upon 9-HOT and protoplast treatment might indicate tight regulation governed by oxylipin signals, among others.

Triacylglycerol lipase and MLT10-like, predicted effectors, are exclusively localized to the M. javanica esophageal glands. Additional effectors that may facilitate plant–nematode interactions through manipulation of the plant defense system, or are required for nematode developmental processes, and which were localized to the esophageal glands upon 9-HOT application, are triacylglycerol (TAG) lipase, which functions in TAG release from lipid droplets by lipolysis in the peroxisome. Interestingly, modulation of plant peroxisomes in giant cells by sedentary RKN has been described previously, and molting cycle MLT-10-like, required for nematode development. Using fluorescence in situ hybridization (FISH), we designed a Cy5-probe to specifically target the spatiotemporal expression of several putative effector-encoding genes derived from the above DEG carrying a signal peptide. FISH results, shown in Fig. 5, localized TAG lipase exclusively to the dorsal and two subventral glands upon J2 exposure to 9-HOT (Fig. 5A1–4), compared to its control (i.e., J2 exposed to MES+ ethanol) that showed no fluorescent signal (Fig. 5A5–8). Similarly, MLT-10 was localized to the subventral glands upon J2 exposure to 9-HOT (Fig. 5B1–4), compared to its control (J2 exposed to MES+ ethanol) with no fluorescent signal.
These results strengthened our assumption that the DEG containing a signal peptide are potential secreted effectors. Additional analysis of TAG lipase and MLT-10-like by qRT-PCR at different stages of *M. javanica* development further correlated their expression with parasitism (Fig. S4).

**Conclusions**

Despite enormous progress in the discovery and identification of nematode effectors in the last decade, less is known about their function and the specific signals required for their induction. Our transcriptomic studies of *M. javanica* provide evidence of transcripts with homology to previously reported plant-parasitic nematode effectors, as well as unknown secreted proteins, all induced by 9-HOT. Together with the oxylipin profile analysis, it seems that oxylipins, while part of the plant's defense response, might play an important signaling role in regulation of the nematode transcriptome. Among the differentially regulated predicted effectors, several were further confirmed by FISH analysis as effectors located within the esophageal glands. Taken together, these results placed oxylipins as early modulators of plant defense signals, as well as important signals regulating nematode parasitism. The implication of G-protein coupled receptor (GPCR) as an oxylipin receptor, shown previously by Affeldt et al. and Lahvic et al., should place this group of nematode receptors as important mediators of parasitic behavior. This interaction remains to be studied.
Methods

Nematode culture and inoculum preparation. M. javanica were multiplied on tomato plants (Solanum lycopersicum cv. Avigail 870) in a greenhouse. Nematode egg masses were extracted by cutting roots into pieces and macerating in 0.05% (v/v) sodium hypochlorite (NaOCl) in a blender\(^1\). The resulting suspension was passed through a set of three sieves (120, 60 and 30 µm). The debris was discarded, while the eggs deposited on the 30-µm sieve were transferred to a 50-ml test tube. Centrifugal flotation with 40% (w/v) sucrose at 6000 rpm for 10 min was performed; the supernatant, containing the eggs, was poured onto a 30-µm sieve and washed with tap water, and eggs were collected in MES buffer\(^3\) and sterilized as described by van Vuuren and Woodward\(^4\).

Subsequently, eggs were transferred onto a 30-µm sieve and suspended in 5 ml MES buffer in a petri dish. The petri dish was placed in a growth chamber at 26 °C under dark conditions till hatching (5–6 days)\(^5\).

J2 exposure to protoplast treatment. Sterilized tomato seedlings were grown at 26 °C under 16 h day-light in sterile plates on standard-strength Gamborg’s B5 Salt medium (DUCHEFA, Haarlem, The Netherlands), supplemented with 2% (w/v) sucrose and solidified with 0.8% (w/v) Gelrite agar (DUCHEFA). Roots were sub-cultured on the same medium with one root section per petri dish (MINIPLAST, Ein Shemer, Israel) and incubated in a growth chamber at 26 °C in the dark for 2 weeks. Protoplasts were released and isolated from about 40 plates of roots using Demidchik and Tester’s protocol. Fresh protoplasts were incubated with 60,000 freshly hatched sterilized J2 at 26 °C in the dark for 3 h.

