RESEARCH NOTE/NOTA DE INVESTIGACIÓN

CATALASE ACTIVITY, LIPID PEROXIDATION, AND PROTEIN CONCENTRATION IN LEAVES OF TOMATO INFECTED WITH *MELOIDOGYNE JAVANICA*

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ABSTRACT

Karanastasi, E., T. Kostara, N. Malamos, and G. Zervoudakis. 2018. Catalase activity, lipid peroxidation, and protein concentration in leaves of tomato infected with *Meloidogyne javanica*. Nematropica 48:15-20.

Plants infected by root-knot nematodes show not only developmental and morphological changes in roots, but also systematic symptoms such as stunted growth, wilting, and susceptibility to other pathogens. The objective of this research was to investigate the effect of different nematode infection levels (0, 1,000, 3,000, and 9,000 second-stage juveniles (J2) of *Meloidogyne javanica* per seedling) on leaf oxidative stress and total protein concentration (TPC) of tomato plants (*Solanum lycopersicum* cv. Belladonna). Catalase (CAT) activity and lipid peroxidation (LP) were used as oxidative stress indexes. Leaf CAT activity was enhanced by the nematode infection in comparison to the untreated plants. In the 1,000 and 3,000 J2-infection levels, 45 and 41% increases in the leaf CAT activity were observed, respectively, while CAT activity increased only 14% at the 9,000 J2-infection level. On the other hand, leaf LP decreased with nematode infection. The maximum decrease (20%) occurred at the 9000 J2 infection level. The TPC decreased with nematode infection. At the 1,000 and 3,000 J2-infection levels, 36 and 29% decreases were observed, respectively, while TPC decreased only 9% at the 9,000 J2-infection level. The increased leaf CAT activity probably represents a part of the plant's antioxidative defense against the possible leaf oxidative stress caused by nematode infection reducing the potent cellular damage and corresponding to the LP decrease. The observed leaf TPC decrease is possibly another plant response against the nematode infection.

Key words: catalase, lipid peroxidation, nematode, oxidative stress, protein, tomato

RESUMEN

Karanastasi, E., T. Kostara, N. Malamos and G. Zervoudakis. 2018. Actividad de catalasa, peroxidación de lípidos y concentración de proteínas en hojas de tomate infectadas con *Meloidogyne javanica*. Nematropica 48:15-20.

Las plantas infectadas por nematodos de agallas muestran no solo cambios de desarrollo y morfológicos en las raíces, sino también síntomas sistemáticos tales como retraso en el crecimiento, marchitez y susceptibilidad a otros patógenos. El objetivo de esta investigación fue investigar el efecto de diferentes niveles de infección por nematodos (0, 1000, 3000 y 9000 juveniles de segunda etapa (J2) de *Meloidogyne javanica* por plántula) sobre el estrés oxidativo foliar y la concentración total de proteína (TPC) de las plantas de tomate (*Solanum lycopersicum* cv. Belladonna). La actividad de catalasa (CAT) y la peroxidación lipídica (LP) se usaron como índices de estrés oxidativo. La actividad CAT de la hoja se vio potenciada por la infección por nematodos en comparación con las plantas no tratadas. En los niveles de infección 1000 y 3000 J2, se observaron aumentos de 45 y 41% en la actividad CAT de la hoja, respectivamente, mientras que la actividad CAT aumentó solo 14% en el nivel de infección de 9000 J2. Por otro lado, la hoja LP disminuyó con la infección por nematodos. La disminución máxima (20%) ocurrió en el nivel de infección de 9000 J2. El TPC disminuyó con la infección por nematodos. En los niveles de infección 1000 y 3000 J2, se observaron 36 y 29% de disminución respectivamente, mientras que TPC disminuyó solo 9% en e l nivel de infección de 9000 J2. El aumento de la actividad de la hoja de

CAT probablemente represente una parte de la defensa antioxidante de la planta, contra el posible estrés oxidativo de la hoja causado por la infección por nematodos, reduciendo el potente daño celular y que corresponde a la disminución del LP. La disminución observada de TPC en la hoja es posiblemente otra respuesta de la planta contra la infección por nematodos.

