Hortic. Environ. Biotechnol. 56(4):546-554. 2015.
DOI 10.1007/s13580-015-0146-6

ISSN (print) : 2211-3452
ISSN (online) : 2211-3460

Research Report

Protective enzymes and genes related to the JA pathway are involved in the response to root-knot nematodes at high soil temperatures in tomatoes carrying *Mi-1*

Canli Hu[1†], Wenchao Zhao[1†], Jingwei Fan[1], Zilong Li[1], Rui Yang[2], Fukuan Zhao[3], JianLi Wang[2], and Shaohui Wang[1*]

[1]*College of Plant Science and Technology, Beijing University of Agriculture, No. 7 BeiNongRoad, Changping District, Beijing 102206, P. R. China*
[2]*Beijing Key Laboratory for Agricultural Application and New Technique, Beijing University of Agriculture, No. 7 BeiNongRoad, Changping District, Beijing 102206, P. R. China*
[3]*Biological Science and Technology College, Beijing University of Agriculture, No. 7 BeiNongRoad, Changping District, Beijing 102206, P. R. China*

*Corresponding author: wangshaohui@bac.edu.cn
†These authors contributed equally to this work.

Received December 3, 2014 / Revised May 28, 2015 / Accepted July 7, 2015

Abstract. Root-knot nematodes (RKNs; *Meloidogyne* spp.) are obligate endoparasites that infect a large number of crop plants and cause severe yield losses. Tomato cultivars carrying the *Mi-1* gene conferring root-knot nematode resistance have been widely used, but this gene loses its effectiveness at soil temperatures above 28°C. In this study, the mechanism of the loss of resistance to RKNs at high soil temperatures was examined using LA0655 (*Solanum lycopersicum* cv. Anahu), which contains the *Mi-1* gene. It was found that high soil temperatures delayed the expression of the *Mi-1* gene and reduced the activities of superoxide dismutase, peroxidase, chitinase, and β-1,3-glucanase. Although genes in the jasmonic acid (JA) pathway exhibited an obvious response at high soil temperatures, this response could not prevent the invasion of RKNs; indeed, at 30 days after inoculation with RKNs, the plants produced large numbers of root knots and egg masses at a soil temperature of 32°C.

***Additional key words*:** gene expression, inoculation, mRNA level, resistance

Introduction

Root-knot nematodes (RKNs; *Meloidogyne* spp.) are obligate endoparasites that parasitize the root systems of a wide variety of crops, and they are considered to be among the most damaging nematode crop pests worldwide (Koenning et al., 1999; Liu, 2000; Williamson, 1988). In tomatoes, resistance to three RKN species (*M. arenaria, M. incognita* and *M. javanica*) is conferred by the *Mi-1* gene (Cortada et al., 2009; Roberts, 1986). *Mi-1*-mediated resistance to RKNs in tomato is characterized by a localized hypersensitive response (HR) that occurs when the nematode attempts to initiate a feeding site (Dropkin, 1969); however, this gene loses its effectiveness at soil temperatures of above 28°C (Devran et al., 2010; Dropkin, 1969; Holtzman, 1965).

Existing evidence suggests that reactive oxygen metabolism is effective under the stress of nematode invasion (Tan and Ye, 2003). Some studies have shown that peroxidase (POD) activity is decreased after the inoculation of plants with *M. incognita* and that susceptibility to this RKN is increased (Guo et al., 2005). A previous study has found that superoxide dismutase (SOD) activity is negatively correlated with resistance to nematodes (Zacheo and Bleve-Zacheo, 1988). Chitinase (CHT) and β-1,3-glucanase (GLU) are important pathogenesis-related proteins (Wang et al., 2010) that commonly exist in higher plants; however, under normal conditions, the activities of these two enzymes are low (Zuo et al., 2009). When challenged by external factors, their activities increase to cope with RKN stress and rhizomania (Jia, 2012; Zhang et al., 2005). Zuo et al. (2009) examined plant varieties that were susceptible to nematodes and found that the activities of CHT and GLU were lower in these varieties than in a resistant variety. Previous studies have shown that jasmonic acid (JA) is involved in plant defense against RKNs (Cooper

et al., 2005; Fujimoto et al., 2011; Zhang et al., 2011), and JA has been shown to increase plant resistance to RKNs (Bhattarai et al., 2008). To date, it is unclear whether the above mentioned indices are altered in response to higher soil temperatures and whether these alterations play roles in the loss of resistance at these high temperatures in LA0655. Thus, the current study is focused on these issues.

