RESEARCH/INVESTIGACIÓN

TRANSCRIPT ACCUMULATION OF DEFENSE GENES IN TOMATO INFECTED BY THE FALSE ROOT-KNOT NEMATODE *NACOBBUS ABERRANS*

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ABSTRACT

Cabrera-Hidalgo, A. J., E. Valadez-Moctezuma, A. G. Bustamante-Ortíz, M. Camacho-Tapía, and N. Marbán-Mendoza. 2021. Transcript accumulation of defense genes in tomato infected by the false root-knot nematode *Nacobbus aberrans*. Nematropica 51:17-26.

Nacobbus aberrans is a migratory-sedentary endoparasitic nematode that forms galls in the roots of infected plants. The purpose of this research was to correlate the expression of four defense-related genes to the *N. aberrans* life cycle in tomato roots. Transcript accumulation of pathogenesis-related protein PR-1, β-1,3-glucanase (PR-2), peroxidases (POX), and phenylalanine ammonia lyase (PAL) in tomato roots after inoculation with *N. aberrans* were estimated by qRT-PCR at 2, 7, 14, 21 and 28 days post-inoculation (dpi). The number of juveniles found in the radical system of tomato increased from 56 to 83 at 7 and 14 dpi, respectively, and to more than 95 juveniles at 21 dpi. Gene quantification revealed that the expression of pathogenesis-related genes varied during the evaluation time, confirming that syncytia formation and maintenance are very complex processes. At 2 dpi, gene expression changes were not statistically significant. At 7 dpi, PR-1 was the only up-regulated gene in infected plants compared to non-inoculated plants. At 14 dpi, the expression of PR-2, PAL and POX was increased in the inoculated plants, and at 21 dpi, the expression of PR-2 was decreased while PR-1 transcript accumulation exhibited a slight increase. Finally, at 28 dpi, the expression of PAL was increased, and the expression of POX was decreased. The gene expression alterations induced by *N. aberrans* could be necessary to ensure the successful completion of the nematode's life cycle and to create suitable conditions for its establishment and development in tomato roots.

Key words: β-1,3-glucanase, *Nacobbus aberrans*, pathogenesis-related protein PR-1, peroxidases, phenylalanine ammonia lyase, *Solanum lycopersicum*

RESUMEN

Cabrera-Hidalgo, A. J., E. Valadez-Moctezuma, A. G. Bustamante-Ortíz, M. Camacho-Tapía, and N. Marbán-Mendoza. 2021. Acumulación de transcritos de genes de defensa en tomate infectado por el falso nematodo agallador *Nacobbus aberrans*. Nematropica 51:17-26.

Nacobbus aberrans es un nematodo endoparásito migratorio-sedentario que forma agallas en las raíces de las plantas infectadas. El objetivo de esta investigación fue correlacionar la expresión de cuatro genes relacionados con la defensa de la planta con el ciclo de vida de *N. aberrans* en raíces de tomate. La acumulación de transcritos de proteínas relacionadas con la patogénesis PR-1, β-1,3-glucanasa (PR-2), peroxidasas (POX) y la fenilalanina amonio liasa (PAL) en raíces de tomate después de la inoculación con *N. aberrans* se estimó con qRT-PCR a los 2, 7, 14, 21 y 28 días después de la inoculación (ddi). El número de juveniles encontrados en el sistema radical de tomate incrementó de 56 a 83 a los 7 y 14 ddi, respectivamente, y a más de 95 juveniles a los 21 ddi. La cuantificación de genes reveló que la expresión de genes relacionados con la patogénesis varió durante el tiempo de evaluación, confirmando que la formación y mantenimiento del sincitio son procesos muy complejos. A los 2 ddi, los cambios en la expresión génica no fueron estadísticamente significativos. A los 7 ddi PR-1 fue el único gen que aumentó en las plantas infectadas comparadas con las plantas sin inocular. A los 14 ddi, la expresión de PR-2, PAL y POX incrementó en las plantas inoculadas, y a los 21 ddi, la expresión de PR-2 disminuyó mientras que la acumulación del transcrito PR-1 aumento ligeramente. Finalmente, a los 28 ddi, la expresión de PAL incrementó, y la expresión de POX disminuyó. Las alteraciones de la expresión génica inducidas por *N. aberrans* podrían ser necesarias para asegurar la finalización exitosa del ciclo de vida del nematodo y crear las condiciones adecuadas para su establecimiento y desarrollo en raíces de tomate.