Palabras claves: catalasa, estrés oxidativo, nematodos, peroxidación de lípidos, proteínas, tomate

Root-knot nematodes (RKN), *Meloidogyne* spp., infect a wide variety of plant species. Root-knot nematodes are abundant in tropical, subtropical and Mediterranean regions, inducing significant economic damages (Mai, 1985; Luc *et al*., 2005). As sedentary endoparasites, RKN enter the roots of their host as second-stage juveniles (J2) and then migrate to developing vascular cells. After choosing their feeding site, J2 stimulate the formation of the giant cells, i.e., large feeding cells that are formed by repeated nuclear division in the absence of cell division (Jones and Goto, 2011). The nematodes feed on these giant cells throughout their lives.

Production of Reactive Oxygen Species (ROS) is common in plants even under optimum growth conditions. Under biotic and abiotic stresses, enhanced generation of ROS disturbs the normal redox environment of plant cells and damages the cellular components causing cell dysfunction (Tewari *et al*., 2007; Demidchik, 2015). Therefore, one of the most rapid defense responses of plants against RKN infection is the production of ROS (called "oxidative burst") at the site of the invasion (Nikoo *et al*., 2014). Hydrogen peroxide (H_2O_2) is a common ROS involved in plant responses not only against nematodes but also against bacterial and fungal pathogen infections (Sahebani and Hadavi, 2009). Catalase (CAT) is one of the most important ROS-scavenging enzymes of plants (Nikoo *et al*., 2014; Demidchik, 2015). Increased ROS concentration causes oxidative damage to cell biopolymers such as the lipid peroxidation (LP) of cell membranes' unsaturated fatty acids (Vasil'eva *et al*., 2004).

Nematode invasion into the plant has a marked effect on root protein synthesis. Giant cells contain more protein and amino acids than normal cells while the protein synthesis rate is correlated with the nematode rate growth (Bird, 1961). Moreover, the nematode root infection may induce changes in leaf gene expression inducing pathogenesis-related proteins (Williamson and Hussey, 1996) while total leaf protein has been reported to decrease (Sun *et al.*, 2010).

Solanum lycopersicum is one of the most important vegetable crops grown throughout the world (Singh *et al*., 2013). In the present study, we examined the effect of three different nematode population infections on leaf oxidative status of tomato plants. The CAT activity and the LP were used as indexes of the antioxidative defense and oxidative damage, respectively. Considering that the RKN infection induces the increase of root giant cells' TPC, we examined the leaf TPC as another possible plant response against the nematode infection.

Five-week-old tomato seedlings (*S. lycopersicum* cv. Belladonna) were transplanted into transparent 300-ml plastic beakers containing steam-sterilized soil and maintained at 25°C, 80% humidity. After transplanting, the roots of each plant (10 plants per treatment) were inoculated with 0, 1,000, 3,000, or 9,000 J2 of *Meloidogyne javanica*. The nematode inoculum was collected from cultures maintained at the Laboratory of Plant Protection and Pharmacology, Technological Educational Institute of Western Greece, originally collected from a tomato glasshouse cultivation in Amaliada, Peloponissos, Greece. The plants were grown in the chamber for 6 wk (12 hr:12 hr photoperiod) until the first egg masses were clearly visible on the roots. Plants were watered as required.

At the appearance of eggs masses, leaf samples were collected from the plants. Samples consisted of the 4th or/and 5th leaf of each plant, depending on availability and size. The leaf samples were cut from the plant, wrapped in plastic bags, and transferred immediately to the laboratory for CAT and LP assays, determination of TPC, and dry weight. The leaf samples were washed gently with deionized H_2O and blotted with paper. Each sample was divided into two parts. The first one was dried to constant weight in an oven at 85°C and the second part was homogenized for one of the following methods.

