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Constitutive and Inducible Expression of Genes Related to Salicylic Acid and Ethylene Pathways in a Moderately Resistant Tomato Cultivar Leads to Delayed Development of *Meloidogyne javanica*

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Abstract: Knowledge of the molecular changes in resistant and susceptible cultivars during nematode attack is essential for developing plant resistance. Increased expression of genes related to the synthesis and signaling of salicylic acid, jasmonic acid, and ethylene is known to induce expression of genes related to defense against plant parasitic nematodes. Here, we inoculated approximately 3000 s-stage juveniles (J2s) of *Meloidogyne javanica* to moderately resistant and highly susceptible tomato cultivars (ALYSTE F-1 and Dutch Mobil, respectively) to compare the developmental disease stages. The roots of each cultivar were collected daily until 30 days after inoculation (DAI). The roots were stained with acid-fuchsin and dissected under a microscope. The results showed that a few parasitic J2s were converted to J3s in the moderately resistant cultivar at 14 DAI, at which time, the highly susceptible cultivar had the highest number of J3s. Comparison of hormonal pathways in the two cultivars revealed that the expression of genes related to the ethylene pathway in ALYSTE F-1 was more strongly upregulated than in Dutch Mobil at 14 DAI. Moreover, the jasmonic acid pathway in the roots of both cultivars decreased at 14 DAI. The expression of genes related to salicylic acid synthesis and signaling was not significantly different between the two cultivars with regard to their non-inoculated controls, respectively, but ALYSTE F-1 in general showed constitutively higher levels of these genes compared to Dutch Mobil at 14 DAI. These results suggest that constitutive and induced expression of genes related to the salicylic acid pathway and ethylene pathway, respectively, delay the development of *M. javanica* J2s in ALYSTE F-1.

Keywords: acid-fuchsin; disease development stages; gene expression; highly susceptible cultivar; hormonal cycles; jasmonic acid pathway

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1. Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are the most destructive group of plant parasitic nematodes worldwide and are known to cause high yield losses in vegetable crops, including tomatoes [1]. These sedentary endoparasites can hijack host machinery through secreting effector molecules to form a complex feeding site, which results in the formation of typical root knots or galls in infected plants [2]. The effector molecules include peptides, enzymes, small metabolites, and other biomolecules, and play an important role in plant parasitism [3,4]. Some of these effectors can be recognized by resistance (R) proteins in the plants, which can activate the plant's immune system [5]. In response to nematode infection, R genes belonging to resistant cultivars repress one or more critical steps in nematode parasitism and affect their reproduction rate. Generally, mechanisms of

resistance can be induced pre- or post-infection [6]. Mechanisms of pre-infection restrict the penetration of second-stage juveniles (J2s) and involve pre-existing morphological factors or the production of root exudates that do not attract, or may repel, J2s [6,7]. Regarding post-infection mechanisms, the activation of molecular and physiological processes in the plant prevents the formation of feeding sites, and inhibits or delays the development of J2s and/or reproduction of the adult females [6,8,9].

Plants have developed a range of morphological and physiological responses to stressors, which allows them to deal with the prevailing environmental conditions. Plants must realize various stressors, process the signals, and activate the optimal stress response to maximize resistance while minimizing costs and side effects [10]. Phytohormones, as signal molecules, play a crucial role in regulating plant growth and development processes, as well as plant response to biotic and abiotic stresses [11–13]. Transcriptomic and microarray analyses have shown that genes responding to several hormones, including jasmonic acid (JA), auxin, salicylic acid (SA), ethylene (ET), gibberellic acid (GA), brassinosteroids (BRs), and abscisic acid (ABA), are differentially expressed in nematode-infected roots [11–13]. Several studies have revealed that the signaling pathways of SA, JA, BRs, ABA, and ET are involved in plant defense against nematodes [14–19]. Ethylene is known to play an important role in nematode attraction and migration, as well as the formation of feeding sites and plant defense during the early stages of nematode infestation [20–23], but its role may differ for the interaction of plants with RKNs and cyst nematodes [24]. Indeed, an intact ET pathway is required for plant resistance against RKN [17,25,26]. On the other hand, there are conflicting results regarding the attraction of cyst nematodes in plants treated with ET inhibitors or roots of ET-insensitive mutants. Some studies have shown that the ET pathway promotes cyst nematode parasitism, as evidenced by the significant reduction of cyst nematodes (*Heterodera schachtii* and *Globodera rostochiensis*) inside the roots of ET-insensitive mutants or wild-type plants treated with ET inhibitors [23,27]. Another study has shown that the attraction of soybean cyst nematode (SCN), *H. glycines*, to the roots of *Arabidopsis* (non-host for SCN) is diminished in ET-overproducing mutants and enhanced in ET-insensitive mutants [28]. In the ET biosynthesis pathway, S-adenosyl-L-methionine (SAM) is first converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC-synthase (ACS), and then ACC is converted into ethylene by ACC-oxidase (ACO) [29]. After accumulation, ET is sensed by endoplasmic reticulum (ER)-localized receptors, which negatively regulate the ET signaling pathway [30]. When ET binds to ET receptors, the Raf-like kinase CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) associated with the receptors is inactivated, which leads to the dephosphorylation of the ER-localized ETHYLENE INSENSITIVE2 (EIN2). After dephosphorylation, the C-terminal domain (CEND) of EIN2 is released and enters the nucleus to activate the EIN3 transcription factor [31–33]. EIN3 activates the expression of ET-responsive transcription factors such as *OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59* (*ORA59*) and *ETHYLENE RESPONSE FACTOR 1* (*ERF1*), which directly activate the expression of ERF-branch marker genes, such as the defense gene *PLANT DEFENSIN1.2 (PDF1.2*) [34,35].

Salicylic acid is a major defense hormone against biotrophic and hemibiotrophic pathogens [36]. The application of SA, or chemicals with similar actions, can reduce nematode infection, and in many cases, nematodes are able to suppress the SA pathway [5,15,35,37–42]. Previous studies have shown that mutants or transgenics with lower SA levels or signaling are more sensitive to nematode attack [37,38], whereas increased SA levels or signaling reduces nematode infestation [43–46]. Although the phenylpropanoid and the isochorismate pathways are involved in SA biosynthesis, pathogen-induced SA accumulation is mainly induced using the isochorismate pathway, in which chorismate is converted to isochorismate by isochorismate synthase 1 (ICS1). Isochorismate pyruvate lyase (IPL) is responsible for converting isochorismate to SA, and accumulation leads to activation of NPR1 (non-expresser of pathogenesis-related genes1), a master regulator of downstream SA signaling [47–49]. As a transcriptional coactivator, NPR1 (also known as NIM1) interacts with TGAs (TGACG-binding factors) in SA-responsive promoters [36]

and activates pathogenesis-related proteins (PRs) such as PR1, PR2, and PR5, which are related to defense responses [50].

In the past decade, more than 20 papers have introduced JA as a defense molecule against RKNs [28,46,51]. Application of exogenous methyl jasmonate (MeJA) to rice (*Oryza sativa*), soybeans (*Glycine max*), and tomatoes (*Solanum lycopersicum*) has been shown to reduce RKN infestations [38,52–56], while inhibitors of JA biosynthesis increase susceptibility to RKN infections [38,55]. Biosynthesis of JA and its derivatives have been studied in various monocotyledonous and dicotyledonous plants, but most studies involve the model plants *Arabidopsis thaliana* and *Lycopersicon esculentum* (tomato) [57]. At least two JA biosynthetic pathways are known to exist, including the octadecane pathway starting from α-linolenic acid (α-LeA, 18:3) and the hexadecane pathway starting from hexadecatrienoic acid (16:3). In these pathways, the 18:3 and 16:3 unsaturated fatty acids, using 13-lipoxygenases (13-LOXs), allene oxide synthases (AOSs), and allene oxide cyclases (AOCs), are converted to 12-oxo-phytodienoic acid (12-OPDA) and dinor-12-oxo-phytodienoic acid (dnOPDA) in the chloroplast, respectively. 12-OPDA and dn-OPDA are then converted to JA by a series of β-oxidation reactions in the peroxisome [58]. JA then enters the cytoplasm and is metabolized into various structures with different biological activities, such as MeJA, 12-hydroxyjasmonic acid (12-OH-JA), and JA-isoleucine (JA-Ile) [57]. Among these, JA-Ile is the most bioactive form of JA in plants, and conversion of JA to JA-Ile is mediated by jasmonoyl-isoleucine synthetase (JAR1) forms [59]. JA-Ile is then transferred to the nucleus and activates several key transcription factors (TFs), such as MYC2, to induce expression of JA-responsive genes. When JA-Ile levels are low, the master TFs, such as MYC2, are repressed through direct interaction with JASMONATE ZIM DOMAIN (JAZ) proteins at the promoter regions of JA-responsive genes [60]. Nuclear JA-Ile acts as "molecular glue" to promote the creation of the SCF^{COI1}-JAZ co-receptor complex and subsequent ubiquitination of JAZ proteins and degradation by the 26S proteasome [61]. The *COI1* (*coronatine insensitive1*) gene encodes an F-box protein, which associates with SKP1 and Cullin proteins to form the SKP1-CULLIN1-F-box-type (SCF) E3 ubiquitin ligase complex (SCF^{COI1}); this complex facilitates the ubiquitination of target proteins (JAZs) and consequently their degradation by the 26S proteasome [62,63]. JAZ degradation releases the inhibitory effect on TFs, such as MYC2. The activation of these TFs triggers the expression of JA-responsive genes, such as protease inhibitors (PIs) and pathways producing secondary metabolites involved in defense responses [28]. Molecular evidence shows that the transcription factor WRKY57 (a negative regulator of JA signaling) reduces resistance to *Botrytis cinerea* infection by increasing the expression of *JAZ1* and *JAZ5* [64].

In terms of post-infection resistance of RKNs, depending on the cultivar and plant species, the resistance mechanisms can occur at the early or late stages of infection, which ultimately activate molecular and physiological processes in the plant [65]. To the best of our knowledge, most molecular studies on RKN-plant interactions have been performed at the early stages of infection. As such, there is limited information on the molecular changes that occur in plants at the later stages of infection. The results of the present study showed that ALYSTE F-1, as a moderately resistant tomato cultivar, disrupted nematode penetration, delayed J2 development, and decreased the reproductive rate of *M. javanica*. Unlike ALYSTE F-1, in Dutch Mobil, a highly susceptible cultivar, RKN could easily complete its life cycle in the infected roots. The hormonal cycles can be investigated to determine the underlying mechanisms responsible for the resistance of ALYSTE F-1 to *M. javanica* at the early stages of infection. Therefore, we compared the transcriptional changes of genes related to the ET (*SlACO1*, *SlACO4*, and *SlERF1*), SA (*SlICS1*, *SlNPR1*, *SlPR1*, *SlPR1a*, and *SlPR1a2*) and JA (*SlLOXD*, *SlAOS3*, *SlCOI1*, *SlJAZ1*, *SlJAZ2*, *SlJAZ3*, *SlMYC2*, and *SlWRKY57*) pathways in Dutch Mobil and ALYSTE F-1 (as highly susceptible and moderately resistant tomato cultivars, respectively) at 14 days after inoculation (DAI) with *M. javanica*. To the best of our knowledge, this is the first study to investigate the expression of these genes in highly susceptible and moderately resistant cultivars in the tomato-*M. javanica* pathosystem at 14 DAI, as well as the first report of the involvement of *WRKY57* in plant-RKN interactions.

2. Material and Methods

2.1. Nematode Inoculum

The RKN-infected roots were collected from a greenhouse in Tirtash-Mazandaran, Iran. Then, the RKN was purified and multiplied on susceptible tomatoes (*S. lycopersicum* L. cv. Early Urbana) as described by Hussey and Barker (1973) [66]. The eggs were extracted from tomato roots [67] and retained for hatching in a double-layered tissue paper placed in a sieve and submerged in a container containing distilled water. Freshly hatched J2s were used for all of the experiments.

2.2. Identification of RKN Species

The RKN species was identified based on morphological and morphometrical examinations of J2s, adult females, and female perineal patterns [68] as well as molecular analysis using species-specific SCAR primers OPAFjav (5′-GGTGCGCGATTGAACTGAGC-3′) and OPARjav (5′-CAGGCCCTTCAGTGGAACTATAC-3′) [69].

2.3. Plant Materials

Based on our previous study [70], ALYSTE F-1 was a moderately resistant tomato cultivar, having the lowest number of egg masses/root system, galls/root system, J2s/soil, and eggs/root system and, consequently, the lowest number of the nematode population; on the other hand, Dutch Mobil was a highly susceptible tomato cultivar, having the highest amounts of nematode reproduction traits. Therefore, in the present study, ALYSTE F-1 and Dutch Mobil were selected to compare the nematode development stages and hormonal cycles to investigate the reason for the difference in the resistance and susceptibility of the two cultivars to RKN.

2.4. Disease Development Stages

The tomato seeds were placed in seed trays containing an equal proportion of cocopeat, perlite, and vermiculite under greenhouse conditions (22 ± 3 °C, with a 16/8 h light/dark photoperiod). After 3 weeks, tomato seedlings were transferred singly to pots containing 1.5 kg of sterile soil, sand, and vermiculite (1:1:1) and allowed to establish for 2 weeks before inoculation. After 2 weeks, approximately 3000 J2s were inoculated to each plant. The roots of each cultivar were collected daily until 30 DAI for staining. Three replicates for each cultivar were considered in each experiment, and the experiment was performed twice independently. The roots were stained according to the method described by Daykin and Hussey (1985) [71]. Briefly, the roots were washed using a gentle water stream to remove soil and debris and then treated with 1.5% NaOCl for 4 min with occasional agitation. The roots were thoroughly rinsed with tap water and kept in tap water for 15 min to remove residual NaOCl. To make the stock acid-fuchsin solution, 1 g acid-fuchsin was dissolved in 100 cc water. Then, glycerin, lactic acid, and water were mixed in equal proportions. Nineteen units of this solution were mixed with one unit of acid-fuchsin stock solution and the mixture was heated to begin boiling. Next, the root samples were placed in the staining solution for 30 s before washing in running water to remove the residual staining solution. The samples were then transferred to a decolorizing solution containing equal proportions of glycerol and water with a few drops of lactic acid. Finally, the roots were dissected under a microscope to identify the different developmental stages of RKN. Photographs were taken under an Olympus BX51 microscope.