Key words: β-1,3-glucanase, *Nacobbus aberrans*, pathogenesis-related protein PR-1, peroxidases, phenylalanine ammonia lyase, *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important crop worldwide. Mexico is the ninth largest producer of tomatoes with 4.2 million tons and the first exporter of tomatoes in the world with 1.7 million tons in 2017 (Faosat, 2020). However, tomato production is threatened by the increased incidence of diseases, insect pests and nematodes. Plant-parasitic nematodes represent an enormous threat to global food security, destroying at least 12.3% of global food production annually (Hassan *et al*., 2012). The losses were estimated to be more than $157 billion worldwide (Hassan *et al*., 2012). The most economically damaging plant-parasitic nematode species, and consequently, the most widely studied, are those that feed as they migrate destructively through host roots causing necrotic lesions (migratory endoparasites) and those that modify host root tissue to create a nutrient sink from which they feed (sedentary endoparasites) (Jones *et al*., 2013). The false root-knot nematode, *Nacobbus aberrans,* is the only known species to have both migratory and sedentary endoparasitic stages within its life cycle (Eves-van den Akker *et al*., 2014).

Nacobbus aberrans form galls in the roots of infected plants and causes economic losses in several countries on the American continents. This nematode has a wide host range, parasitizing mainly potato (*Solanum tuberosum*), tomato, sugar beet (*Beta vulgaris*), pepper (*Capsicum annuum*), and bean (*Phaseolus vulgaris*). *Nacobbus aberrans* is considered an A1 quarantine pest and at least 40 countries have implemented quarantine measures to prevent its introduction (Manzanilla-López, 2010). Yield loss due to *N. aberrans* was estimated to be 36% and 12-83% on beans and tomato, respectively, in Mexico (Manzanilla-López *et al*., 2002; Cristóbal-Alejo *et al*., 2006). However, yield loss statistics are not available for most crops. *Nacobbus aberrans* is widely distributed in Mexico across production regions. The distribution of this nematode has expanded since its discovery in Chapingo, Mexico (Brunner de Magar, 1967), and currently *N. aberrans* is found in more than a third of the country's states, affecting crops of economic importance (Manzanilla-Lopez, 2010; Cabrera-Hidalgo *et al*., 2015).

During infection, plants generally respond by activating broad-spectrum defense responses both locally and systemically in addition to their basal resistance (Portillo *et al*., 2013). Elicitors from pathogens induce a cascade of defense responses in the plant cell that include rigidification of cell walls with further deposition of callose, lignin and suberin, acceleration of cell death (hypersensitive reaction; HR), production of reactive oxygen

species (ROS), phytoalexins, phenolic compounds, pathogenesis-related (PR) proteins and many other defense-related proteins (Sels *et al*., 2008). Transcriptomic analyses revealed that the induction of feeding cells by nematodes involves an extensive reprogramming of gene expression within the targeted root cells (Caillaud *et al*., 2008; Portillo *et al*., 2013). However, specific responses of plants to attack by specific pathogens are far from being completely understood.

The induction of pathogenesis-related (PR) proteins is considered as an indicator of plant-induced defense responses. They are induced by various types of pathogens such as viruses, bacteria, fungi, and nematodes (Van Loon *et al*., 2006). PR-proteins are generally presented constitutively and increase during infection. Based on their amino acid sequences and biochemical characterization, PR-proteins are classified into 17 families (van Loon *et al*., 2006). Thus, β-1,3-glucanases (PR-2) are well known to be more efficient against numerous nematodes, bacterial and fungal pathogens (Van Loon *et al*., 2006). Also, peroxidases (PR-9) are expressed to limit cellular spread of the infection through the establishment of structural barriers or the generation of highly toxic environments by massively producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Almagro *et al*., 2009). Moreover, phenylalanine ammonia lyase (PAL) is an enzyme induced in the phenyl-propanoid pathway in the biosynthesis of various natural products of phenylpropanoid such as lignin, pigment, flavonoid, and phytoalexin which act as a defense response to microbial infections and abiotic stress (Vogt, 2010).