Leaf sample of about 0.5 g fresh weight was ground at 4°C under dim light (to prevent artificial LP) in a porcelain mortar with homogenization buffer containing 50 mM Na_2HPO_4, pH 7.2, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM butylated hydroxyanisole (BHA), and 0.15% ethanol. The homogenization was carried out using 5-ml buffer per gram fresh sample weight. The homogenate was assayed for LP products by a modified thiobarbituric acid (TBA)-based method (Buege and Aust, 1978). Specifically, 0.5-ml

homogenate was mixed with 0.5-ml TBA reagent containing 0.5% (w/v) TBA, 20% (w/v) trichloroacetic acid (TCA), and 0.33 N HCl. A 5-µl aliquot 2% (w/v) of the lipid antioxidant BHA (in absolute ethanol) was added to prevent artificial LP during the assay. The mixture was incubated at 100°C for 15 min and brought to room temperature (RT). One ml of 1-butanol was added, the solution mixed by vigorous vortexing, centrifuged at 15,000 g for 3 min, and the absorbance determined with a Shimadzu UV-1601 spectrophotometer (Shimadzu Corp., Japan) of the upper butanol layer measured at 535 and 600 nm (subtracting the non-specific absorbance) against a sample blank. The sample blank consisted of 0.5-ml sample mixed with 0.5 ml 20% TCA in 0.33 N HCl and with 5 µl 2% w/v BHA and a reagent blank (0.5-ml homogenization buffer mixed with 0.5-ml TBA reagent and 5 µl 2% w/v BHA). Absorbance difference A (535–600) was converted to malondialdehyde (MDA) equivalents with the molar extinction coefficient $1.56 \times 10^5 M^{-1} cm^{-1}$. Lipid peroxidation was defined as TBA-reactive substances (TBARS) and expressed in nmol MDA equivalents per gram dry weight.

Leaf sample of about 0.5-g fresh weight was ground at 4°C in a porcelain mortar with homogenization buffer containing 100 mM K_2HPO_4 pH 7.0, 1 mM EDTA, 0.5-mM phenylmethylsulfonyl fluoride (PMSF), and 0.3% ethanol. The homogenization was carried out using 5-ml buffer per gram fresh sample weight. The homogenate was centrifuged at 3,300 g for 5 min at 4°C. The resulting supernatant was subsequently used for both CAT activity and protein concentration estimation. Catalase was assayed by depletion of H_2O_2 measured at 240 nm (Blum and Fridovich, 1983).

The CAT assay consisted of mixing (in a 30°C water bath) 0.85-ml sample (proper dilution of the homogenate supernatant), 0.05 ml of the homogenization buffer and 0.1 ml 0.09 M H_2O_2 stock solution (made fresh in homogenization buffer). The linear absorbance decrease (vs time) of the assay mixture was measured at 240 nm in a Shimadzu UV-1601 spectrophotometer. The absorbance decrease rate of consumed H_2O_2 was converted to CAT units from a pure CAT standard curve.

The protein concentration was determined by a modification of a Coomassie Brilliant Blue-based method (Assimakopoulos *et al.*, 2008). Specifically, 0.063 ml of the above homogenate supernatant (or an appropriate dilution) was mixed with 0.02 ml of 0.5% (v/v) Triton X-100 and 0.017 ml of 6 N HCl. The mixture was incubated at 100°C for 10 min, brought to RT, mixed with 0.9 ml of 0.033% (w/v) Coomassie Brilliant Blue G-250 stock reagent (made in 0.5 N HCl, stirred for 30 min, filtered through Whatman #1 filter paper by water pump aspiration, and stored in the dark) and incubated for 5 min at RT. The absorbance at 620 nm (against appropriate sample and reagent blanks) of the mixture was converted to mg of protein from a Bovine Serum Albumin standard curve, using a Shimadzu UV-1601 spectrophotometer.