2.5. Expression Analysis of Genes Related to JA, SA, and ET Pathways 14 Days following RKN Attack in Highly Susceptible and Moderately Resistant Tomato Cultivars

2.5.1. RNA Extraction and cDNA Synthesis

The roots of Dutch Mobil and ALYSTE F-1 tomato cultivars were inoculated with the 3000 J2s of *M. javanica*. The whole roots were collected at 14 DAI, quickly washed with deionized water, dried with sterile paper towels, and then frozen in liquid nitrogen to avoid RNA degradation. The root samples were kept at −80 °C until required for RNA extraction.

Five biological replicates for each treatment were considered, and each biological replicate contained three technical replicates. Total RNA was isolated from the root of infected and non-infected tomato seedlings using a Column RNA Isolation Kit (Denazist Asia, Iran), with additional on-column DNase I digestion. The quality and quantity of the extracted RNA were examined using NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA) and 1.2% denaturing agarose gel electrophoresis.

For the first-strand of cDNA synthesis, 2 µL of 10 pmol oligo dT primer was added to 10 ng RNA, and the volume was made up to 11 µL using RNAse-free water (Denazist Asia, Iran), before incubating for 5 min at 65 °C. After placing the samples on ice to cool, a mixture containing 4 µL of 5x RT Buffer, 2 µL of 10 mM dNTPs, 1 µL RNase Inhibitor (20 U/µL), and 2 µL Thermo- Resistant H- MuLV Reverse Transcriptase (Parsitous, Iran) was added to each sample; the final volume of the reaction mixture was 20 µL. The samples were incubated at 25 °C for 10 min, before incubating at 45 °C for 60 min. The reaction was stopped by heating at 70 °C for 10 min. The samples were immediately chilled on ice. The quality of the cDNA was assessed by a standard PCR using *Tubulin alpha-3 chain* (*TAC*) as the reference gene, and by checking the products on a 1.2% agarose gel. cDNA was used for amplification of several genes associated with hormonal cycles, including *SlLOXD*, *SlAOS3* (JA biosynthesis), *SlCOI1*, *SlJAZ1*, *SlJAZ2*, *SlJAZ3* (JA signaling), *SlMYC2* (key transcription factor of the JA-signaling pathway), *SlWRKY57* transcription factor (negative regulator of the JA pathway), *SlACO1*, *SlACO4* (ET biosynthesis), *SlERF1* (ET response), *SlICS1* (key enzyme in the SA biosynthesis), *SlNPR1* (the central coactivator of TGA transcription factors in the SA-signaling pathway), and *SlPR1*, *SlPR1a*, and *SlPR1a2* (PR proteins induced by the SA pathway). The list of primers used for real-time PCR with details is shown in Table 1. The primers were designed using Oligo7, Oligo Analyzer, and Gene Runner software.

Table 1. List of primers used for gene expression analysis by quantitative real-time PCR.

Gene	Forward Primer (5′to 3′)	Reverse Primer (5′to 3′)	Locus ID (solgenomics.net) 8 May 2022	Amplicon Length (bp)	Reference
SlCOI1 [a]	GGGTACAAGGATACAGGGCAT	GGCAAGAGAATAGTAGGCAAGT	Solyc05g052620.2	173	This study
SlMYC2 [a]	ATGCTTCCAAATCTATGCCGTT	TAATAACCATCTCCCCAACCCA	Solyc08g076930.1	163	This study
SlWRKY57 [a]	GGACTTATCAATCACGAAGCAT	CATCTGGTTGACTTGTTTCTGG	Solyc05g012500.2	190	This study
SlJAZ1 [a]	GTGATTCATCGTCGTCATCGTC	TCATTTGTGCCTTCTCTGGTTG	Solyc12g009220.1	147	This study
SlJAZ2 [a]	TCAGAGTTCATTTGGGACTTTC	CTGGCTTAATCTGGAGGTGTT	Solyc03g122190.2	134	This study
SlJAZ3 [a]	GGAATGAAGGCTGAGTCGGAAC	GAAACTCGGAACCACCAAATCG	Solyc07g042170.2	186	This study
SlJAR1 [a]	GCCATTTATAAGAAAGGAGGGA	CAGCATCTTTAGTCAACACCT	Solyc10g011660.2	109	This study
SlAOS3 [a]	CACTTTCCCTCTACCTTACATCCT	AACCGCCATACGAATTGAATCC	Solyc10g007960.1	170	[72]
SlLOXD [a]	ATCCCTGACGAGAACGATCC	TCCAAGTAGACGGTTGCTGT	Solyc03g122340.2	178	This study
SlERF1 [a]	GGGTCCTTGGTCTCTACTCA	TCTCTTGTGCTTGACTCTTCTA	Solyc05g051200.1	142	This study
SlACO1 [a]	TGAGTTGGTGAACCATGGAA	AGTAGGAAGATGGCGCAAGA	Solyc07g049530.2	190	[73]
SlACO4 [a]	CGCAGGAGGCATCATACTTC	CCGAGTCCCATCTGTTTGTG	Solyc07g049550.2	196	[72]
SlICS1 [a]	GTTCCTCTCCAAGAAATGTCC	TCCTTCAAGCTCATCAAACTC	Solyc06g071030.2	142	[74]
SlNPR1 [a]	TACCAAGTCTACAGAGGAAGGA	CAAATCATCGCCTGCCATAG	Solyc07g040690.2	133	This study
SlPR 1a [a]	GCTGTGAAGATGTGGGTTGATG	CGTTGTCCTCTCCAGTTACCT	Solyc01g106620.2	200	[72]
SlPR1a2 [a]	TTGGGATGCCGACTTGGAAT	CCGCTAACACATTCATTCGTATCG	Solyc09g007020.1	192	[72]
SlPR1 [a]	TGTCCGAGAGGCCAAGCTATAAC	AATGAACCACCATCCGTTGTTGC	Solyc00g174340.1	143	[75]
SlTAC [b]	CTCACGCATTGACCACAAGT	CAGCACCAACCTCCTCATAATC	Solyc08g006890.2	149	[72]

[a] Target gene; [b] Reference gene.

2.5.2. Quantitative Real-Time PCR (qRT-PCR)

qRT-PCR was conducted on a Bio-Rad CFX96 (Bio-Rad, Hercules, CA, USA). The reaction mixture contained 1 µL of cDNA, 7.5 µL of 2X SYBR® Green Real Time PCR Master Mix (Parstous, Iran), 0.3 µL (10 µM) of each primer, and RNAse-free water was added to a

total volume of 15 µL. All PCR cycles began with 5 min at 95 °C, followed by 40 three-step cycles comprising 95 °C for 15 s, 58 to 62 °C for 30 s and 72 °C for 30 s, and finally 72 °C for 5 min. After the PCR reaction, a melting curve was created by gradually increasing the temperature to 95 °C to test for specificity of amplification. *TAC* was used as a reference gene for the normalization of qRT-PCR data (Table 1 from [72]). To determine the relative level of gene expression in the two tomato cultivars, the average ΔCT values were obtained by calculating the difference between the Ct mean of the target gene and reference gene. The transcript levels were calculated according to the $2^{-\Delta CT}$ method and the fold change values were calculated according to the $2^{-\Delta\Delta CT}$ method [76,77]. The non-inoculated treatment was considered as the control. To confirm the results, the expression of genes was examined in another two independent experiments.

2.6. Statistical Analysis

Analysis of variance (ANOVA) was performed by Minitab version 17 in a completely randomized design. The results are reported as significant or non-significant based on Fisher's LSD ($p \leq 5\%$). All diagrams were drawn using Microsoft Excel 2013.

3. Results

3.1. Comparison of M. javanica Invasion, Development, and Reproduction in Highly Susceptible and Moderately Resistant Tomato Cultivars

The highest number of pre-parasitic (invasive) J2s (338.33 ± 12.73), parasitic J2s (367.52 ± 21.36), J3s (310.35 ± 8.40), J4s (303.78 ± 6.96), and adult females (326.67 ± 12.91) for the highly susceptible cultivar Dutch Mobil were found at 3, 7, 14, 20, and 28 DAI, respectively (Table 2 and Figure 1. In contrast, the highest number of invasive J2s (29 ± 0.58), parasitic J2s (30 ± 0.58), J3s (16 ± 2.51), J4s (14 ± 0.6), and adult females (3.40 ± 1.20) for the moderately resistant cultivar ALYSTE F-1 were found at 4, 9, 19, 25, and 30 DAI, respectively (Table 2 and Figure 1). The number of egg masses in the roots of Dutch Mobil and ALYSTE F-1 were 34 ± 1.45 and 0, respectively, at 30 DAI (Table 2). Compared to Dutch Mobil, ALYSTE F-1 had less invasive J2s and delayed the life period of *M. javanica*, which led to a decrease in nematode reproduction (Table 2). ALYSTE F-1 disturbed the penetration, development, and reproduction of *M. javanica* (Table 2). Unlike in ALYSTE F-1, the RKN could easily complete its life cycle in the infected roots of Dutch Mobil (Figure 2).

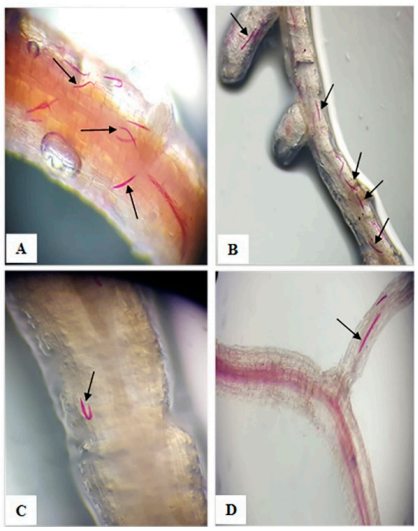

Figure 1. Comparison of the number of *Meloidogyne javanica* larvae in the highly susceptible cultivar Dutch Mobil (**A**,**B**) and the moderately resistant cultivar ALYSTE F-1 (**C**,**D**) in a part of the root. Arrows show nematode larvae in the roots. Acid-fuchsin was used to stain root-knot nematodes in the roots.

Table 2. Comparison of *Meloidogyne javanica* invasion, development, and reproduction in highly susceptible and moderately resistant tomato cultivars (Dutch Mobil and ALYSTE F-1, respectively).

	Invasion by J2s		**Parasitic J2s**		**J3s**		**J4s**		**Females**	
Days post inoculation	Dutch Mobil	ALYSTE F-1	Dutch Mobil	ALYSTE F-1	Dutch Mobil	ALYSTE F-1	Dutch Mobil	ALYSTE F-1	Dutch Mobil	ALYSTE F-1
1	35.67 ± 8.09 e	1.67 ± 0.88 e	0	0	0	0	0	0	0	0
2	269 ± 16.26 c	9.33 ± 1.20 d	0	0	0	0	0	0	0	0
3	338.33 ± 12.73 a	22.67 ± 2.90 b	0	0	0	0	0	0	0	0
4	296 ± 7.77 b	29 ± 0.58 a	42.33 ± 15.39 fg	0	0	0	0	0	0	0
5	163 ± 18.90 d	29.67 ± 1.33 a	157.35 ± 17.57 d	0.5 ± 0.34 j	0	0	0	0	0	0
6	22.33 ± 1.45 ef	19.67 ± 1.20 b	293 ± 9.81 b	10.33 ± 1.20 ghi	0	0	0	0	0	0
7	0.5 ± 0.34 f	15.33 ± 1.77 c	367.52 ± 21.36 a	14 ± 2.08 efgh	0	0	0	0	0	0
8	0	9 ± 0.58 d	354.40 ± 17.68 a	19 ± 1.15 d	0	0	0	0	0	0
9	0	0	289.57 ± 12.98 b	30 ± 0.58 a	10.57 ± 3.48 jk	0	0	0	0	0
10	0	0.33 ± 0.33 e	242.40 ± 23.82 c	25 ± 2.52 bc	56 ± 7.81 gh	0	0	0	0	0
11	0	0	252 ± 16.59 c	27 ± 2.52 abc	48.62 ± 8.64 hi	0	0	0	0	0
12	0	0	153.65 ± 24.51 d	24.63 ± 3.38 bc	138.43 ± 22 e	2 ± 1.15 h	0	0	0	0
13	0	0	87 ± 19.05 e	26 ± 0.58 abc	234.70 ± 28.91 c	2.57 ± 0.83 gh	0	0	0	0
14	0	0	54.48 ± 10.68 ef	23.54 ± 2.40 c	310.35 ± 8.40 a	4.63 ± 2.03 fg	0	0	0	0
15	0	0	27 ± 2 fgh	29 ± 1.15 ab	280.39 ± 11.83 ab	7.33 ± 1.20 ef	24.63 ± 1.45 ghi	0	0	0
16	0	0	14.40 ± 3.84 gh	26.52 ± 2.18 abc	245.67 ± 19.27 c	9 ± 1.52 cde	39.34 ± 7.53 ghi	0	0	0
17	0	0	0.5 ± 0.34 h	18.37 ± 0.88 de	259 ± 19.08 bc	12.33 ± 1.20 abc	74.33 ± 13.96 f	0	0	0
18	0	0	0	16.75 ± 0.33 def	198.89 ± 8.95 d	14.33 ± 1.33 ab	119.70 ± 16.19 e	0	0	0
19	0	0	0	14.63 ± 1.45 defg	110 ± 7.5 ef	16 ± 2.51 a	232.33 ± 3.93 bc	0	0	0
20	0	0	0	14 ± 1.73 efgh	80 ± 3.76 fg	12 ± 2.31 abc	303.78 ± 6.96 a	4.73 ± 1.86 bcde	0	0
21	0	0	0	12 ± 1.52 ghi	34 ± 4.05 hig	9.33 ± 1.45 cde	299.30 ± 11.29 a	8.67 ± 1.20 bcde	12 ± 1.15 f	0
22	0	0	0	12.66 ± 2.33 fghi	24 ± 3 ijk	11.67 ± 1.53 bcd	255 ± 15.40 b	5.76 ± 1.45 e	32.34 ± 4.84 ef	0
23	0	0	0	11.62 ± 2.03 ghi	11.83 ± 2.73 jk	12 ± 1.45 abc	230 ± 11.54 bc	6 ± 0.60 de	55 ± 4.16 e	0
24	0	0	0	9.36 ± 0.37 i	1.65 ± 0.88 k	7.38 ± 2.35 ef	214.37 ± 1.76 c	12 ± 2.30 abc	88.67 ± 6.98 d	0
25	0	0	0	11 ± 0.58 ghi	0	5.34 ± 0.32 efg	167 ± 9.07 d	14 ± 0.6 a	139.70 ± 18.52 c	0
26	0	0	0	9 ± 0.52 i	0.5 ± 0.22 k	7 ± 2.31 ef	78 ± 9.29 f	13.70 ± 2.73 a	231 ± 4.93 b	0
27	0	0	0	9.63 ± 1.45 hi	0	6 ± 1 efg	47.34 ± 8.87 g	12.36 ± 2.97 ab	245.33 ± 7.96 b	1.67 ± 0.89 b
28	0	0	0	9 ± 1.15 i	0	5.67 ± 1.76 efg	7 ± 1.15 i	11 ± 1.53 abc	326.67 ± 12.91 a	1.67 ± 0.5 ab
29	0	0	0	10.33 ± 0.88 ghi	0	7.69 ± 0.88 def	12 ± 4.04 i	10 ± 1.53 abcd	319 ± 9.85 a	0
30	0	0	0	9.42 ± 1.20 i	0	7.33 ± 0.88 ef	13.67 ± 3.84 hi	8.40 ± 1.20 cde	307.58 ± 10.48 a	3.40 ± 1.20 a
Number of egg masses 30 days after inoculation										
Dutch Mobil							ALYSTE F-1			
34 ± 1.45							0			