Nacobbus aberrans causes morphological, cellular and biochemical changes in host plants; the induction of specialized feeding sites (syncytia) being one of the most important changes in root tissues (López-Martínez *et al*., 2011; Godinez-Vidal *et al*., 2013). In the present research, the alterations that occur during the compatible interaction and the changes induced by the false root-knot nematode *N. aberrans* in tomato plants were studied. The information generated will contribute to the knowledge of gene reprogramming that occurs during parasitism by *N. aberrans*. Therefore, the main objective of this study was to investigate the effects of *N. aberrans* on tomato defense responses based on the relative expression of selected genes including β-1,3-glucanases (PR-2), peroxidases (POX), PR-1 and PAL.

MATERIALS AND METHODS

Nematode, plant material, and inoculation

Four-week-old tomato (cv. Rio Grande) seedlings susceptible to *N. aberrans* were used. Plants were transplanted into pots containing a substrate mixture tezontle-agrolite in a 1:1 ratio. The seedlings were maintained under greenhouse conditions (28 ± 3 °C, 13 hours of light and 40% relative humidity) during the study.

Galls, eggs and juveniles of *N. aberrans* were obtained from tomato roots collected from greenhouses in the experimental agricultural field 'San Martin' at the Autonomous University Chapingo (Texcoco, State of Mexico). Root galls were excised, washed and disinfested with a 2% sodium hypochlorite solution. *Nacobbus aberrans* eggs were extracted with the sieving technique (Cobb, 1918) and juveniles by washing in a nebulizer chamber (EPPO, 2013).

Eight days before transplanting, 15,000 *N. aberrans* eggs were inoculated into soil in each pot, and at the time of the transplant 1,200 *N. aberrans* second-stage juveniles (J2) were inoculated into the root zone of each plant. A total of 30 plants were used in this assay (15 inoculated plants and 15 non-inoculated plants). Roots from inoculated plants were collected at 2, 7, 14, 21, and 28 days post-inoculation (dpi). Additionally, non-inoculated plants were used as controls. Three plants from each treatment were selected for counting *N. aberrans* within the roots after staining with sodium hypochlorite-acid fuchsin (Byrd *et al*., 1983). The number of *N. aberrans* per gram root was recorded (average three replicates), and the juvenile and adult stages were observed with an Olympus® CX31 microscope (Olympus, Tokyo, Japan).

RNA isolation, cDNA synthesis, and qRT-PCR

Three technical replicates were completed for inoculated and non-inoculated plants at 2, 7, 14, 21, and 28 dpi. RNA was isolated from 100 mg of bulked root from four tomato plants for each replicate. The tissue samples were ground in liquid nitrogen using a porcelain mortar. Total RNA was extracted using automated Maxwell® 16 Total RNA Purification Kit (Promega, Madison, WI