J2 exposure to 9-HOT treatment. The 9-HOT oxylipin used in this study was purchased from CAYMAN CHEMICAL Company, diluted with MES buffer to a final concentration of 10 µM. Freshly hatched sterilized J2 were incubated in vials (500 J2 per vial for a total of 15,000 juveniles for each oxylipin treatment) containing 9-HOT or 0.01 M MES + ethanol (to a final concentration of 10 mM, as a control) for 3 h at 26 °C in the dark.

cDNA library preparation and high-throughput sequencing. Total RNA extraction from freshly hatched J2, and J2 exposed to protoplasts, 9-HOT and MES + ethanol, was performed with Trizol, using total RNA extraction from the Caenorhabditis elegans bioprotocol (2011) with steps 10–12 being replaced by the use of the Arcturus PicoPure RNA Isolation Kit, Applied Biosystems (Foster City, CA, USA), and stored frozen at − 80 °C till further processing\(^6\). Quality measurements for total RNA were performed using TapeStation 2200 (Agilent). The RIN (RNA integrity number) values of all 8 samples were between 7 and 10.

Library preparation and data generation. Eight RNA-Seq libraries were produced using the NEB-NEXT ULTRA Directional RNA Library Prep Kit for Illumina (NEB, cat no. E7420) according to the manufacturer's protocol and starting with 100 ng of total RNA. The mRNA pull-up was performed using the Magnetic Isolation Module (NEB, cat no. E7490). The eight libraries were mixed in a single tube at equal molar concentrations. RNA-Seq data were generated using the Illumina NextSeq Machine and Nextera Preparation Kit at the Technion—Institute of Technology in Haifa, Israel. Eight paired-end RNA-Seq libraries of two replicates for each condition were sequenced.

Differential expression analysis. The sequences were trimmed for adaptor and low-quality sequence removal using Trimomatic software\(^2\). Cleaned sequences were mapped using Bowtie2\(^8\) and quantified using the RSEM method\(^9\) to the reference genome of M. javanica (accession no. GCA_90003945.1). The annotated proteins of the nematode were searched for signal peptides using the software SignalP 5.0\(^10\).

DEG were identified using the DESeq2 R package\(^11\). To create the Venn diagrams, we used Venny website http://bioin fogp.cnb.csic.es/tools/venny/\(^12\). All RNA-Seq datasets were uploaded to the SRA NCBI database under BioProject Accession No. PRJNA480605.

CAZyme annotation. The sequences for and functional annotation of CAZymes (automated carbohydrate-active enzyme annotation) was performed using the CAZy database (http://www.cazy.org/) according to Lombard et al.\(^13\). We assigned the M. javanica (accession no. GCA_90003945.1) proteins to the CAZy database using the dbCAN2 meta server (http://dbcan2.cshl.edu/dbCAN2/index.php).

Plant material and growth conditions. Tomato (Solanum lycopersicum cv. Avigail 870) seeds were sterilized with 1.4% (v/v) NaOCl for 10 min, washed three times with sterile water for 5 min each, and then planted on standard strength Gamborg’s B5 salts medium (DUCHEFA), supplemented with 2% sucrose and solidified with 0.8% Gelrite agar (DUCHEFA). Seeds were kept in a growth chamber at 26 °C under a 16/8-h photoperiod at 120 µmol/m²/s for 2 weeks until cotyledons appeared.