Means ± standard errors are presented. Different letters within any parameter (pre-parasitic J2s, parasitic J2s, J3s, J4s, females, and egg masses) are significantly different ($p \leq 0.05$) according to the Fisher's least significant difference (LSD) test.

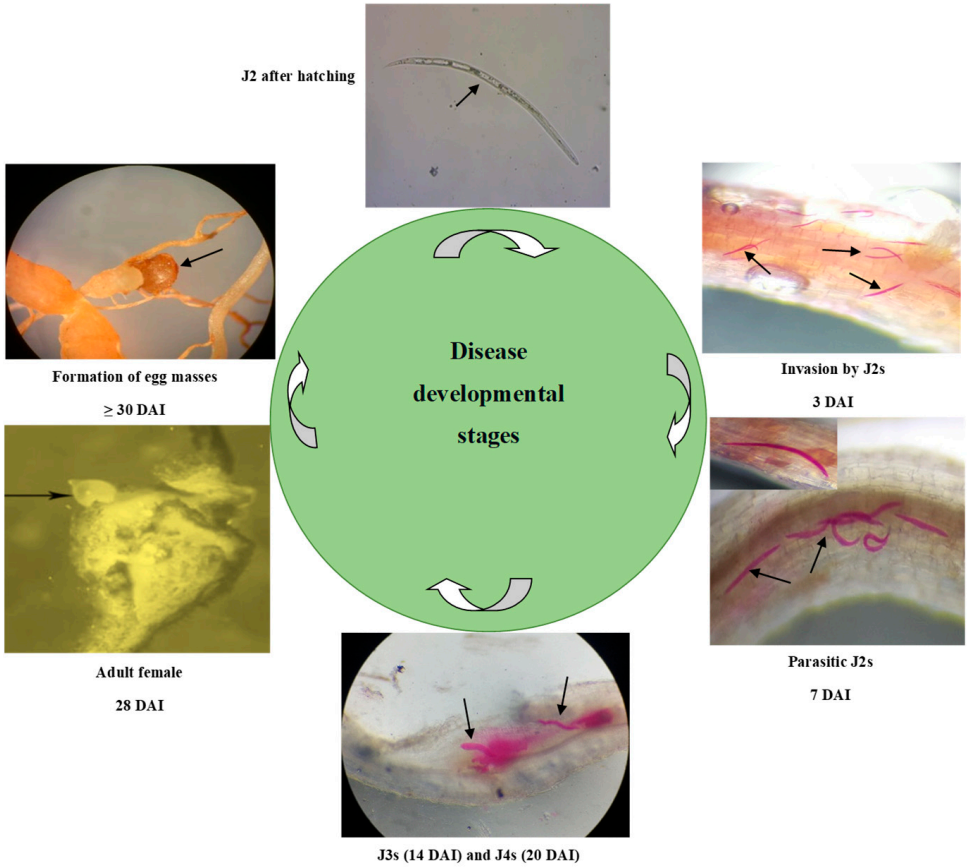

Figure 2. Meloidogyne javanica life cycle in the roots of the highly susceptible tomato cultivar, Dutch Mobil. The highest number of invasive J2s, parasitic J2s, J3s, J4s, and adult females in Dutch Mobil were observed at 3, 7, 14, 20, and 28 DAI, respectively. Arrows show the life stages of the nematode in the root. Roots were stained with acid-fuchsin. J2s, J3s, and J4s: juveniles in the second, third, and fourth stages of development, respectively; DAI: days after inoculation.

Our results showed that a few parasitic J2s were converted to J3s in the moderately resistant cultivar at 14 DAI, while the highly susceptible cultivar had the highest number of J3s at this time (Table 2). Therefore, we next conducted molecular comparisons between the highly susceptible and moderately resistant cultivars at 14 DAI in an attempt to understand the reasons for the delayed development of J2s in the moderately resistant cultivar.

3.2. ET-Related Responses in the Roots of ALYSTE F-1 and Dutch Mobil at 14 DAI with M. javanica (Gene Bank Accession Number: OM281060)

SlACO1 and *SlACO4* (enzymes involved in ET biosynthesis), and *SlERF1* (ET-inducible gene), were used to investigate the ET-dependent responses.

The mRNA levels of *SlACO1* and *SlACO4* compared to corresponding control (1.30-fold and 2.04-fold, respectively) did not significantly change in the roots of Dutch Mobil at 14 DAI (Figure 3). In contrast, transcripts of *SlACO1* (6.52-fold) and *SlACO4* (6.65-fold) were markedly ($p \leq 5\%$) upregulated in the roots of ALYSTE F-1 (Figure 3). Generally, inoculated ALYSTE F-1 tended to have higher expression levels of *SlACO1* and *SlACO4* than inoculated Dutch Mobil at 14 DAI (Figure 3).

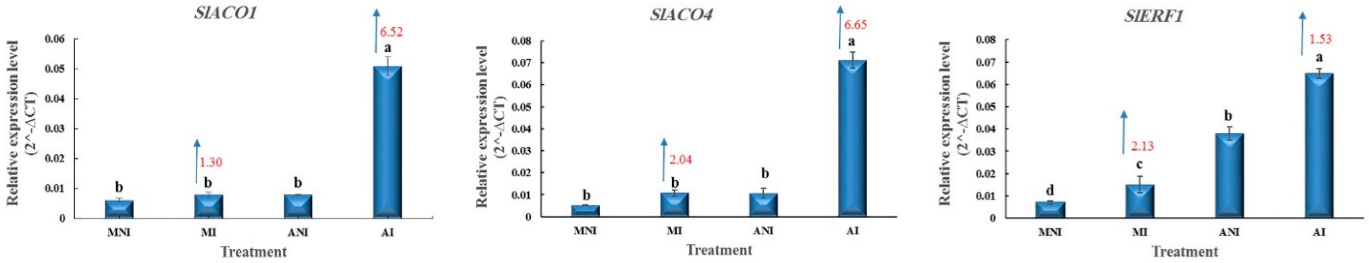

Figure 3. Analysis of the expression levels of ethylene-related genes in the roots of Dutch Mobil and ALYSTE F-1 (as highly susceptible and moderately resistant tomato cultivars, respectively) at 14 days after inoculation with *Meloidogyne javanica*. Gene expression was measured using quantitative real-time PCR (qRT-PCR). Five biological replicates for each treatment were considered in an experiment, and each biological replicate contained three technical replicates. The experiment was performed three times independently. *Tubulin alpha-3 chain* (*TAC*) was used as a reference gene for the normalization of qRT-PCR data. The transcript levels were calculated according to the $2^{-\Delta CT}$ method. The bars represent means $2^{-\Delta CT} \pm$ SE. The numbers displayed in the graph (in red) with blue arrows indicate the fold change (increase or decrease in the expression of genes of each cultivar compared to the corresponding control based on $2^{-\Delta\Delta CT}$). The means with different letters within each column are significantly ($p \leq 5\%$) different according to the Fisher's least significant difference (LSD) test. MNI: Dutch Mobil non-inoculated, MI: Dutch Mobil inoculated, ANI: ALYSTE F-1 non-inoculated, AI: ALYSTE F-1 inoculated, *ACO1*: *ACC- oxidase 1*, *ACO4*: *ACC- oxidase 4*, *ERF1*: *ethylene response factor 1*.

ET-responsive TFs such as EIN3, EIN3-LIKE 1 (EIL1), ORA59, and ERF1 activate the downstream defense gene *PDF1.2* [78]. The expression level of *SlERF1* in the *M. javanica*-infected roots of ALYSTE F-1 (1.53-fold) and Dutch Mobil (2.13-fold) was significantly upregulated compared to corresponding controls at 14 DAI (Figure 3). Additionally, the expression level of *SlERF1* in inoculated ALYSTE F-1 was significantly higher ($p \leq 5\%$) than that in the inoculated Dutch Mobil at 14 DAI (Figure 3).

3.3. SA-Related Responses in the Roots of ALYSTE F-1 and Dutch Mobil at 14 DAI with M. javanica

SlICS1 (key enzyme in SA biosynthesis), *SlNPR1* (interacts with TGA transcription factors in the SA-signaling pathway), and *SlPR1*, *SlPR1a*, and *SlPR1a2* (PR proteins induced by the SA pathway) were used as the marker genes for investigating SA-related responses.

In the isochorismate pathway, ICS1, as a key enzyme, converts chorismate to isochorismate, which is then converted to SA by IPL [47,48]. At 14 DAI, the expression of *SlICS1* did not change significantly in the roots of inoculated ALYSTE F-1 (1.07-fold) and Dutch Mobil (1.26-fold) compared to corresponding non-inoculated controls (Figure 4). However, the inoculated ALYSTE F-1 had constitutively higher expression levels of *SlICS1* compared to the inoculated Dutch Mobil (Figure 4).

SA accumulation leads to the activation of NPR1, a master regulator of downstream SA signaling [49]. At 14 DAI, the mRNA levels of *SlNPR1* in inoculated ALYSTE F-1 and Dutch Mobil were not significantly different to those of the corresponding non-inoculated controls (Figure 4). The results also showed that the expression level of *SlNPR1* in ALYSTE F-1 was constitutively higher than in Dutch Mobil (Figure 4).

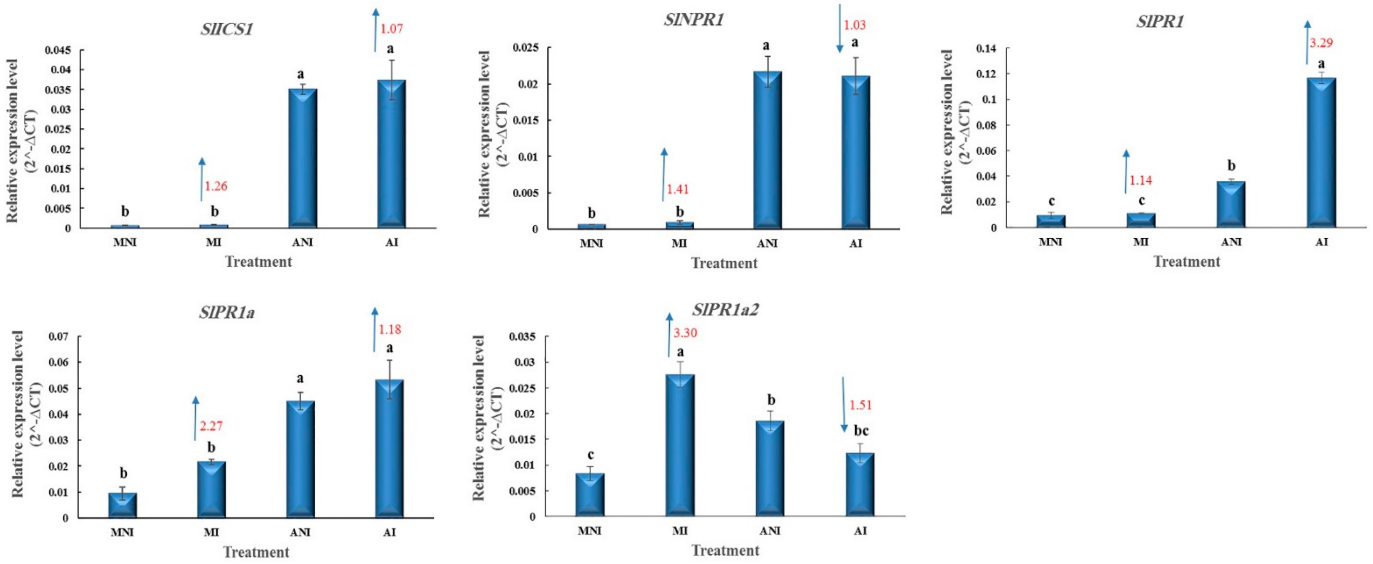

Figure 4. Analysis of the expression levels of salicylic acid-related genes in the roots of Dutch Mobil and ALYSTE F-1 (as highly susceptible and moderately resistant tomato cultivars, respectively) at 14 days after inoculation with *Meloidogyne javanica*. Gene expression was measured using quantitative real-time PCR (qRT-PCR). Five biological replicates for each treatment were considered in an experiment, and each biological replicate contained three technical replicates. The experiment was performed three times independently. *Tubulin alpha-3 chain* (*TAC*) was used as a reference gene for the normalization of qRT-PCR data. The transcript levels were calculated according to the $2^{-\Delta CT}$ method. The bars represent the means $2^{-\Delta CT} \pm$ SE. The numbers displayed in the graph (in red) with blue arrows indicate the fold change (increase or decrease in the expression of genes of each cultivar compared to the corresponding control based on $2^{-\Delta\Delta CT}$). The means with different letters within each column are significantly ($p \leq 5\%$) different according to the Fisher's least significant difference (LSD) test. MNI: Dutch Mobil non-inoculated, MI: Dutch Mobil inoculated, ANI: ALYSTE F-1 non-inoculated, AI: ALYSTE F-1 inoculated, *ICS1: isochorismate synthase1*, *NPR1: non-expresser of pathogenesis-related genes1*, PRs (*PR1, PR1a, and PR1a2*): pathogenesis-related proteins.