USA) following the manufacturer's instructions. RNA was verified by 1% agarose gel electrophoresis and quantified using a NanoDropTM1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). First-strand cDNA was synthesized from 1 µg of total RNA using the RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific) with oligo(dT)$_{18}$ primer, following the manufacturer's instructions. Expression quantification of defense-related genes was performed by real-time PCR in a final volume of 20 µL containing 10 µL SsoAdvancedTM Universal SYBR® Green Spermix (Bio-Rad, California, CA USA), 10 pM of each primer, and 50 ng of cDNA. The PCR cycling parameters were as follows: pre-incubation at 95°C for 2 min, 40 cycles for alignment and extension stage at 60°C for 1 min. The qRT-PCRs were performed in triplicate using a CFX96 instrument (Bio-Rad). The specificity of the PCR products was verified by dissociation melting curve analysis after 40 cycles. The primers used were as follows: for the PR-1 (P4) gene (GenBank accession no. NM_001247594.2): forward primer 5' CCAGACTATAACTACGCTACCAACC 3' and reverse primer 5' GTAAAGAACCTAGCCACGATACC 3'; for the PR-2 gene (accession no. NM_001312890.1): forward primer 5' CACCAACATTCACATAACAGAGG 3' and reverse primer 5' AGTAACAGGGCTGATTTCATTACC 3'; for the POX gene (accession no. NM_001302921.2): forward primer 5' GTTGCTAGAGATGCAGTTGTGG 3' and reverse primer 5' CCCGGTGAAATTGTATAGACG 3'; for the PAL gene (accession no. XM_026029821.1): forward primer 5' CTTTGTCCTATATTGCTGGTTTGC 3' and reverse primer 5' TTCTGAGCTACCTTCACATAAGAGC 3'. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (accession no. U93208.1; forward primer 5'GAGAAGGAATACAACCCAGAGC 3' and reverse primer 5' TGGTAGCACTTTCCCTACAGC 3') was used as internal reference to normalize expression level, and non-inoculated plants were used to calibrate the transcript levels of the gene of interest. Transcript levels were expressed as fold-change due to treatment in relation to the transcript basal levels in non-inoculated plants. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

Data were subjected to analysis of variance for a completely randomized model using RStudio (RStudio Team, 2020). When significantly different, means were compared using Tukey's test ($P \leq 0.05$). Two types of variance analysis were carried out: i) to compare the expression of each gene between the inoculated and non-inoculated plants at each time, and ii) for each gene when combined across the five sampling times.

RESULTS

The first *N. aberrans* J2 were found in the tomato root system at 7 dpi with 56 (± 8.5) J2/g of root (Fig. 1A and a); at 2 dpi, no J2 were found. At 14 dpi, 83 (± 15.3) J2/g root were found (Fig. 1B). The number of J2 increased over time, reaching their highest density of 95 (± 9.4) J2/g root at 21 dpi. The juveniles adopted their characteristic 'C' form categorizing third-stage juveniles (J3) at 21 dpi (Fig. 1C and c). At the last sampling date, 28 dpi, J2 and J3 were no longer found, however, females were found in the galls, with 1-2 females per gall (Fig. 1D).

Changes in expression of different defense-related genes including three PR-proteins and PAL genes in the tomato/*N. aberrans* interaction over time were analyzed by qRT-PCR (Fig. 2). There were no changes in gene expression at 2 dpi for any of the genes between inoculated and non-inoculated plants ($P \leq 0.05$). At 7 dpi, the expression of PR-1 exhibited a 13.87-fold increase in tomato roots inoculated with *N. aberrans* compared with the expression in non-inoculated plants ($P \leq 0.05$; Fig. 2). However, the increase of PR-2 (0.24-fold) and the decrease of POX (-0.31-fold) and PAL (-0.26-fold) were not significant different in inoculated vs. non-inoculated plants ($P \leq 0.05$). At 14 dpi, the genes PR-2, PAL and POX were up-regulated, with a fold change between 1.23 and 9.23 compared to their expression in non-inoculated plants. PR-2 exhibited the greatest increase, followed by PAL with a 4.19-fold increase compared to the non-inoculated control ($P \leq 0.05$). However, PR-1 exhibited a slight non