Plasmid construction and generation of transgenic hairy roots. All PCR amplifications used for plasmid construction were performed with Recombinant Taq DNA polymerase (THERMO SCIENTIFIC, Paisley, UK) according to the manufacturer’s instructions. To amplify LOX1.2, AOSI, OPR2 and aDOXI promoter regions, tomato genomic DNA was used as a template for PCR, with the following primers: LOX1.2 FOR: 5′-TAT CAT ATA AGT GAG CTC GGA CCT ACT-3′, REV: 5′-AGG TGA CTG GCC CGG GTT TTC CTC AGA AAA AGT TTC-3′, with SacI and Smal restriction sites; AOSI FOR: 5′-CGT TTT TAC CAC AGG TCG AAT TCA ACG CGG T-3′, REV: 5′-AGG TAC CTA GCC CGG GTT CTA TTA GAA AAA AAT CAA-3′, with EcoRI and Smal restriction sites; OPR2 FOR: 5′-CTT TTA TGA ATG GTG GTA CCC TTT TCA CCA-3′, REV: 5′-AGG TAC CTA
were weighed and all of the data were normalized to the relative weight of those frozen tissues. Biological replicates with a total of 100–130 mg of gall samples from inoculated and noninoculated tomato roots and galls were collected 5, 15 and 28 DAI. The noninoculated roots were collected as controls. Five independent J2 M. javanica B5 medium and kept in the dark for 2 weeks. Root systems were inoculated with freshly hatched described by Chinnapandi et al. 125, and assayed histochemically for GUS activity at the designated times after 18S ATCC 15,834 strain was used for the transformation by heat-shock method124. Individual cotyledons were excised from 15- to 20-day-old tomato seedlings grown as described above and immersed in a 2-day-old R. rhizogenes suspension for incubation at 28 °C for 2 h, with agitation at 100 rpm. The excised cotyledons then were placed on standard-strength Gamborg's B5 salt media for 3 days for co-cultivation, and then transferred to B5 agar media supplemented with the antibiotics kanamycin (50 mg/mL) (DUCHEFA, Haarlem, the Netherlands) and timentin (15:1) at 300 mg/mL. (DUCHEFA, Haarlem, the Netherlands). After 7–10 days of incubation in the dark at 25 °C, roots emerged from the wounded surface of the cotyledons. Hairy roots were transferred to Gamborg's B5 medium containing 0.8% Gelrite and kanamycin (50 mg/mL). For nematode-infection experiments, transformed roots were subcultured in antibiotic-free media for 2 weeks, and 300 freshly hatched sterile M. javanica juveniles were used to inoculate the transgenic root lines, and root samples were taken at the designated time points for GUS assessment.

GUS bioassay. Two-week-old hairy root lines carrying the promoter GUS constructs were inoculated as described by Chinnapandi et al. 125, and assayed histochemically for GUS activity at the designated times after infection with 300 sterile freshly hatched pre-parasitic M. javanica J2.

Plant oxylipin and hormone extraction. Tomato (Solanum lycopersicon cv. Avigail 870) seeds were sterilized and planted as described. Emerging roots were cut into 2-cm long segments, subcultured on Gamborg's B5 medium and kept in the dark for 2 weeks. Root systems were inoculated with freshly hatched M. javanica J2 and galls were collected 5, 15 and 28 DAI. The noninoculated roots were collected as controls. Five independent biological replicates with a total of 100–130 mg of gall samples from inoculated and noninoculated tomato roots at the selected time points were collected into a 1.5-mL tube and kept at −80 °C until further analysis. Samples were weighed and all of the data were normalized to the relative weight of those frozen tissues.

Oxylipins/hormones were extracted from each sample in liquid N2 by using the phytohormone-extraction protocol reported by Yang et al. 126 with the following modifications: 500 µL of phytohormone extraction buffer (1-propanol:water:HCl at 2:1:0.002 v/v) containing 500 nM deuterated internal standards: d-ABA ([2H6](+)-cis, trans-ABA; OLCHEM), d-IAA ([3H] indole-3-acetic acid, OLCHEM), d-JA (2,4,4-d3; acetyl-2,2-d2 JA; CDN Isotopes), and d-SA (d-d-SA, SIGMA) were added to 100–130 mg gall tissue127. The galls were homogenized at 6000 rpm for 30 s, twice. Samples were agitated for 30 min at 4 °C in the dark and then 500 µL dichloromethane was added and samples were agitated again for 30 min at 4 °C in the dark. Samples were centrifuged at 13,000 g for 5 min and the lower layer was collected into a glass vial for complete evaporation under a N2 gas stream. Samples were resuspended in 150 µL methanol, shaken for 1 min and then centrifuged in a 1.5-mL microcentrifuge tube at 14,000 g for 2 min to pellet any debris. Supernatant (100 µL) was collected into an autosampler vial for injection into a SCIEX API 3200 LC–MS/MS with a C18 column for chromatography and electrospray ionization. Peaks were integrated using Analyst 1.6.2 software and metabolites were quantified against internal standards41.127. Identification of fatty acid peaks was verified by comparison of the mass spectra to authentic standards. Noninoculated tomato roots served as controls.