Previous studies have shown that SA regulates the expression of *PR1*, *PR2*, and *PR5*, while the JA pathway regulates the expression of *PR3*, *PR4*, and *PR12* (*PDF1.2*) [50]. Our results showed that the expression level of *SlPR1* in Dutch Mobil was slightly upregulated (1.14-fold) at 14 DAI with *M. javanica*. However, the transcripts of *SlPR1* in inoculated ALYSTE F-1 were markedly upregulated (3.29-fold) compared to those of the corresponding control at 14 DAI (Figure 4). The expression level of *SlPR1* in the roots of inoculated ALYSTE F-1 was significantly higher than that in the inoculated Dutch Mobil (Figure 4). The transcript levels of *SlPR1*a in the roots of ALYSTE F-1 (1.18-fold) and Dutch Mobil (2.28-fold) were not significantly upregulated compared to those in the corresponding controls (Figure 4). However, the expression levels of *SlPR1a* in the inoculated ALYSTE F-1 were significantly higher than those in the inoculated Dutch Mobil at 14 DAI (Figure 4). The expression level of *SlPR1a2* in the roots of Dutch Mobil was significantly upregulated (3.30-fold) at 14 DAI, but was slightly downregulated in ALYSTE F-1 (Figure 4). The mRNA levels of *SlPR1a2* in the roots of inoculated Dutch Mobil were significantly higher than those in inoculated ALYSTE F-1 at 14 DAI (Figure 4). The inoculated ALYSTE F-1 often had higher levels of PRs, both constitutively and in inductive responses, compared to the inoculated Dutch Mobil (Figure 4).

3.4. JA-Related Responses in the Roots of ALYSTE F-1 and Dutch Mobil at 14 DAI with M. javanica

SlLOXD, *SlAOS3* (JA biosynthesis), *SlCOI1*, *SlJAZ1*, *SlJAZ2*, *SlJAZ3* (JA signaling), *SlMYC2* (a key transcription factor of JA-signaling pathway), and *SlWRKY57* transcription

factor (a negative regulator of the JA pathway) were used as the marker genes for studying JA-related responses.

At 14 DAI, the transcript levels of *SlLOXD* in Dutch Mobil were significantly upregulated (4.53-fold) compared to those of the corresponding control (Figure 5). At 14 DAI, the mRNA levels of *SlLOXD* were significantly downregulated in the roots of ALYSTE F-1 (1.54-fold), but were still higher than those in Dutch Mobil (Figure 5).

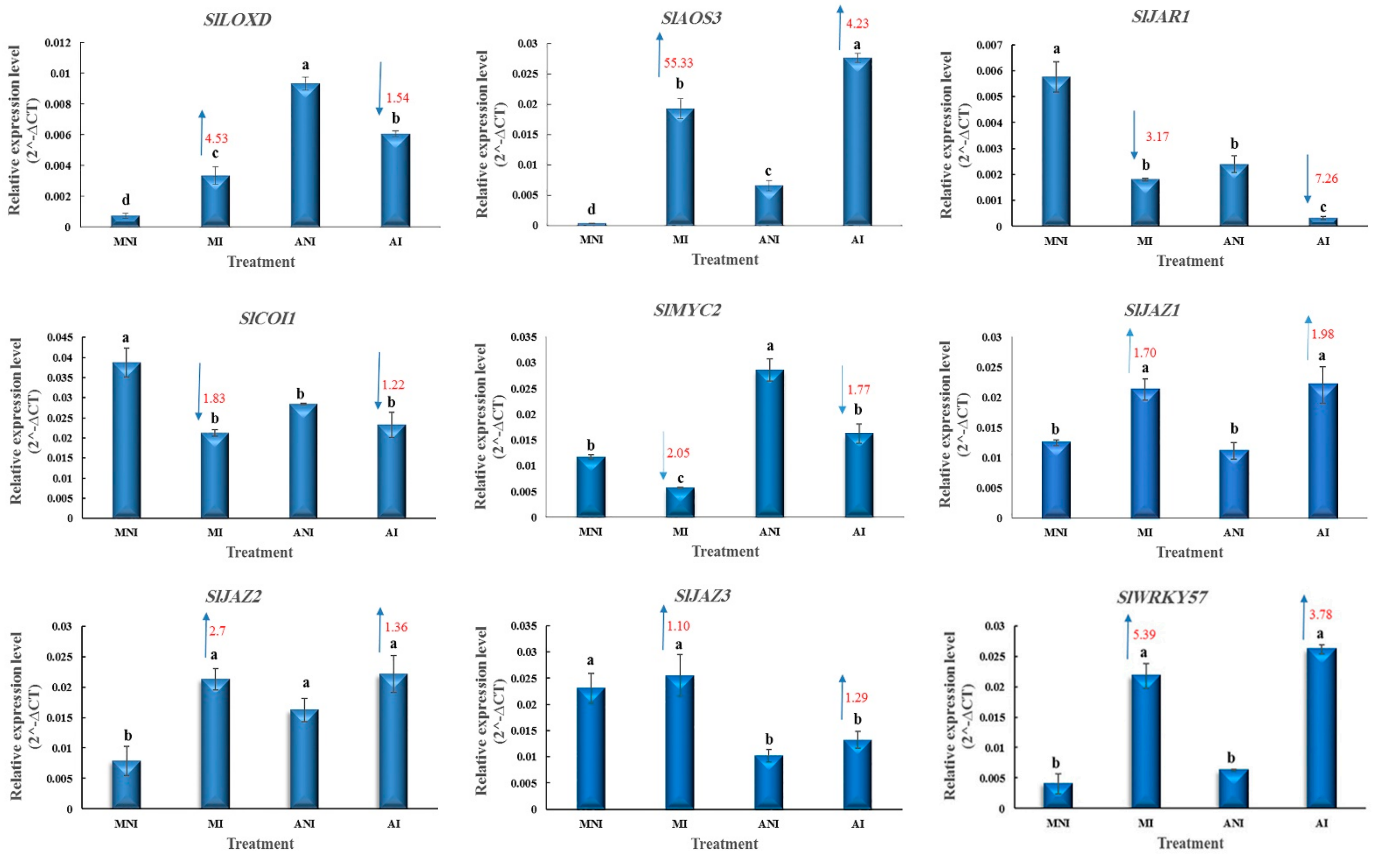

Figure 5. Analysis of the expression levels of jasmonic acid-related genes in the roots of Dutch Mobil and ALYSTE F-1 (as highly susceptible and moderately resistant tomato cultivars, respectively) at 14 days after inoculation with *Meloidogyne javanica*. Gene expression was measured using quantitative real-time PCR (qRT-PCR). Five biological replicates for each treatment were considered in an experiment, and each biological replicate contained three technical replicates. The experiment was performed three times independently. *Tubulin alpha-3 chain* (*TAC*) was used as a reference gene for the normalization of qRT-PCR data. The transcript levels were calculated according to the $2^{-\Delta CT}$ method. The bars represent the means $2^{-\Delta CT} \pm$ SE. The displayed numbers in the graph (in red) with blue arrows indicate the fold change (increase or decrease in the expression of genes of each cultivar compared to the corresponding control based on $2^{-\Delta\Delta CT}$). The means with different letters within each column are significantly ($p \leq 5\%$) different according to the Fisher's least significant difference (LSD) test. MNI: Dutch Mobil non-inoculated, MI: Dutch Mobil inoculated, ANI: ALYSTE F-1 non-inoculated, AI: ALYSTE F-1 inoculated, *LOXD: lipoxygenase D*, *AOS3: allene oxide synthase3*, *JAR1: jasmonoyl-isoleucine synthetase1*, *COI1: coronatine insensitive1*, *MYC2: transcription factor MYC2*, *JAZ: jasmonate ZIM domain*, *WRKY57: WRKY transcription factor 57*.

The transcript levels of *SlAOS3* were remarkably upregulated in the roots of both ALYSTE F-1 (4.23-fold) and Dutch Mobil (55.33-fold) at 14 DAI with RKN (Figure 5). Despite the higher induction of *SlAOS3* in Dutch Mobil (55.33-fold), ALYSTE F-1 still had significantly higher transcript levels of *SlAOS3* than in Dutch Mobil at 14 DAI (Figure 5).

Conversion of JA to JA-Ile is mediated by JAR1 forms [59]. JA-Ile is then transferred to the nucleus and activates MYC2 to induce the expression of JA-responsive defense genes such as *VEGETATIVE STORAGE PROTEIN2* (*VSP2*) [60]. In this research, the transcripts of *SlJAR1* were significantly downregulated in the roots of both ALYSTE F-1 (7.26-fold) and Dutch Mobil (3.17-fold) at 14 DAI. Our findings showed that the lowest expression level of *SlJAR1* was observed in inoculated ALYSTE F-1 (Figure 5).

COI1 encodes an F-box protein, which associates with SKP1 and Cullin proteins to form the SKP1-CULLIN1-F-box-type (SCF) E3 ubiquitin ligase complex (SCF^{COI1}) [62,63]. After entering the nucleus, JA-Ile functions as "molecular glue" to promote the creation of the SCF^{COI1}-JAZ co-receptor complex, and consequently, degradation of JAZ proteins [61]. Master TFs, such as MYC2, are repressed through direct interaction with JAZ proteins at the promoter regions of JA-responsive genes [60]. The degradation of JAZ proteins activates these TFs to express JA-responsive genes [60,61]. Our results showed that the expression level of *SlCOI1* in the highly susceptible cultivar Dutch Mobil was significantly downregulated (1.83-fold), which was in contrast to the mRNA level of *SlCOI1* in ALYSTE F-1, which was not differentially downregulated at 14 DAI (Figure 5). The expression levels of *SlCOI1* in the roots of inoculated ALYSTE F-1 and Dutch Mobil were not significantly different at 14 DAI (Figure 5).

The mRNA levels of *SlMYC2* were remarkably downregulated in the roots of both ALYSTE F-1 (1.77-fold) and Dutch Mobil (2.05-fold) at 14 DAI (Figure 5). The expression level of *SlMYC2* in the roots of inoculated ALYSTE F-1 was significantly greater than that in inoculated Dutch Mobil at 14 DAI (Figure 5).

The expression level of *SlJAZ1, SlJAZ2*, and *SlJAZ3* was investigated at 14 DAI. The transcript levels of *SlJAZ1* were significantly upregulated in the roots of both ALYSTE F-1 (1.98-fold) and Dutch Mobil (1.70-fold). The expression levels of *SlJAZ1* in the roots of inoculated ALYSTE F-1 and Dutch Mobil were not significantly different at 14 DAI (Figure 5). The mRNA level of *SlJAZ2* in the roots of Dutch Mobil was significantly upregulated (2.7-fold) at 14 DAI, while only a minor upregulation of *SlJAZ2* (1.36-fold) noted in the roots of ALYSTE F-1 at 14 DAI. The expression level of *SlJAZ2* was not significantly different between inoculated ALYSTE F-1 and Dutch Mobil (Figure 5). The results showed that the transcripts of *SlJAZ3* were slightly expressed in the roots of ALYSTE F-1 (1.29-fold) and Dutch Mobil (1.10-fold) compared to the corresponding controls at 14 DAI, although the inoculated Dutch Mobil had higher levels of *SlJAZ3* expression than the inoculated ALYSTE F-1 (Figure 5).

The transcription factor WRKY57 decreases the expression of JA-responsive genes by increasing the expression of *JAZ1* and *JAZ5* [64]. The transcript levels of *SlWRKY57* were differentially expressed in the roots of ALYSTE F-1 (3.78-fold) and Dutch Mobil (5.39-fold) compared to corresponding controls at 14 DAI (Figure 5). However, the expression levels of *SlWRKY57* were not significantly different between inoculated ALYSTE F-1 and Dutch Mobil (Figure 5).

4. Discussion

4.1. Disease Development Stages

Resistant cultivars and inducers of systemic acquired resistance (SAR), such as *β-Aminobutyric* acid (BABA), inhibit or delay the development of RKNs in the roots of plants [65,79]. Kumari et al. (2016) [80] observed that the development of *M. graminicola* was delayed in the resistant rice cultivar Vandana compared to the susceptible cultivar Pusa 1121. They suggested that lignin and callose deposition in the resistant cultivar prevented the penetration of J2s and subsequently delayed the development and reproduction of *M. graminicola*. Similarly, our results showed that the moderately resistant cultivar ALYSTE F-1 not only disrupted the penetration of the nematode, but also delayed the development of J2s, thereby decreasing the reproductive rate of *M. javanica*.