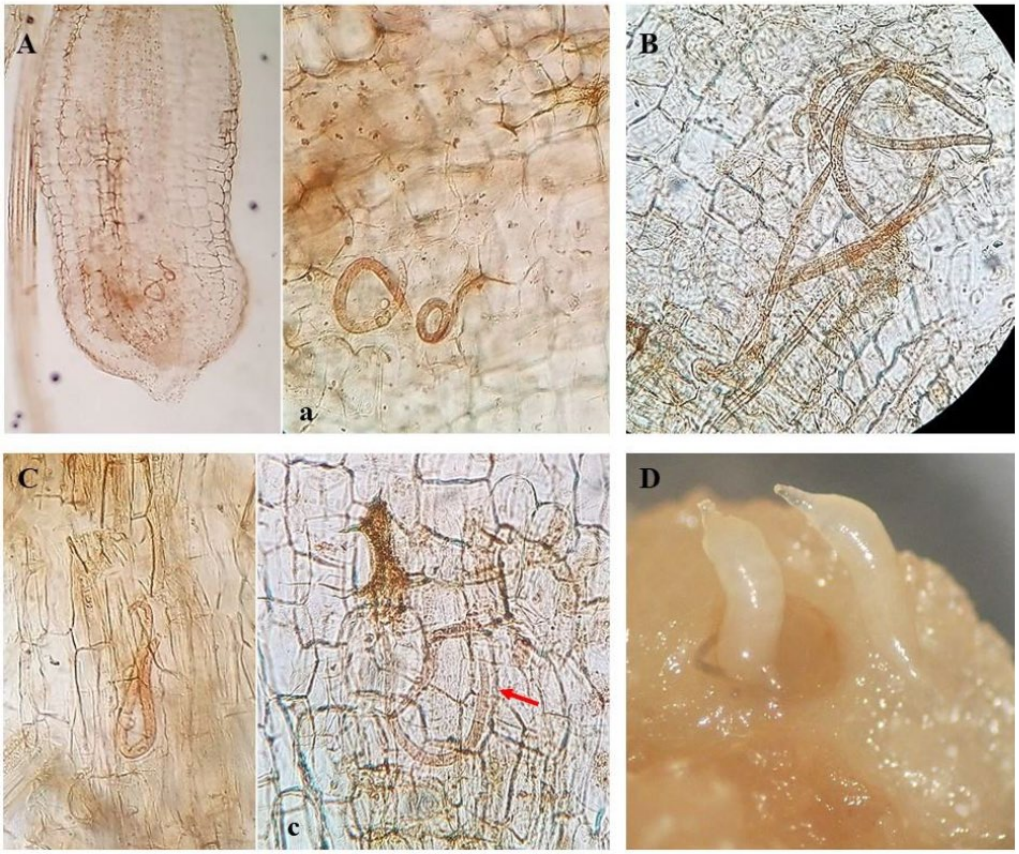

Figure 1. Infection process of *Nacobbus aberrans* in tomato roots. A) Second-stage juveniles (J2) at 7 dpi penetrating the epidermal tissue, a) Root penetration detailed. B) Mass of J2 at 14 dpi in the vascular tissue. C) Third-stage juveniles (J3) in the vascular tissue at 21 dpi with a characteristic form of "C" (c, red arrow). D) Females in galls of tomato root at 28 dpi.

significant increase of 0.87-fold compared to expression in the non-inoculated plants ($P \leq 0.05$; Fig. 2). At 21 dpi, the expression of the PR-2 gene decreased -7.24-fold in tomato plants inoculated with *N. aberrans* compared to non-inocluated plants. Conversely, PR-1 exhibited an increase of 2.19-fold in inoculated vs. non-inoculated plants ($P \leq 0.05$; Fig. 2). The slight decrease of PAL gene (-1.18-fold) and increase of POX gene (0.59-fold) at 21 dpi were not statistically significant different between inoculated and non-inoculated plants ($P \leq 0.05$). At 28 dpi, PAL gene was up-regulated 1.58-fold and POX down-regulated 2.58-fold in inoculated vs. non-inoculated plants ($P \leq 0.05$; Fig. 2). At this same time, the increase of PR-2 (2.61-fold) and decrease of PR-1 (-1.42-fold) were not significantly different between inoculated and non-inoculated plants ($P \leq 0.05$).

The expression of the four genes combined across the evaluation times (2, 7, 14, 21 and 28 dpi) varied depending on the gene. PR-2 and PAL genes displayed higher expression at 14 dpi and lower expression at 21 dpi ($P \leq 0.05$; Fig. 2). The POX gene had similar expression during the first 4 sampling times and a decline at 28 dpi. The PR-1 gene had constant expression except at 7 dpi ($P \leq 0.05$; Fig. 2).