The results were obtained from six independent measurements (six plants randomly selected from each treatment) and expressed as mean ± standard error of mean (SEM). The normality of the data distribution was verified graphically for each treatment, by plotting the normal probability of the empirical distribution function for each treatment using Weibull plotting positions, against the corresponding normal distribution function (Malamos and Koutsoyiannis, 2015). Statistical differences between the means of the different indices of three nematode population infections were calculated by implementing a Student's t-test in MS-Excel for statistical level of significance $\alpha = 0.05$.

Catalases are highly active enzymes that do not require cellular reductants as they primarily dismutate H_2O_2 to H_2O and O_2 ($2H_2O_2 \rightarrow 2H_2O + O_2$) (Willekens *et al*., 1995; Mhamdi *et al*., 2010). Therefore, CAT activity is usually used as an antioxidative defense index. The root CAT activity has been used in studies about the effect of nematode infection to the tomato plants (Molinari, 2008; Sahebani and Hadavi, 2009; Nikoo *et al*., 2014), considering that the root is the directly infected plant organ. However, the root samples from the infected plants are not free from nematode cells, and consequently, it is quite questionable if CAT activity measurements represent only the plant cells antioxidative response and not the contribution of the nematode cells enzyme. Other researchers have investigated Catalase (CAT) activity from plant shoots (Kaur *et al*., 2014; Molinari *et al*., 2014) or leaves (Abd-Elgawad *et al*., 2012; Singh *et al*., 2013) since plants infected by root-knot nematodes show systematic symptoms just as stunted growth, wilting, and susceptibility to other pathogens in addition to developmental and morphological changes in roots (Williamson and Hussey, 1996).

According to our results, the tomato leaf CAT activity was elevated with nematode infection (Fig. 1). Catalase (CAT) activity was more intense in the low and medium inoculation levels with 45 and 41% increases, respectively. However, in the high inoculation level, CAT activity increased only 14% compared to the uninoculated plants. Previous findings also suggest increased leaf CAT activity in nematode-infected tomato plants (Sun *et al*.,

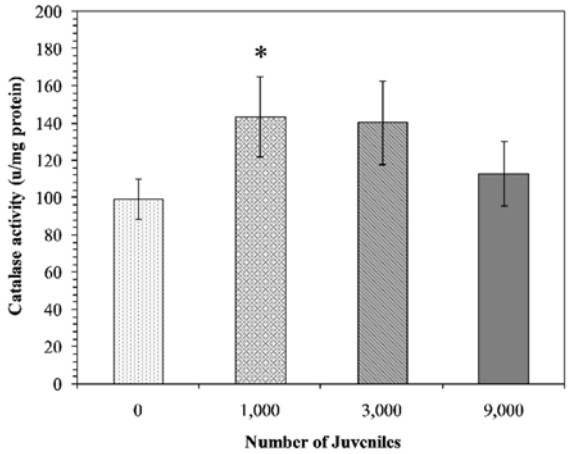

Fig. 1. Effect of infection by *Meloidogyne javanica* on leaf catalase activity of tomato plants at different inoculation levels. Vertical bars represent mean ± SEM (n = 6). Means with asterisk are significantly different from the untreated plants (P<0.05).

2010; Singh *et al*., 2013). It has been suggested that the shoot CAT activity was unaffected in susceptible tomato cultivars, while CAT activity decreased in resistant cultivars (Kaur *et al*., 2013, 2014; Molinari *et al*., 2014). It is also quite interesting that the leaf enzyme activity of untreated resistant tomato cultivars has been reported to be two-fold higher than that of untreated susceptible ones (Abd-Elgawad *et al*., 2012) suggesting that the increased leaf CAT activity is not only a possible plant response against the nematode infection, but it can be an inherent evolutionary characteristic for some plants.