4.2. ET-Related Responses in the Roots of ALYSTE F-1 and Dutch Mobil at 14 DAI

The differential expression of plant defense proteins and other changes in plant development are mainly regulated by phytohormones such as the general plant defense hormones SA, ET, and JA, and other less-studied hormones such as cytokinin, ABA, and auxin [81]. Therefore, investigation into the hormonal cycles in resistant and susceptible cultivars will improve understandings of the mechanisms underlying the prevention and delay of RKN development in resistant cultivars. In this study, the expression levels of genes related to the ET pathway, including *SlACO1*, *SlACO4*, and *SlERF1*, were investigated at 14 DAI. The results showed that the expression of most ET-related genes was slightly upregulated in Dutch Mobil compared to the corresponding controls, but was more strongly upregulated in the moderately resistant cultivar ALYSTE F-1. Moreover, inoculated ALYSTE F-1 tended to show higher transcript levels of *SlACO1*, *SlACO4*, and *SlERF1* than inoculated Dutch Mobil. Several studies across multiple plant species have shown that ET prohibits RKN infestations, probably through reducing the attraction of nematodes to plant roots [22,38,82]. Studies have shown that ET signaling influences the attraction of plant-parasitic nematodes, which may be mediated by modulating the composition of root exudates. In this regard, Dyer et al. (2019) [83] demonstrated that root exudates were significantly more attractive for *Globodera pallida* and *M. incognita* after knockdown of *ERF-E2* in tomato, while gas chromatography-mass spectrometry analysis showed major changes in the composition of root exudates of transgenic plants compared to the controls. Dyer et al. (2019) [83] also highlighted the potential value in engineering plant root exudates to control parasites via modulating plant genes. The findings demonstrated that plants that are resistant to RKN infection have higher transcript levels of ET biosynthesis and response genes than susceptible cultivars [5,80]. Furthermore, Iberkleid et al. (2014) [84] investigated the expression level of *SlAOS2*, *SlACO1*, and *SlOPR3* in resistant (*Mi*-carrying) and susceptible (non-*Mi*-carrying) tomato cultivars at 48 h and 5 days after attack with *Mi*-avirulent and virulent isolates of *M. javanica*. The results revealed upregulation of *ACO1* in roots carrying *Mi*, whereas only a slight change was detected in the non-*Mi*-carrying roots after inoculation with both isolates. Kyndt et al. (2012) [81] studied the expression of genes related to ET biosynthesis and signaling, including *OsACS1*, *OsACO7*, *OsEIN2*, and *OsERF1*, in a susceptible rice cultivar infected with *M. graminicola*. The results showed that *M. graminicola* suppressed the ET pathway in both local and systemic tissues at 3 DAI. Therefore, it can be concluded that the increase in susceptibility of rice to RKN is associated with the decrease in the expression of ET-related genes. Kumari et al. (2016) [80] also investigated the expression level of *OsACS1*, *OsACO7*, *OsEIN2*, and *OsERF1* genes in the root and shoot tissues of susceptible and resistant rice cultivars at 2 and 6 DAI with *M. graminicola*. The results demonstrated a positive correlation between ET-inducible genes in rice and the overall defense against *M. graminicola*. Moreover, transcriptomic analysis performed by Shukla et al. (2018) [5] demonstrated the induction of ABA and ET signaling pathways and the suppression of SA and JA pathways at later stages of infection during tomato-*M. incognita* compatible interaction, as well as the induction of ABA and ET signaling pathways at the early stages during incompatible interactions. These previous studies confirmed the role of the ET pathway in defense against RKNs. Similarly, our results showed that the expression levels of genes related to ET synthesis and signaling in the moderately resistant cultivar were significantly higher than those in the highly susceptible cultivar. An earlier survey showed that silicon (as a resistance inducer) decreased the number of *M. graminicola* RKNs in rice roots and delayed nematode development through upregulation of ET-related genes, including *OsERF1*, *OsEIN2*, and *OsACS1* [85]. In this regard, our results confirmed the upregulation of ET-related genes and the delayed development of *M. javanica* in a moderately resistant cultivar.

4.3. SA-Related Responses in the Roots of ALYSTE F-1 and Dutch Mobil at 14 DAI

Our findings showed that there was no significant change in the expression of genes related to SA synthesis and signaling in the two tested cultivars. Therefore, it can be

concluded that the SA pathway is unlikely to be induced in the two cultivars at 14 DAI. Considering the lack of significant difference in the genes related to SA synthesis and signaling (*SlICS1* and *SlNPR1*), the expression of PR proteins in the two cultivars was not expected to be significantly different from the corresponding controls. However, our results showed that the transcripts of *SlPR1* in ALYSTE F-1 and *SlPR1a2* in Dutch Mobil were significantly upregulated at 14 DAI. Thomma et al. (1998) [50] demonstrated that the pathogen-inducible genes *PR-1*, *PR-2*, and *PR-5* require SA signaling for activation. Nevertheless, several studies have shown that not only genes related to the SA-dependent pathway, but also those related to other hormone pathways, are involved in the regulation of SA-associated PR proteins. For example, *OsPR1b* is an SA-inducible gene in rice [86,87], but is also known to be regulated by ABA, JA, and the ET precursor ACC [86–88]. Mei et al. (2006) [88] demonstrated that the exogenous application of JA induced the upregulation of *PR1a*, *PR3*, and *PR5*, and enhanced resistance against the rice blast fungus (*Magnaporthe grisea*). Therefore, considering the different expression patterns of *SlPR1* in ALYSTE F-1 and *SlPR1a2* in Dutch Mobil, it is likely that genes related to other hormonal pathways are also involved in the regulation of these PR proteins. The results also showed that most PR proteins (*SlPR1* and *SlPR1a*) were expressed at higher levels in inoculated ALYSTE F-1 than in inoculated Dutch Mobil, demonstrating that the resistance of ALYSTE F-1 is higher than that of Dutch Mobil at 14 DAI. PR proteins, such as peroxidases, proteinase inhibitors, and chitinases, are a series of defense proteins that are activated in response to infection [81]. Tirumalaraju et al. (2011) [89] observed that the number of induced proteins involved in defense responses and plant stress to *M. arenaria*, including patatin-like proteins, putative PR proteins, and other stress-related proteins, was higher in resistant peanut cultivars than that in the susceptible cultivars. Kumari et al. (2016) [80] also showed that the expression levels of *OsPR1a*, *OsPR1b*, and *OsPR10* were higher in the local and systemic tissues of a resistant rice cultivar infected with *M. graminicola* than in those of a susceptible cultivar.

As the expression levels of genes related to SA synthesis and signaling in ALYSTE F-1 were constitutively higher than those in Dutch Mobil, the expression of most PR proteins (as SA-responsive genes) was also constitutively higher in ALYSTE F-1. Based on these results, it is likely that the expression levels of other SA-responsive genes are also constitutively higher in ALYSTE F-1 at 14 DAI, which ultimately delays the development of *M. javanica* and reduces the nematode reproduction rate (Table 2). Several studies have shown that the application of SA, or chemicals with a similar action, can reduce nematode infection [23,37–39,41,42]. Qtu et al. (1997) [90] described an important role of chitinases in a resistant soybean cultivar during the *M. incognita* infection, and concluded that JA and SA pathways are likely involved in soybean resistance response. Furthermore, studies on *A. thaliana* indicated that *M. incognita* activated both JA- and SA-dependent pathways in the infected roots, which was confirmed by the high expression levels of *PR-2*, *PR-3*, and *PR-5* in the soybean roots infected with nematode [91]. Guimaraes et al. (2015) [92] also demonstrated that wild peanut (*Arachis stenosperma*) resistance to *M. arenaria* was induced by the expression of both JA- dependent and SA-dependent defense genes and their regulators in the root. Another study demonstrated increased expression of genes encoding transcription factors related to the hormonal signaling of SA, ET, JA, and ABA, as well as PR proteins in the roots of resistant sweet potato cultivar under *M. incognita* treatment [93]. The survey of Ojeda-Rivera et al. (2022) [46] showed that plant defense responses constitutively were activated in the resistant cotton cultivar NemX, and these responses correlated with increased levels of JA and SA. Taken together, these findings demonstrate the importance of the SA pathway in defense against RKNs.

4.4. JA-Related Responses in the Roots of ALYSTE F-1 and Dutch Mobil at 14 DAI

The analysis of the JA pathway in ALYSTE F-1 at 14 DAI showed that the mRNA transcripts of *SlLOXD*, *SlJAR1*, and *SlMYC2* were downregulated, and only *SlAOS3* was upregulated. Downregulation of *SlJAR1* in the moderately resistant cultivar ALYSTE F-1 has likely reduced JA-Ile synthesis and subsequent formation of the SCF^{COI1}-JAZ complex.

This reduction in the SCF^{COI1}-JAZ complex probably increased the inhibitory effect of JAZ proteins on TFs related to JA, such as MYC2, and ultimately decreased the expression of JA-responsive genes. Additionally, the increased expression of JA pathway inhibitors *SlJAZ1* and *SlWRKY57*, and decreased expression of *SlMYC2* and *SlLOXD*, proves that the JA-responsive genes were downregulated in the roots of ALYSTE F-1 at 14 DAI. The analysis of the JA pathway in the roots of Dutch Mobil revealed the upregulation of the JA biosynthesis genes, *SlAOS3* and *SlLOXD*, at 14 DAI. Despite this, the genes related to JA signaling, *SlJAR1*, *SlMYC2*, and *SlCOI1*, were downregulated in the roots of Dutch Mobil. The mRNA transcripts of *SlJAZ1*, *SLJAZ2*, and *SlWRKY57*, as inhibitors of the JA pathway, were also upregulated at 14 DAI. The recent study proved that the expression of JA-responsive genes was downregulated in the roots of Dutch Mobil at 14 DAI. Many studies have introduced JA as a defense molecule against RKNs [28,46,51]. Fan et al. (2015) [94] showed that endogenous JA and exogenous MeJA play important roles in the induction of systemic defense in tomato roots against *M. incognita* attack. Moreover, Fujimoto et al. (2011) [53] found that the number of egg masses of *M. incognita* decreased in tomato plants overexpressing the JA-responsive genes, *multicystatin* (*MC*) and *PIs*. Therefore, they concluded that these genes may be important for RKN invasion and infection. Fan et al. (2015) [94] showed that the mRNA level of *PI-II* in JA overexpressed transgenic tomato 35S::prosystemin (35S::PS) gradually increased with time after inoculation with RKN. They also showed that the production of phytohormones such as JA leads to the enclosure of the invasive nematodes at the infection site, thereby inhibiting nematode reproduction and spread. A study on soybeans (*Glycine max*) infected with *M. incognita* showed that all members of the AOS family and other gene encoding enzymes involved in the JA signaling pathway were significantly downregulated after RKN infection [95]. Another study showed that ABA synthesis and the signaling pathways related to JA and ET were downregulated in susceptible cultivars of peanuts (*Arachis hypogea*) after infection with *M. arenaria*, indicating that downregulation of the JA/ET signaling pathways may increase the susceptibility of peanuts to RKNs [96]. Song et al. (2021) [97] showed that the *M. javanica* effector Mj2G02 inhibited cell death and promoted parasitism in *Arabidopsis* by interfering with the JA signaling pathway. RNA-Seq and qRT-PCR analyses revealed that Mj2G02 was capable to suppress the plant immune response through upregulating JAZ genes and downregulating *JAR1* and four JA-responsive genes, including *MYC3*, *UPI* (*Serine protease inhibitor 2C potato inhibitor I-type family protein*), *THI2.1* (*Thionin 2.1*), and *WRKY75*. Taken together, these results highlight the importance of the JA pathway in defense against RKNs. The present research showed a decrease in the JA signaling pathway, followed by a decreasing expression of JA-responsive genes in the roots of both highly susceptible and moderately resistant cultivars at 14 DAI. Despite higher expression of genes related to the ET and SA defense pathways in the moderately resistant cultivar, we did not expect to observe a nematode population in the roots. However, a small population of nematodes was observed in the roots of the moderately resistant cultivar, which was likely facilitated by the decrease in expression of JA-responsive genes. In line with the results of previous studies, the present research proved that the decrease in expression of JA-responsive genes increased the plant susceptibility to the RKN.

Our results showed that the expression levels of genes involved in JA biosynthesis, *SlAOS3* and *SlLOXD*, in the moderately resistant cultivar were higher than those in the highly susceptible cultivar. The biosynthesis pathway of oxylipins (lipid-derived compounds) branches into many metabolites (e.g., JA, OPDA, MeJA, and JA-Ile), each with different levels of toxicity to RKNs, with key enzymes, including AOS and LOX, known to play an important role in this pathway [98,99]. Due to the creation of intermediate compounds, these enzymes are not only effective in the JA biosynthesis pathway, but also in other pathways leading to the synthesis of various metabolites with different levels of toxicity to RKNs. Considering the higher expression levels of *SlAOS3* and *SlLOXD* in the moderately resistant cultivar, it is likely that other pathways associated with these enzymes have higher levels of toxic metabolites, which may underlie the defense of the moderately

resistant cultivar to RKN. Gleason et al. (2016) [100] also showed that the intermediate OPDA is much more important than JA for defense against RKN. Moreover, numerous studies have shown a positive correlation between JA biosynthesis enzymes (LOX and AOS) and plant defense against RKNs. Indeed, 9-LOX derivatives are among the most active oxylipins in terms of antimicrobial and/or antifungal activity and are also involved in regulating programmed cell death reactions [101–106]. Ozalvo et al. (2014) [107] stated that LOX4 (13-LOXs) in *Arabidopsis* plays an important role in controlling plant defense against *M. javanica* infection. Macharia et al. (2020) [108] investigated transcriptomic changes in potato roots during a compatible interaction with *M. javanica* at *0, 3,* and *7 DAI*, and showed that the expression levels of *AOS* (two genes), *AOC* (one gene), *LOX* (four genes), and *12-OPR* (*12-oxophytodienoate*, three genes) were downregulated at 3 and 7 DAI, which led to an increase in potato susceptibility to RKN. Consistent with these studies, our study also shows that resistance to RKNs is associated with high levels of LOX and AOS enzymes.