DISCUSSION

Nacobbus aberrans J2 densities found in the radical tomato system increased over time. A similar trend was reported by Godinez-Vidal *et al*. (2013), with 138, 246, 110 and 9 *N. aberrans* J2 in the roots of pepper 'CM334' at 2, 7, 14 and 21 dpi, respectively,. Villar-Luna *et al*. (2015a) reported 200, 300 and 150 J2 in pepper 'CM334' at 2, 7 and 21 dpi, respectively,. The difference in J2 density was probably due to the differences in parasitism of *N. aberrans* in tomato and pepper, and to the initial quantity of the inoculum used for inoculation. In the present study, 1,200 *N. aberrans* J2 were used as initial inoculum while Godinez-Vidal *et al*. (2013) used 2,000 *N. aberrans* J2 and

Villar-Luna *et al*. (2015a) applied 3,000 J2 as initial inoculum per plant. In our study, the first *N. aberrans* J2 were observed at 7 dpi, located mainly in epidermal tissue, where they pierced the cell wall with their stylet and formed feeding tubes. Similar results were reported by Manzanilla-López *et al*. (2002). At 14 dpi, *N. aberrans* J2 moved near the vascular tissue and formed the feeding sites (false knot of 2 mm in diameter) where they are expected to obtain their greatest source of food (Chavarro-Carrero *et al*., 2017). In our study, the induction of the feeding sites of *N. aberrans* was observed in half of the time reported by Inserra *et al*. (1983), with the first feeding sites detected 25 dpi in beet. Similarly, Castillo and Marbán-Mendoza (1984) detected syncytia up to 30 days after *N. aberrans* penetration of *C. annuum* and *C. baccatum* roots. The differences in the days of the appearance of syncytia in previous studies could be associated with environmental conditions, and to the specific *N. aberrans*/plant interaction. The highest density of *N. aberrans* J2 was found at 21 dpi coinciding with the period that the nematode reduces its glandular activity and could enter quiescence and diapause (Manzanilla-López *et al*., 2002). The juveniles of *N. aberrans* adopted their characteristic form of "C" at 21 dpi indicative of J3, similar to what was reported by Villar-Luna *et al*. (2015b) in roots of pepper 'CM334'. At the last sampling (28 dpi), females were found in galls, a specific characteristic of false root-knot nematode species (Godinez-Vidal *et al*., 2013)

Plants possess defense mechanisms to respond to pathogen attack, which usually involve the transcriptional activation of several defense-related genes that subsequently lead to the de novo synthesis of various proteins and antimicrobial compounds. Among the unknown responses when the tomato plant is parasitized by *N. aberrans* are the expressions profiles of pathogenesis-related protein PR-1, β-1,3-glucanase (PR-2) and peroxidases (PR-9, POX), and phenylalanine