ROS can disrupt normal cell metabolism through oxidative damage to lipids, proteins, and nucleic acids (Chen *et al*., 2009). Therefore, LP measured as MDA equivalents can be used as a marker of the cellular damage caused by oxidative stress. Responding to different nematode infection levels, tomato leaf LP was slightly decreased in comparison to uninoculated plants. LP decreased 20% at the 9,000 J2 inoculation level (Fig. 2). Inadequate information exists on the effect of nematode infection to plant LP. It has only been reported that the nematode infection increases tomato root LP (Zhou *et al*., 2015). Another study estimated tomato LP from infected and uninfected tomatoes but focused on the effect of furostanol glycosides on LP. It is unclear if LP increases or not due to nematode infection (Vasil'eva *et al*., 2004).

Total leaf protein has been reported to decrease in three different tomato genotypes in response to nematode infection (Sun *et al*., 2010). We found similar decreases (Fig. 3). The decrease in TPC was greater at the low and medium inoculation levels with 36 and 29% decreases, respectively, compared to 9% at the highest inoculation level.

The slight LP decrease combined with increased CAT activity indicates that the plant leaf antioxidative defense is activated against potential nematode-induced leaf oxidative stress. Therefore, the increased CAT activity copes effectively with the possible cellular oxidative damages, not only retaining the LP levels of the infected plants near to the control ones but perhaps inhibiting them. The decrease in the LP content with the simultaneous increase of CAT activity suggests that leaf cells may have different partitioning patterns between growth and defense against nematode infection.

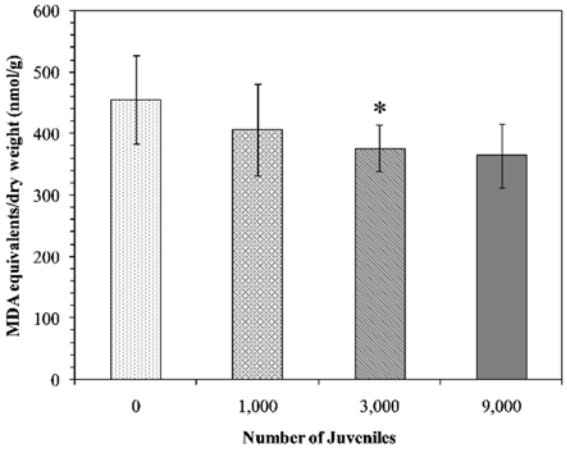

Fig. 2. Effect of infection by *Meloidogyne javanica* on leaf lipid peroxidation of tomato plants at different inoculation levels. Vertical bars represent mean ± SEM (n = 6). Means with asterisk are significantly different from the untreated plants (P < 0.05).

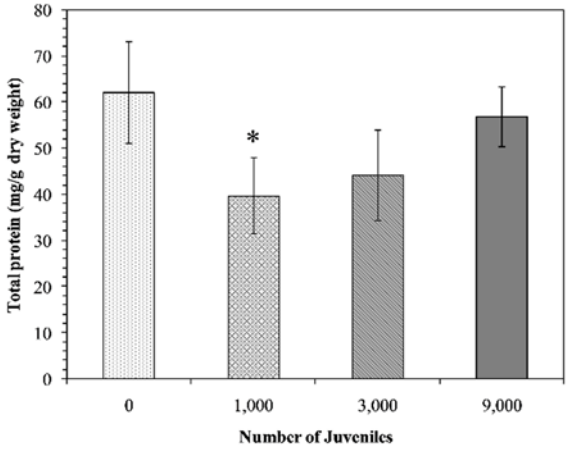

Fig. 3. Effect of infection by *Meloidogyne javanica* on leaf total protein concentration of tomato plants at different inoculation levels. Vertical bars represent mean ± SEM (n = 6). Means with asterisk are significantly different from the untreated plants (P< 0.05).

Further research concerning other antioxidative defense or cellular oxidative damage indexes will clarify the oxidative role of nematode infection in leaves.

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