The transcription factor WRKY57 is known to decrease the expression of JA-responsive genes by increasing the expression of *JAZ1* and *JAZ5* [64]. Our results also confirmed the simultaneous upregulation of *SlWRKY57* and *SlJAZ1* at 14 DAI. Therefore, increasing the expression of *SlWRKY57* in two tomato cultivars may lead to increasing the expression of *SlJAZ1* (a negative regulator of JA signaling), followed by decreasing the expression of JA-responsive genes.

5. Conclusions

Tomatoes are attacked by several pathogens and pests, including fungi, bacteria, and nematodes, among which, RKNs cause considerable damage to the quality and yield of tomato plants [70,109]. The most effective method of managing RKNs is by employing tolerant and resistant cultivars [70]. The present study showed that the moderately resistant cultivar ALYSTE F-1 delayed the development of *M. javanica* at 14 DAI, while the highly susceptible cultivar Dutch Mobil allowed RKN to complete its life cycle in the infected roots. The comparison of the hormonal pathways between the two cultivars revealed that the genes related to the ET pathway were more strongly expressed in ALYSTE F-1 than in Dutch Mobil, and the JA pathway in the roots decreased at 14 DAI in both cultivars. Although we found no significant change in the expression of genes related to SA synthesis and signaling between the two cultivars, in general, ALYSTE F-1 showed constitutively higher levels of these genes at 14 DAI with *M. javanica* (Figure 6). Previous studies show that MYC transcription factors (TFs) inhibit ERF TFs within the JA signaling pathway to antagonize ET signaling. To coordinate plant defense and development reactions, EIN3 and MYC2, the two main TFs of the ET and JA signaling pathways, physically interact and mutually prevent each other's transcriptional activity [110–112]. Our results prove that the ET and JA pathways acted antagonistically at 14 DAI (Figure 6), and suggest that the downregulation of *SlMYC2* observed in the two cultivars reduces the inhibitory effects on EIN3/EIL1 TFs and leads to the upregulation of *SlERF1* and other ERFs (Figure 6).

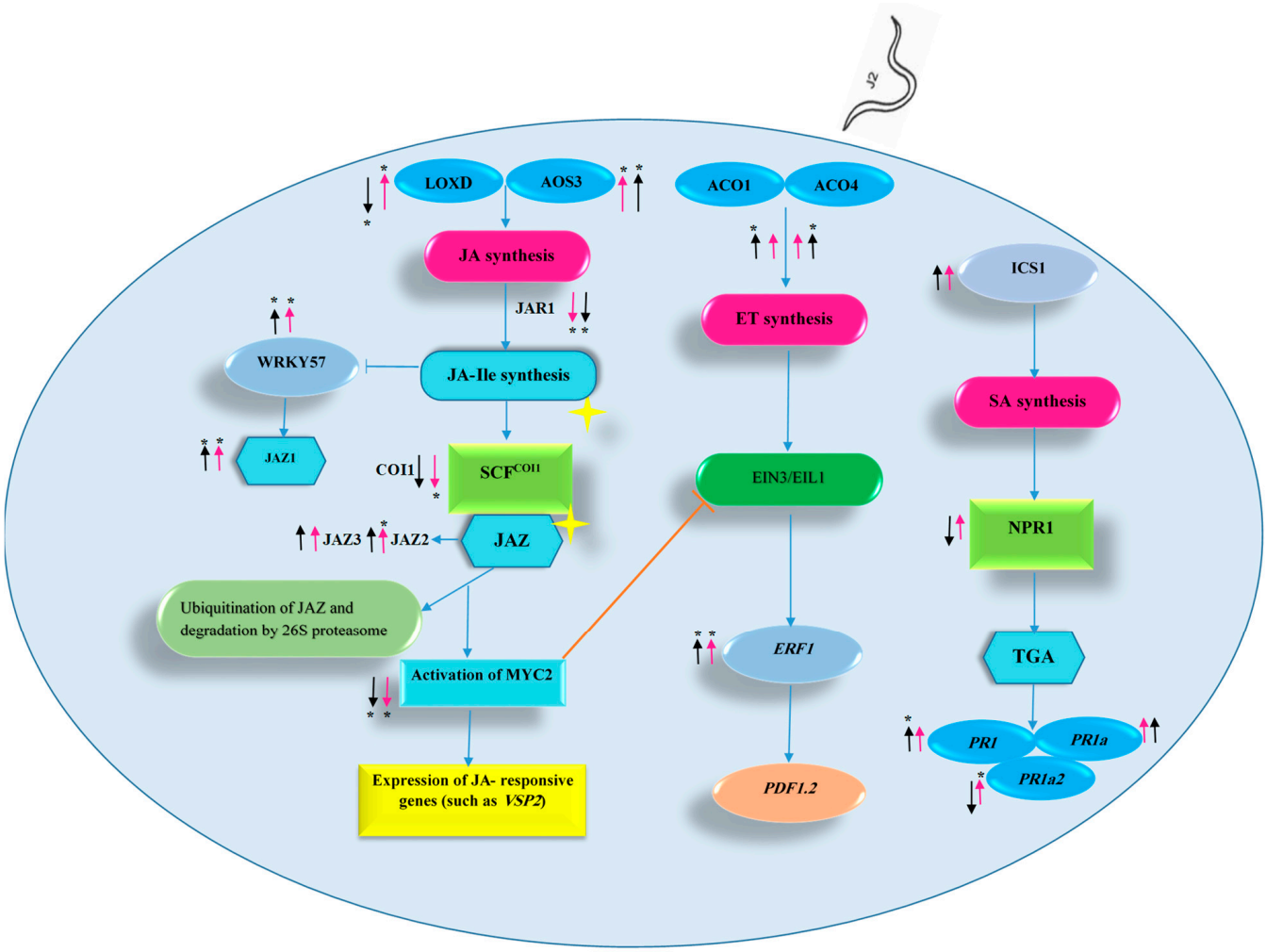

Figure 6. Model depicting the changes in the expression of genes related to the ethylene (ET), salicylic acid (SA), and jasmonic acid (JA) pathways in the roots of Dutch Mobil and ALYSTE F-1 (as highly susceptible and moderately resistant tomato cultivars, respectively) at 14 days after inoculation (DAI) with *Meloidogyne javanica*. The mRNA transcripts of genes related to the JA pathway in the two cultivars demonstrated decreased expression of JA-responsive genes. The transcription factor (TF) WRKY57, as a negative regulator of JA signaling, enhanced the expression level of *SlJAZ1*. Most of the genes related to the ET pathway in ALYSTE F-1 were more strongly expressed than in Dutch Mobil. Although. There was no significant change in the expression of genes related to SA synthesis and signaling in the two cultivars; ALYSTE F-1 showed constitutively higher levels of these genes. The ET and JA pathways acted antagonistically at 14 DAI. It is possible that the downregulation of *SlMYC2* decreased the inhibitory effect on the EIN3/EIL1 TFs and led to the upregulation of *SlERF1* and other ERFs. The pink and black arrows show a decrease or increase in the expression of genes in Dutch Mobil and ALYSTE F-1, respectively. The asterisks on each arrow indicate significant differential expression ($p \leq 5\%$) compared to uninfected plants. JA: jasmonic acid, ET: ethylene, SA: salicylic acid, LOXD: lipoxygenase D, AOS3: allene oxide synthase3, JA-Ile: JA-isoleucine, JAZ: jasmonate ZIM domain, COI1: *coronatine insensitive1*, SCF^{COI1}: SKP1-CULLIN1-F-box-type (SCF) E3 ubiquitin ligase complex, VSP2: vegetative storage protein2, ACO: ACC- oxidase, EIN3: ethylene insensitive3, EIL1: EIN3-like1, ERF1: ethylene response factor1, PDF1.2: plant defensin1.2, ICS1: isochorismate synthase1, NPR1: non-expresser of pathogenesis-related genes1, TGA: TGACG-binding factor, PRs: pathogenesis-related proteins.

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References

1. Janati, S.; Houari, A.; Wifaya, A.; Essarioui, A.; Mimouni, A.; Hormatallah, A.; Sbaghi, M.; Dababat, A.; Mokrini, F. Occurrence of the root-knot nematode species in vegetable crops in Souss region of Morocco. *Plant Pathol. J.* **2018**, *34*, 308. [CrossRef] [PubMed]
2. Abad, P.; Williamson, V.M. Plant nematode interaction. A sophisticated dialogue. In *Advances in Botanical Research*; Kader, J.C., Delseny, M., Eds.; Elsevier: Philadelphia, PA, USA, 2010; pp. 147–192.
3. Mitchum, M.G.; Hussey, R.S.; Baum, T.J.; Wang, X.; Elling, A.A.; Wubben, M.; Davis, E.L. Nematode effector proteins: An emerging paradigm of parasitism. *New Phytol.* **2013**, *199*, 879–894. [CrossRef] [PubMed]
4. Shukla, N.; Kaur, P.; Kumar, A. Molecular aspects of plant-nematode interactions. *Indian J. Plant Physiol.* **2016**, *21*, 477–488. [CrossRef]
5. Shukla, N.; Yadav, R.; Kaur, P.; Rasmussen, S.; Goel, S.; Agarwal, M.; Jagannath, A.; Gupta, R.; Kumar, A. Transcriptome analysis of root-knot nematode (*Meloidogyne incognita*)-infected tomato (*Solanum lycopersicum*) roots reveals complex gene expression profiles and metabolic networks of both host and nematode during susceptible and resistance responses. *Mol. Plant Pathol.* **2018**, *19*, 615–633. [CrossRef]
6. Huang, C.S. Formation, anatomy and physiology of giant cells induced by root-knot nematodes. In *An Advanced Treatise on Meloidogyne*; Sasser, J.N., Carter, C.C., Eds.; NCSU and USAID Cooperative Publication: Raleigh, NC, USA, 1985; pp. 155–164.
7. Jatala, P.; Russel, C.C. Nature of sweet potato resistance to *Meloidogyne incognita* and the effects of temperature on parasitism. *J. Nematol.* **1972**, *4*, 1.
8. Giebel, J. Mechanism of resistance to plant nematodes. *Ann. Rev. Phytopathol.* **1982**, *20*, 257–279. [CrossRef]
9. Anwar, S.A.; Mc Kenry, M.V. Penetration, development and reproduction of *Meloidogyne arenaria* on two new resistant *Vitis* spp. *Nematropica* **2000**, *30*, 9–17.
10. Leon-Reyes, A.; Spoel, S.H.; De Lange, E.S.; Abe, H.; Kobayashi, M.; Tsuda, S.; Millenaar, F.F.; Welschen, R.A.; Ritsema, T.; Pieterse, C.M. Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. *Plant Physiol.* **2009**, *149*, 1797–1809. [CrossRef]
11. Ithal, N.; Recknor, J.; Nettleton, D.; Maier, T.; Baum, T.J.; Mitchum, M.G. Developmental transcript profiling of cyst nematode feeding cells in soybean roots. *Mol. Plant Microbe Interact.* **2007**, *20*, 510–525. [CrossRef]
12. Swiecicka, M.; Filipecki, M.; Lont, D.; Van Vliet, J.; Qin, L.; Goverse, A.; Bakker, J.; Helder, J. Dynamics in the tomato root transcriptome on infection with the potato cyst nematode *Globodera rostochiensis*. *Mol. Plant Pathol.* **2009**, *10*, 487–500. [CrossRef]
13. Cabrera, J.; Barcala, M.; Fenoll, C.; Escobar, C. Transcriptomic signatures of transfer cells in early developing nematode feeding cells of Arabidopsis focused on auxin and ethylene signaling. *Front. Plant Sci.* **2014**, *5*, 107. [CrossRef] [PubMed]
14. Bhattarai, K.K.; Xie, Q.G.; Mantelin, S.; Bishnoi, U.; Girke, T.; Navarre, D.A.; Kaloshian, I. Tomato susceptibility to root-knot nematodes requires an intact jasmonic acid signaling pathway. *Mol. Plant Microbe Interact.* **2008**, *21*, 1205–1214. [CrossRef] [PubMed]
15. Uehara, T.; Sugiyama, S.; Matsuura, H.; Arie, T.; Masuta, C. Resistant and susceptible responses in tomato to cyst nematode are differentially regulated by salicylic acid. *Plant Cell Physiol.* **2010**, *51*, 1524–1536. [CrossRef]
16. Kyndt, T.; Denil, S.; Haegeman, A.; Trooskens, G.; Bauters, L.; Van Criekinge, W.; De Meyer, T.; Gheysen, G. Transcriptional reprogramming by root knot and migratory nematode infection in rice. *New Phytol.* **2012**, *196*, 887–900. [CrossRef] [PubMed]
17. Nahar, K.; Kyndt, T.; Nzogela, Y.B.; Gheysen, G. Abscisic acid interacts antagonistically with classical defense pathways in rice–migratory nematode interaction. *New Phytol.* **2012**, *196*, 901–913. [CrossRef]