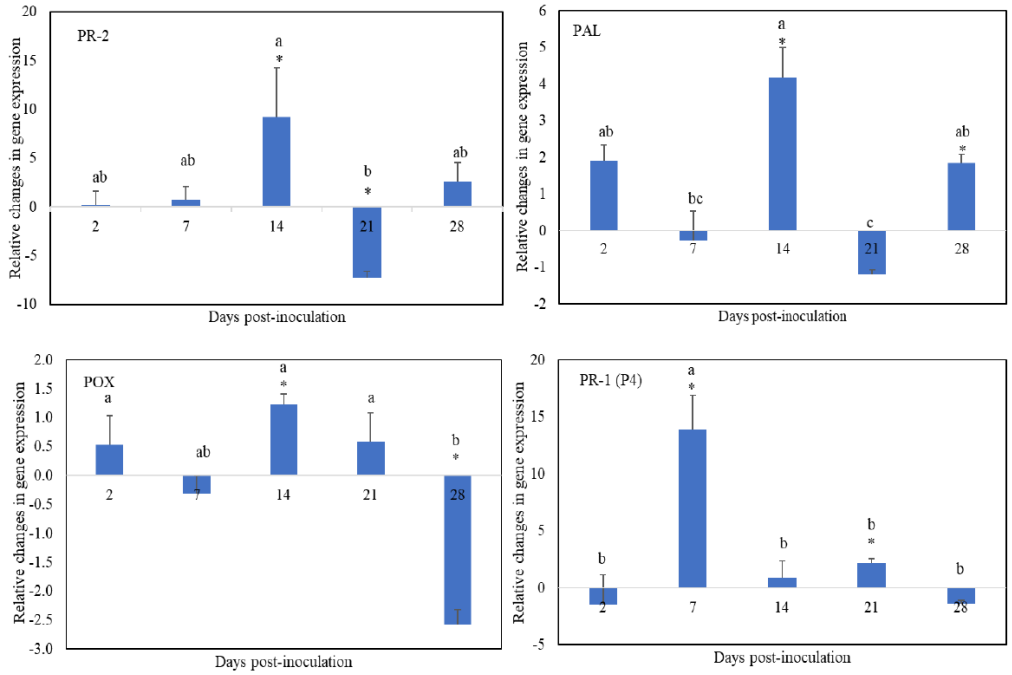

Figure 2. Relative expression of four defense-related genes in the tomato-*Nacobbus aberrans* interaction at 2, 7, 14, 21, and 28 days post-inoculation. PR-2: β-1,3-glucanase; PAL: phenylalanine ammonia lyase; POX: peroxidases; PR-1 (P4): pathogenesis-related protein PR-1. Relative expression was calculated using the 2-ΔΔCt method (Livak and Schmittgen, 2001). Glyceraldehyde-3-phosphate dehydrogenase gene was used as internal reference to normalize expression. Non-inoculated plants were used to calibrate the transcript levels of the gene of interest, which were expressed as fold-change due to treatment in relation to the basal level of transcripts in control plants (1x). * indicates statistically significant differences ($P \leq 0.05$). Different letters indicate significant differences for a gene expression at each time point ($P \leq 0.05$, Tukey test). Bars represent the standard error (n = 3).

ammonia lyase (PAL). The expression patterns of these genes quantified by qRT-PCR enabled us to gain insight into the defense mechanism that tomato uses in response to *N. aberrans* parasitism. The quantification results revealed that the expressions of these genes varied throughout the evaluation time. It has been documented that defense genes are present constitutively and increased expression is related to the presence of stresses (Van Loon *et al*., 2006). At 2 dpi, no change in gene expression of any of the four genes was found. This could be related to the absence of *N. aberrans* J2 and feeding sites in the roots of tomato plants at this time, since *N. aberrans* J2 could be in the migratory phase. In resistant plants, up-regulation of plant defense responses were activated very early, and expression levels remained high during the period of syncytium expansion when tomato plants were infected by cyst nematode *Globodera rostochiensis* (Swiecicka *et al*., 2009).

At 7 dpi, the PR-1 gene was up-regulated in tomato roots inoculated with *N. aberrans*. Despite this, it did not prevent the development of *N. aberrans* J2 in the roots of tomato plants. The PR-1 family is strongly conserved and appears to be represented in many plant species. Thus, the PR-1 proteins are often used as markers of the enhanced defensive state conferred by pathogen-induced systemic acquired resistance (SAR), but their biological activity has remained elusive (Van Loon *et al*., 2006; Uehara *et al*., 2010). In tomato, PR-1 was used as marker for resistance conferred by Hero A against *G. rostochiensis* (Uehara *et al*., 2010). However, no resistance role of Hero A against *N. aberrans* is known.