Real-time qPCR analysis. For the qRT-PCR experiments, we removed contaminating genomic DNA from RNA with the Turbo DNA-Free Kit from AMBION (APPLIED BIOSYSTEMS). DNA-free RNA (1 µg) was converted to first-strand cDNA using the Verso cDNA Synthesis Kit (ABGENE, Epsom, UK), and reactions were performed using ABsolute SYBR Green ROX Mix (ABGENE). Primers for qRT-PCR experiments were designed with Primer Express software (APPLIED BIOSYSTEMS, Table 1S). A total volume of 10 µL contained 3.4 µL cDNA, consisting of 1× SYBR-Green ROX Mix (ABGENE) and 150 nM forward primer and 150 nM reverse primer subjected to real-time PCR (Rotor-Gene RG-3000, CORBETT RESEARCH) using 0.1 mL 4-tube strips & caps (AXYGEN, Union City, CA, USA). All PCR cycles began with 2 min at 50 °C, then 10 min at 95 °C, followed by 40 cycles of 10 s at 95 °C and 1 min at 60 °C. After the PCR, a melting curve was generated by gradually increasing the temperature to 95 °C to test for amplicon specificity. For qRT-PCR, a mixture of all cDNAs was used for all treatments as a template for calibration curves designed for each pair of primers. Each reaction was performed in triplicate and the results represent the mean of two independent biological experiments. Two reference genes, EF-1α (GenBank accession no. U94493.1) and 18S (GenBank accession no. AF442193.1), were used as endogenous controls for gene-expression analysis. Transcript levels were normalized for each sample with the geometric mean of the corresponding selected reference genes. All of the reference genes were confirmed to display minimal variation across the treatment and were the most stable reference genes from a set of tested genes in a given cDNA sample. Values were expressed as the increase or decrease in level relative to a calibration sample. A negative control PCR without cDNA template was also run to confirm the absence of
nonspecific PCR products (NTC) No Template Control. The same process was performed for qRT-PCR of the expression of the nematode effectors TAG lipase and MLT-10.

**FISH.** Freshly hatched pre-parasitic *M. javanica* J2 were exposed to 9-HOT diluted in MES buffer to a final concentration of 10 mM, or to 0.01 M MES buffer, for 3 h; all samples were washed with 0.01 M MES buffer. The FISH procedure followed the method of Sakurai et al. with slight modifications. The J2 were dissected manually with a razor blade and transferred to Carnoy’s fixative (chloroform:ethanol:glacial acetic acid, 6:3:1, v/v) and fixed overnight. The samples were then decolorized in 6% (v/v) hydrogen peroxide in ethanol for 2 h and hybridized overnight in hybridization buffer (20 mM Tris–HCl pH 8.0, 0.9 M NaCl, 0.01% w/v SDS, 30% v/v formamide) containing 10 pmol fluorescent probe/mL. Based on the transcriptome sequences of interest, DNA probes were designed using Primer Express 3.0.1 software and checked for specificity using BLASTn (NCBI); TAG lipase (nematode effector) Cy5 (5′-Cy5-AATGTAGTGGTGACTCCAGATCG-3′) and MLT-10-like Cy5 (5′-Cy5'-AGACAAAAAGGGTGCAGAGCA-3′) were used as probes to target *M. javanica* J2. The stained samples were submersed in hybridization buffer supplemented with DAPI (0.1 mg/mL in 1X PBS) and transferred to a slide with liquid blocker, covered, sealed with nail polish and viewed under a confocal microscope. Detection specificity was confirmed using *M. javanica* exposed to 0.01 M MES buffer only as a control.

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Author contributions
S.B., N.F., E.B., M.K., conceived and designed the experiment. N.F. and N.S. isolated and acquired the RNA-Seq data. N.F. and N.S. performed the transcriptomic alignment with the M. javanica genome. N.F., E.B. and M.K. performed the oxylipin analysis. N.F. and S.B. wrote the manuscript. E.B. performed all of the confocal microscopy work. P.B. and X.Q. helped with the data analysis. All authors made substantial contributions to the final text. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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