18. Matthews, B.F.; Beard, H.; MacDonald, M.H.; Kabir, S.; Youssef, R.M.; Hosseini, P.; Brewer, E. Engineered resistance and hypersusceptibility through functional metabolic studies of 100 genes in soybean to its major pathogen, the soybean cyst nematode. *Planta* **2013**, *237*, 1337–1357. [CrossRef]
19. Li, R.; Rashotte, A.M.; Singh, N.K.; Weaver, D.B.; Lawrence, K.S.; Locy, R.D. Integrated signaling networks in plant responses to sedentary endoparasitic nematodes: A perspective. *Plant Cell Rep.* **2015**, *34*, 5–22. [CrossRef]
20. Wubben, M.J.E., II; Su, H.; Rodermel, S.R.; Baum, T.J. Susceptibility to the sugar beet cyst nematode is modulated by ethylene signal transduction in *Arabidopsis thaliana*. *Mol. Plant Microbe Interact.* **2001**, *14*, 1206–1212. [CrossRef]
21. Wubben, M.J.E., II; Rodermel, S.R.; Baum, T.J. Mutation of a UDP-glucose-4-epimerase alters nematode susceptibility and ethylene responses in *Arabidopsis* roots. *Plant J.* **2004**, *40*, 712–724. [CrossRef]
22. Fudali, S.L.; Wang, C.; Williamson, V.M. Ethylene signaling pathway modulates attractiveness of host roots to the root-knot nematode *Meloidogyne hapla*. *Mol. Plant Microbe Interact.* **2013**, *26*, 75–86. [CrossRef]
23. Kammerhofer, N.; Radakovic, Z.; Regis, J.M.; Dobrev, P.; Vankova, R.; Grundler, F.M.; Siddique, S.; Hofmann, J.; Wieczorek, K. Role of stress-related hormones in plant defense during early infection of the cyst nematode *Heterodera schachtii* in *Arabidopsis*. *New Phytol.* **2015**, *207*, 778–789. [CrossRef]
24. Hu, Y.; You, J.; Li, C.; Williamson, V.M.; Wang, C. Ethylene response pathway modulates attractiveness of plant roots to soybean cyst nematode *Heterodera glycines*. *Sci. Rep.* **2017**, *7*, 41282. [CrossRef] [PubMed]
25. Huang, W.K.; Ji, H.L.; Gheysen, G.; Debode, J.; Kyndt, T. Biochar-amended potting medium reduces the susceptibility of rice to root-knot nematode infections. *BMC Plant Biol.* **2015**, *15*, 267. [CrossRef] [PubMed]
26. Goode, K.; Mitchum, M.G. Pattern-triggered immunity against root-knot nematode infection: A minireview. *Physiol. Plant.* **2022**, *174*, e13680. [CrossRef] [PubMed]
27. Goverse, A.; Overmars, H.; Engelbertink, J.; Schots, A.; Bakker, J.; Helder, J. Both induction and morphogenesis of cyst nematode feeding cells are mediated by auxin. *Mol. Plant Microbe Interact.* **2000**, *13*, 1121–1129. [CrossRef] [PubMed]
28. Gheysen, G.; Mitchum, M.G. Phytoparasitic nematode control of plant hormone pathways. *Plant Physiol.* **2019**, *179*, 1212–1226. [CrossRef]
29. Houben, M.; Van de Poel, B. 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO): The enzyme that makes the plant hormone ethylene. *Front. Plant Sci.* **2019**, *695*. [CrossRef]
30. Ju, C.; Chang, C. Mechanistic insights in ethylene perception and signal transduction. *Plant Physiol.* **2015**, *169*, 85–95. [CrossRef]
31. Alonso, J.M.; Hirayama, T.; Roman, G.; Nourizadeh, S.; Ecker, J.R. EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. *Science* **1999**, *284*, 2148–2152. [CrossRef]
32. Ju, C.; Yoon, G.M.; Shemansky, J.M.; Lin, D.Y.; Ying, Z.I.; Chang, J.; Garrett, W.M.; Kessenbrock, M.; Groth, G.; Tucker, M.L.; et al. CTR1 phosphorylates the central regulator EIN2 to control ethylene hormone signaling from the ER membrane to the nucleus in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 19486–19491. [CrossRef]
33. Qiao, H.; Shen, Z.; Huang, S.S.C.; Schmitz, R.J.; Urich, M.A.; Briggs, S.P.; Ecker, J.R. Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. *Science* **2012**, *338*, 390–393. [CrossRef] [PubMed]
34. Solano, R.; Stepanova, A.; Chao, Q.; Ecker, J.R. Nuclear events in ethylene signaling: A transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev.* **1998**, *12*, 3703–3714. [CrossRef] [PubMed]
35. Pré, M.; Atallah, M.; Champion, A.; De Vos, M.; Pieterse, C.M.; Memelink, J. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. *Plant Physiol.* **2008**, *147*, 1347–1357. [CrossRef]
36. Li, N.; Han, X.; Feng, D.; Yuan, D.; Huang, L.J. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: Do we understand what they are whispering? *Int. J. Mol. Sci.* **2019**, *20*, 671. [CrossRef]
37. Wubben, M.J.E., II; Jin, J.; Baum, T.J. Cyst nematode parasitism of *Arabidopsis thaliana* is inhibited by salicylic acid (SA) and elicits uncoupled SA-independent pathogenesis-related gene expression in roots. *Mol. Plant Microbe Interact.* **2008**, *21*, 424–432. [CrossRef] [PubMed]
38. Nahar, K.; Kyndt, T.; De Vleesschauwer, D.; Höfte, M.; Gheysen, G. The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. *Plant Physiol.* **2011**, *157*, 305–316. [CrossRef] [PubMed]
39. Molinari, S.; Fanelli, E.; Leonetti, P. Expression of tomato salicylic acid (SA)-responsive pathogenesis-related genes in *Mi-1*-mediated and SA-induced resistance to root-knot nematodes. *Mol. Plant Pathol.* **2014**, *15*, 255–264. [CrossRef] [PubMed]
40. Barcala, M.; García, A.; Cabrera, J.; Casson, S.; Lindsey, K.; Favery, B.; García-Casado, G.; Solano, R.; Fenoll, C.; Escobar, C. Early transcriptomic events in microdissected Arabidopsis nematode-induced giant cells. *Plant J.* **2010**, *61*, 698–712. [CrossRef]
41. El-Shafeey, E.S.I.; Ghareeb, R.Y.; Abd-Elhady, M.A.; Abd-Elhady, S.H.; Salim, M.S. Defense-related genes induced by application of silver nanoparticles, ascorbic acid and salicylic acid for enhancing the immune response system of eggplant against invasion of root–knot nematode, *Meloidogyne Javanica*. *Biotechnol. Biotechnol. Equip.* **2021**, *35*, 917–933. [CrossRef]
42. Sahebani, N.; Gholamrezaee, N. The ability of *Meloidogyne javanica* to suppress salicylic acid-induced plant defense responses. *Nematology* **2022**, *24*, 499–508. [CrossRef]
43. Priya, D.B.; Somasekhar, N.; Prasad, J.S.; Kirti, P.B. Transgenic tobacco plants constitutively expressing *Arabidopsis* NPR1 show enhanced resistance to root-knot nematode, *Meloidogyne incognita*. *BMC Res. Notes* **2011**, *4*, 231. [CrossRef] [PubMed]
44. Lin, J.; Mazarei, M.; Zhao, N.; Zhu, J.J.; Zhuang, X.; Liu, W.; Pantalone, V.R.; Arelli, P.R.; Stewart, C.N., Jr.; Chen, F. Overexpression of a soybean salicylic acid methyltransferase gene confers resistance to soybean cyst nematode. *Plant Biotechnol. J.* **2013**, *11*, 1135–1145. [CrossRef] [PubMed]

45. Youssef, R.M.; MacDonald, M.H.; Brewer, E.P.; Bauchan, G.R.; Kim, K.H.; Matthews, B.F. Ectopic expression of AtPAD4 broadens resistance of soybean to soybean cyst and root-knot nematodes. *BMC Plant Biol.* **2013**, *13*, 67. [CrossRef] [PubMed]
46. Ojeda-Rivera, J.O.; Ulloa, M.; Roberts, P.A.; Kottapalli, P.; Wang, C.; Nájera-González, H.R.; Payton, P.; Lopez-Arredondo, D.; Herrera-Estrella, L. Root-knot nematode resistance in *Gossypium hirsutum* determined by a constitutive defense-response transcriptional program avoiding a fitness penalty. *Front. Plant Sci.* **2022**, *13*, 858313. [CrossRef] [PubMed]
47. Dewdney, J.; Reuber, T.L.; Wildermuth, M.C.; Devoto, A.; Cui, J.; Stutius, L.M.; Drummond, E.P.; Ausubel, F.M. Three unique mutants of *Arabidopsis* identify eds loci required for limiting growth of a biotrophic fungal pathogen. *Plant J.* **2000**, *24*, 205–218. [CrossRef]
48. Wildermuth, M.C.; Dewdney, J.; Wu, G.; Ausubel, F.M. Isochorismate synthase is required to synthesize salicylic acid for plant defense. *Nature* **2001**, *414*, 562–565. [CrossRef]
49. Zhang, X.; Chen, S.; Mou, Z. Nuclear localization of NPR1 is required for regulation of salicylate tolerance, isochorismate synthase 1 expression and salicylate accumulation in *Arabidopsis*. *J. Plant Physiol.* **2010**, *167*, 144–148. [CrossRef]
50. Thomma, B.P.; Eggermont, K.; Penninckx, I.A.; Mauch-Mani, B.; Vogelsang, R.; Cammue, B.P.; Broekaert, W.F. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15107–15111. [CrossRef]
51. Huang, H.; Zhao, W.; Qiao, H.; Li, C.; Sun, L.; Yang, R.; Ma, X.; Ma, J.; Song, S.; Wang, S. SlWRKY45 interacts with jasmonate-ZIM domain proteins to negatively regulate defense against the root-knot nematode *Meloidogyne incognita* in tomato. *Hortic. Res.* **2022**, *9*, uhac197. [CrossRef]
52. Cooper, W.R.; Jia, L.; Goggin, L. Effects of jasmonate-induced defenses on root-knot nematode infection of resistant and susceptible tomato cultivars. *J. Chem. Ecol.* **2005**, *31*, 1953–1967. [CrossRef]
53. Fujimoto, T.; Tomitaka, Y.; Abe, H.; Tsuda, S.; Futai, K.; Mizukubo, T. Expression profile of jasmonic acid-induced genes and the induced resistance against the root-knot nematode (*Meloidogyne incognita*) in tomato plants (*Solanum lycopersicum*) after foliar treatment with methyl jasmonate. *J. Plant Physiol.* **2011**, *168*, 1084–1097. [CrossRef] [PubMed]
54. Zhang, L.; Cheng, J.; Yang, R.; Sun, Z.; Wu, C.; Wang, S. Effects of JA synthesis-related genes *Spr2* and *LePrs* on the resistance to root-knot nematodes in tomato. *Sci. Agric. Sin.* **2011**, *44*, 4022–4028.
55. Zhou, J.; Jia, F.; Shao, S.; Zhang, H.; Li, G.; Xia, X.; Zhou, Y.; Yu, J.; Shi, K. Involvement of nitric oxide in the jasmonate-dependent basal defense against root-knot nematode in tomato plants. *Front. Plant Sci.* **2015**, *6*, 193. [CrossRef]
56. Kyndt, T.; Nahar, K.; Haeck, A.; Verbeek, R.; Demeestere, K.; Gheysen, G. Interplay between carotenoids, abscisic acid and jasmonate guides the compatible rice-*Meloidogyne graminicola* interaction. *Front. Plant Sci.* **2017**, *8*, 951. [CrossRef]
57. Ruan, J.; Zhou, Y.; Zhou, M.; Yan, J.; Khurshid, M.; Weng, W.; Zhang, K. Jasmonic acid signaling pathway in plants. *Int. J. Mol. Sci.* **2019**, *20*, 2479. [CrossRef] [PubMed]
58. Baker, A.; Graham, I.A.; Holdsworth, M.; Smith, S.M.; Theodoulou, F.L. Chewing the fat: β-oxidation in signaling and development. *Trends Plant Sci.* **2006**, *11*, 124–132. [CrossRef]
59. Fonseca, S.; Chini, A.; Hamberg, M.; Adie, B.; Porzel, A.; Kramell, R.; Miersch, O.; Wasternack, C.; Solano, R. (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nat. Chem. Biol.* **2009**, *5*, 344–350. [CrossRef]
60. Liu, H.; Timko, M.P. Jasmonic acid signaling and molecular crosstalk with other phytohormones. *Int. J. Mol. Sci.* **2021**, *22*, 2914. [CrossRef]
61. Thines, B.; Katsir, L.; Melotto, M.; Niu, Y.; Mandaokar, A.; Liu, G.; Nomura, K.; He, S.Y.; Howe, G.A.; Browse, J. JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signaling. *Nature* **2007**, *448*, 661–665. [CrossRef]
62. Xie, D.X.; Feys, B.F.; James, S.; Nieto-Rostro, M.; Turner, J.G. COI1: An Arabidopsis gene required for jasmonate-regulated defense and fertility. *Science* **1998**, *280*, 1091–1094. [CrossRef]
63. Zhai, Q.; Zhang, X.; Wu, F.; Feng, H.; Deng, L.; Xu, L.; Zhang, M.; Wang, Q.; Li, C. Transcriptional mechanism of jasmonate receptor COI1-mediated delay of flowering time in *Arabidopsis*. *Plant Cell.* **2015**, *27*, 2814–2828. [CrossRef] [PubMed]
64. Jiang, Y.; Yu, D. The WRKY57 transcription factor affects the expression of jasmonate ZIM-domain genes transcriptionally to compromise *Botrytis cinerea* resistance. *Plant Physiol.* **2016**, *171*, 2771–2782. [CrossRef] [PubMed]
65. Williamson, V.M.; Roberts, P.A. Mechanisms and genetics of resistance. In *Root-Knot Nematodes*; Perry, R.N., Moens, M., Starr, J.L., Eds.; CAB International: Wallingford, UK, 2009; pp. 301–319.
66. Hussey, R.S.; Barker, K.R. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Rep.* **1973**, *57*, 1025–1028.
67. Stirling, G.; Nicol, J.; Reay, F. *Advisory Services for Nematode Pests (Operational Guidelines)*; Rural Industries Research Development Corporation: Barton, ACT, Australia, 2002; pp. 22–32.
68. Jepson, S.B. *Identification of Root-Knot Nematodes Meloidogyne Species*; CABI Publishing: Wallingford, UK, 1987; pp. 1–265.
69. Zijlstra, C.; Donkers-Venne, D.; Fargette, M. Identification of *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assay. *Nematology* **2000**, *2*, 847–853.
70. Asadi-Sardari, A.; Mahdikhani-Moghadam, E.; Zaki-Aghl, M. The biochemical changes in two moderately resistant and highly susceptible tomato cultivars at the later stages of *Meloidogyne javanica* infection. *Nematology* **2022**, *24*, 1085–1103. [CrossRef]
71. Daykin, M.E.; Hussey, R.S. Staining and histopathological techniques in nematology. In *An Advance Treatise on Meloidogyne*; Barker, K.R., Carter, C.C., Sasser, J.N., Eds.; North Carolina State University Graphics: Raleigh, NC, USA, 1985; pp. 39–48.