At 14 dpi, corresponding to formation and growth of the syncytia and increased *N. aberrans* J2 density, the expression of three genes (PR-2, PAL and POX) were up-regulated. Despite this, it seems that their expression levels were not adequate to prevent the establishment and development of *N. aberrans* in the roots of tomato. Thus, the successful establishment of *N. aberrans* was associated with an effective repression of defense mechanisms (Van Loon *et al*., 2006). Through the induction (formation and growth) of syncytia, *Meloidogyne* spp., *Globodera* sp., *Heterodera* sp., and *N. aberrans* establish an intimate and sophisticated interaction with their susceptible hosts in order to successfully complete their life cycles. In previous studies, the accumulation of transcripts of POX, PR-1 and PR-2 were reduced in the compatible interaction of pepper 'CM334'-*N. aberrans* (Fernández-Herrera *et al*., 2012; Villar-Luna *et al*., 2015a). In root galls induced by *M. javanica* in tomato, the expression of PR genes such as peroxidases were down-regulated, whereas two WRKY isogenes were over-expressed (Bar-Or *et al*., 2005). The over-expression of POX gene that occurred in the compatible interaction could be necessary for restructuring the cell wall of the syncytium, since differentiation of the feeding site is a prerequisite for the development and reproduction of the nematode. Peroxidases appear to contribute to the strengthening of cell walls by lignin and suberin depositions (Van Loon, 1997). Moreover, several reports suggested a key role for PAL-mediated metabolism in nematode resistance (Klink *et al*., 2007; Uehara *et al*., 2010). PAL is an important enzyme in the defense mechanisms of plants because it is involved in the phenylpropanoid pathway that leads to synthesis of chemical and physical barriers for defense of plants against root pathogen (Vogt, 2010). Therefore, the regulation of defense genes differs in the level and timing of their expression between different pathogen-plant interactions.

At 21 dpi, the expression of the PR-2 gene was decrease and PR-1 was increased in tomato plants inoculated with *N. aberrans*. Contrary, in compatible pepper 'CM334'-*N. aberrans* interaction, the expression of PR-1, POX and PR-2 genes in whole roots at 21 dpi was reduced (Fernández-Herrera *et al*., 2012). This is probably because parasitism depends on the plant species and the level of susceptibility, and also depends on the nematode's life cycle in each host. Moreover, at this time, *N. aberrans* J3 and J4 were present, which could have a different effect compared to that of *N. aberrans* J2 on the expression of some genes, such as PR-1 and PR-2 genes.

At 28 dpi, PAL gene was up-regulated and POX was down-regulated in tomato plants inoculated with *N. aberrans*. Contrary, in the pepper 'CM334' infected by *N. aberrans*, the levels of POX and PR-1 genes were slightly enhanced at 60 dpi (Villar-Luna *et al*., 2015b). Fernández-Herrera *et al*. (2012) reported a similar behavior for PR-1, POX and PR-2 genes in the whole root system in the pepper 'CM334'-*N. aberrans* interactions.

Previous reports indicated that *N. aberrans*

causes changes in the expression of genes involved in the defense of the plant to provide adequate conditions that allow it to establish and complete its life cycle successfully in pepper plants (Fernández-Herrera *et al*., 2012; Godínez-Vidal *et al*., 2013; Villar-Luna *et al*., 2015a, 2015b). The up-regulation of some genes and the down-regulation of other genes in tomato plants infected by *N. aberrans* at 2, 7, 14, 21 and 28 dpi, indicate that, during nematode parasitism, these four genes were not coordinately regulated, suggesting that their regulation may be under different control mechanisms across time. Transcriptional reprogramming induced by nematodes in their susceptible hosts implies the overexpression of genes in favor to the nematode (e.g., genes associated with the formation of the specialized feeding site), while genes that restrict nematode establishment and reproduction are repressed (defense genes) (Caillaud *et al*., 2008; Eves-van den Akker *et al*., 2016).

In conclusion, changes in the expression of defense genes PR-1, PR-2, POX and PAL after inoculation of tomato with *N. aberrans* estimated by qRT-PCR were variable depending on the sampling time. The expression of the four genes was not turned-off during *N. aberrans* penetration, migration or creation and proliferation of the syncytia. The transcriptional modulation carried out by *N. aberrans* could be necessary to ensure the successful completion of its life cycle in tomato.

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