72. Kamali, S.; Javadmanesh, A.; Stelinski, L.L.; Kyndt, T.; Seifi, A.; Cheniany, M.; Zaki-Aghl, M.; Hosseini, M.; Heydarpour, M.; Asili, J.; et al. Beneficial worm allies warn plants of parasite attack below-ground and reduce above-ground herbivore preference and performance. *Mol. Ecol.* **2022**, *31*, 691–712. [CrossRef]
73. Lamovšek, J.; Stare, B.G.; Pleško, I.M.; Širca, S.; Urek, G. Agrobacteria enhance plant defense against root-knot nematodes on tomato. *Phytopathology* **2017**, *107*, 681–691. [CrossRef]
74. Rubio, M.B.; Quijada, N.M.; Pérez, E.; Domínguez, S.; Monte, E.; Hermosa, R. Identifying beneficial qualities of *Trichoderma parareesei* for plants. *Appl. Environ. Microbiol.* **2014**, *80*, 1864–1873. [CrossRef]
75. Song, W.; Ma, X.; Tan, H.; Zhou, J. Abscisic acid enhances resistance to *Alternaria solani* in tomato seedlings. *Plant Physiol. Biochem.* **2011**, *49*, 693–700. [CrossRef]
76. Ludwig, A.; Schulte, A.; Schnack, C.; Hundhausen, C.; Reiss, K.; Brodway, N.; Held-Feindt, J.; Mentlein, R. Enhanced expression and shedding of the transmembrane chemokine CXCL16 by reactive astrocytes and glioma cells. *J. Neurochem.* **2005**, *93*, 1293–1303. [CrossRef]
77. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2DDCT method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
78. Goossens, J.; Fernández-Calvo, P.; Schweizer, F.; Goossens, A. Jasmonates: Signal transduction components and their roles in environmental stress responses. *Plant Mol. Biol.* **2016**, *91*, 673–689. [CrossRef]
79. Oka, Y.; Cohen, Y.; Spiegel, Y. Local and systemic induced resistance to the root-knot nematode in tomato by DL-β-amino-n-butyric acid. *Phytopathology* **1999**, *89*, 1138–1143. [CrossRef] [PubMed]
80. Kumari, C.; Dutta, T.K.; Banakar, P.; Rao, U. Comparing the defense-related gene expression changes upon root-knot nematode attack in susceptible versus resistant cultivars of rice. *Sci. Rep.* **2016**, *6*, 22846. [CrossRef]
81. Kyndt, T.; Nahar, K.; Haegeman, A.; De Vleesschauwer, D.; Höfte, M.; Gheysen, G. Comparing systemic defense-related gene expression changes upon migratory and sedentary nematode attack in rice. *Plant Biol.* **2012**, *14*, 73–82. [CrossRef] [PubMed]
82. Mantelin, S.; Bhattarai, K.K.; Jhaveri, T.Z.; Kaloshian, I. *Mi-1*-mediated resistance to *Meloidogyne incognita* in tomato may not rely on ethylene but hormone perception through ETR3 participates in limiting nematode infection in a susceptible host. *PLoS ONE* **2013**, *8*, e63281. [CrossRef]
83. Dyer, S.; Weir, R.; Cox, D.; Cheseto, X.; Torto, B.; Dalzell, J.J. Ethylene Response Factor (ERF) genes modulate plant root exudate composition and the attraction of plant parasitic nematodes. *Int. J. Parasitol.* **2019**, *49*, 999–1003. [CrossRef] [PubMed]
84. Iberkleid, I.; Ozalvo, R.; Feldman, L.; Elbaz, M.; Patricia, B.; Horowitz, S.B. Responses of tomato genotypes to avirulent and *Mi*-virulent *Meloidogyne javanica* isolates occurring in Israel. *Phytopathology* **2014**, *104*, 484–496. [CrossRef]
85. Zhan, L.P.; Peng, D.L.; Wang, X.L.; Kong, L.A.; Peng, H.; Liu, S.M.; Liu, Y.; Huang, W.K. Priming effect of root-applied silicon on the enhancement of induced resistance to the root-knot nematode *Meloidogyne graminicola* in rice. *BMC Plant Biol.* **2018**, *18*, 50. [CrossRef]
86. Agrawal, G.K.; Rakwal, R.; Jwa, N.S. Rice (*Oryza sativa* L.) *OsPR1b* gene is phytohormonally regulated in close interaction with light signals. *Biochem. Biophys. Res. Commun.* **2000**, *278*, 290–298. [CrossRef]
87. Mitsuhara, I.; Iwai, T.; Seo, S.; Yanagawa, Y.; Kawahigasi, H.; Hirose, S.; Ohkava, Y.; Ohashi, Y. Characteristic expression of twelve rice *PR1* family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180). *Mol. Genet. Genom. Med.* **2008**, *279*, 415–427. [CrossRef] [PubMed]
88. Mei, C.; Qi, M.; Sheng, G.; Yang, Y. Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, *PR* gene expression, and host resistance to fungal infection. *Mol. Plant Microbe Interact.* **2006**, *19*, 1127–1137. [CrossRef] [PubMed]
89. Tirumalaraju, S.V.; Jain, M.; Gallo, M. Differential gene expression in roots of nematode-resistant and-susceptible peanut (*Arachis hypogaea*) cultivars in response to early stages of peanut root-knot nematode (*Meloidogyne arenaria*) parasitization. *J. Plant Physiol.* **2011**, *168*, 481–492. [CrossRef]
90. Qtu, J.; Hallmann, J.; Kokalis-Burelle, N.; Weaver, D.B.; Rodríguez-Kábana, R.; Tuzun, S. Activity and differential induction of chitinase isozymes in soybean cultivars resistant or susceptible to root-knot nematodes. *J. Nematol.* **1997**, *29*, 523. [PubMed]
91. Hamamouch, N.; Li, C.; Seo, P.J.; Park, C.M.; Davis, E.L. Expression of *Arabidopsis* pathogenesis-related genes during nematode infection. *Mol. Plant Pathol.* **2011**, *12*, 355–364. [CrossRef]
92. Guimaraes, P.M.; Guimaraes, L.A.; Morgante, C.V.; Silva, O.B., Jr.; Araujo, A.C.G.; Martins, A.C.; Saraiva, M.A.P.; Oliveira, T.N.; Togawa, R.C.; Leal-Bertioli, S.C.M.; et al. Root transcriptome analysis of wild peanut reveals candidate genes for nematode resistance. *PLoS ONE* **2015**, *10*, e0140937. [CrossRef]
93. Lee, I.H.; Shim, D.; Jeong, J.C.; Sung, Y.W.; Nam, K.J.; Yang, J.W.; Ha, J.; Lee, J.J.; Kim, Y.H. Transcriptome analysis of root-knot nematode (*Meloidogyne incognita*)-resistant and susceptible sweet potato cultivars. *Planta* **2019**, *249*, 431–444. [CrossRef]
94. Fan, J.W.; Hu, C.L.; Zhang, L.N.; Li, Z.L.; Zhao, F.K.; Wang, S.H. Jasmonic acid mediates tomato's response to root knot nematodes. *J. Plant Growth Regul.* **2015**, *34*, 196–205. [CrossRef]
95. Ibrahim, H.M.; Hosseini, P.; Alkharouf, N.W.; Hussein, E.H.; Abd El Kader, Y.; Aly, M.A.; Matthews, B.F. Analysis of gene expression in soybean (*Glycine max*) roots in response to the root knot nematode *Meloidogyne incognita* using microarrays and KEGG pathways. *BMC Genom.* **2011**, *12*, 220. [CrossRef]

96. Clevenger, J.; Chu, Y.; Guimaraes, L.A.; Maia, T.; Bertioli, D.; Leal-Bertioli, S.; Timper, P.; Holbrook, C.C.; Ozias-Akins, P. Gene expression profiling describes the genetic regulation of *Meloidogyne arenaria* resistance in *Arachis hypogaea* and reveals a candidate gene for resistance. *Sci. Rep.* **2017**, *7*, 1317. [CrossRef]
97. Song, H.; Lin, B.; Huang, Q.; Sun, T.; Wang, W.; Liao, J.; Zhuo, K. The *Meloidogyne javanica* effector Mj2G02 interferes with jasmonic acid signaling to suppress cell death and promote parasitism in *Arabidopsis*. *Mol. Plant Pathol.* **2021**, *22*, 1288–1301. [CrossRef]
98. Dave, A.; Graham, I.A. Oxylipin signaling: A distinct role for the jasmonic acid precursor cis-(+)-12-oxo-phytodienoic acid (cis-OPDA). *Front. Plant Sci.* **2012**, *3*, 42. [CrossRef] [PubMed]
99. Naor, N.; Gurung, F.B.; Ozalvo, R.; Bucki, P.; Sanadhya, P.; Miyara, S.B. Tight regulation of allene oxide synthase (AOS) and allene oxide cyclase-3 (AOC3) promote *Arabidopsis* susceptibility to the root-knot nematode *Meloidogyne javanica*. *Eur. J. Plant Pathol.* **2018**, *150*, 149–165. [CrossRef]
100. Gleason, C.; Leelarasamee, N.; Meldau, D.; Feussner, I. OPDA has key role in regulating plant susceptibility to the root-knot nematode *Meloidogyne hapla* in *Arabidopsis*. *Front. Plant Sci.* **2016**, *7*, 1565. [CrossRef] [PubMed]
101. Kolomiets, M.V.; Chen, H.; Gladon, R.J.; Braun, E.J.; Hannapel, D.J. A leaf lipoxygenase of potato induced specifically by pathogen infection. *Plant Physiol.* **2000**, *124*, 1121–1130. [CrossRef]
102. Göbel, C.; Feussner, I.; Schmidt, A.; Scheel, D.; Sanchez-Serrano, J.; Hamberg, M.; Rosahl, S. Oxylipin profiling reveals the preferential stimulation of the 9-lipoxygenase pathway in elicitor-treated potato cells. *J. Biol. Chem.* **2001**, *276*, 6267–6273. [CrossRef]
103. Prost, I.; Dhondt, S.; Rothe, G.; Vicente, J.; Rodriguez, M.J.; Kift, N.; Carbonne, F.; Griffiths, G.; Esquerré-Tugayé, M.T.; Rosahl, S.; et al. Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. *Plant Physiol.* **2005**, *139*, 1902–1913. [CrossRef]
104. Vellosillo, T.; Martínez, M.; López, M.A.; Vicente, J.; Cascón, T.; Dolan, L.; Hamberg, M.; Castresana, C. Oxylipins produced by the 9-lipoxygenase pathway in Arabidopsis regulate lateral root development and defense responses through a specific signaling cascade. *Plant Cell.* **2007**, *19*, 831–846. [CrossRef]
105. Hwang, I.S.; Hwang, B.K. The pepper 9-lipoxygenase gene CaLOX1 functions in defense and cell death responses to microbial pathogens. *Plant Physiol.* **2010**, *152*, 948–967. [CrossRef]
106. Christensen, S.A.; Huffaker, A.; Kaplan, F.; Sims, J.; Ziemann, S.; Doehlemann, G.; Ji, L.; Schmitz, R.J.; Kolomiets, M.V.; Alborn, H.T.; et al. Maize death acids, 9-lipoxygenase–derived cyclopente (a) nones, display activity as cytotoxic phytoalexins and transcriptional mediators. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 11407–11412. [CrossRef]
107. Ozalvo, R.; Cabrera, J.; Escobar, C.; Christensen, S.A.; Borrego, E.J.; Kolomiets, M.V.; Castresana, C.; Iberkleid, I.; Brown Horowitz, S. Two closely related members of Arabidopsis 13-lipoxygenases (13-LOXs), LOX3 and LOX4, reveal distinct functions in response to plant-parasitic nematode infection. *Mol. Plant Pathol.* **2014**, *15*, 319–332. [CrossRef]
108. Macharia, T.N.; Bellieny-Rabelo, D.; Moleleki, L.N. Transcriptome profiling of potato (*Solanum tuberosum* L.) responses to root-knot nematode (*Meloidogyne javanica*) infestation during a compatible interaction. *Microorganisms* **2020**, *8*, 1443. [CrossRef]
109. Ghadamgahi, F.; Tarighi, S.; Taheri, P.; Saripella, G.V.; Anzalone, A.; Kalyandurg, P.B.; Catara, V.; Ortiz, R.; Vetukuri, R.R. Plant growth-promoting activity of *Pseudomonas aeruginosa* FG106 and its ability to act as a biocontrol agent against potato, tomato and Taro pathogens. *Biology* **2022**, *11*, 140. [CrossRef] [PubMed]
110. Song, S.; Huang, H.; Gao, H.; Wang, J.; Wu, D.; Liu, X.; Yang, S.; Zhai, Q.; Li, C.; Qi, T.; et al. Interaction between MYC2 and ETHYLENE INSENSITIVE3 modulates antagonism between jasmonate and ethylene signaling in *Arabidopsis*. *Plant Cell* **2014**, *26*, 263–279. [CrossRef] [PubMed]
111. Zhang, X.; Zhu, Z.; An, F.; Hao, D.; Li, P.; Song, J.; Yi, C.; Guo, H. Jasmonate-activated MYC2 represses ETHYLENE INSENSITIVE3 activity to antagonize ethylene-promoted apical hook formation in *Arabidopsis*. *Plant Cell* **2014**, *26*, 1105–1117. [CrossRef] [PubMed]
112. Zheng, Y.; Lan, Y.; Shi, T.; Zhu, Z. Diverse contributions of MYC 2 and EIN 3 in the regulation of *Arabidopsis* jasmonate-responsive gene expression. *Plant Direct.* **2017**, *1*, e00015. [CrossRef] [PubMed]