The goal of our research was to study the mechanism of RKN resistance loss at high soil temperatures in plants carrying the *Mi-1* gene. These results may help to provide a scientific theoretical basis for the regulation of RKN resistance.

Materials and Methods

Plant materials

LA0655 (*S. lycopersicum* cv. Anahu) was provided by the CM Rick Tomato Genetics Resource Center, University of California, Davis, USA. UC82 seeds were provided by Professor V.M. Williamson of the Department of Nematology, University of California, Davis, USA.

Growth conditions

Seeds were surface-sterilized by submergence in 1% NaOCl for 20 min and maintained at 28°C in the dark to allow for germination in Petri dishes containing filter paper. Seedlings were grown in an autoclaved mixture of peat and vermiculite packed in plastic pots (10 × 9 cm) in a growth chamber maintained under 16 h of light at 26°C and 8 h of darkness at 15°C. The plants were watered daily, and nutrient solution was applied once a week.

Nematode cultures

RKNs were maintained on tomato UC82 plants, and egg masses were removed from roots with nematodes using tweezers (Lei et al., 2013). Then, the collected egg masses were incubated in a petri dish containing distilled water at a constant temperature of 26°C.

Infective second-stage juveniles were collected from the liquid suspension after most of the eggs had hatched. The nematodes were quantified by counting under a stereoscopic microscope (MOTIC SMZ-140, China).

The *Mi-1* gene identification

The method of the *Mi-1* gene identification was performed as previously described by Williamson et al. (1994) using the following primers: SCAR (Sequence characterized amplified region) forward primer, 5'-TCGGAGCCTTGGTCTGAATT-3'; SCAR reverse primer, 5'-GCCAGAGATGATTCGTGAGA-3'. The procedures are as follows:

The PCR mix (20 μL) contained 20 ng of DNA, 0.2 μmol·L^{-1} each primer, 1 U rTaq DNA polymerase, and 0.1 mmol·L^{-1} dNTPs. The reaction was carried out under the following conditions: 94°C for 5 min followed by 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min with a final extension at 72°C for 10 min. The reaction was then held at 4°C until sample analysis. The PCR products were detected by electrophoresis on a 1.5% agarose gel.

For detection of the codominant SCAR marker, enzymatic digestion of the PCR products is required (Williamson et al., 1994). To measure the enzymatic digestion products, the following reagents were mixed and incubated for 6 h at 65°C: 10 μL of TaqI (2 U), 1.5 μL of buffer, and 3.3 μL of ddH_2O. The resulting products were detected by electrophoresis on a 2.5% agarose gel (Wang et al., 2009).

Inoculation with RKNs

When the plants had four fully expanded true leaves (30 days after sowing), they were transferred to a growth chamber. After 5 d, the plants were inoculated with RKNs at 25°C or 32°C under the controlled conditions in two different growth chambers.

Detection of the *Mi-1* gene mRNA level in leaves

To measure the expression of the *Mi-1* gene, leaves were collected at 0, 6, 12, 24, and 48 hour after infection (hai) for RNA extraction and qRT-PCR. Total RNA from 200-mg leaf samples was extracted with TRIZOL reagent (Invitrogen) according to the manufacturer's instructions. First-strand cDNA was synthesized with the SYBR PrimeScript reverse transcription-PCR (RT-PCR) kit II.

Measurement of SOD, POD, CHT and GLU activities

Leaves were collected after inoculation with RKNs at 0 h, 6 h, 12 h, 24 h, 72 h, 144 h, 10 d, and 15 d to measure the enzyme activities of SOD and POD (Zhao et al., 2000), CHT (Boller et al., 1983), and GLU (Yu et al., 2007).

The activity of SOD was tested using the NBT reduction assay (Zhao et al., 2000). Enzyme extraction solution and phosphate buffer were each added to two separate tubes. Subsequently, reaction liquid was added to each tube. Control tubes were placed in the dark, and experimental tubes were placed in the light (4000 lx) for 30 min. After the reaction, the absorbance values were determined at 560 nm using a spectrophotometer (Tian Mei UV1101, China).

The activity of POD was assayed by the guaiacol method (Zhao et al., 2000). Enzyme extraction solution was added to each reaction mixture, and enzyme activity was determined dynamically within 30 s. Enzyme activity data are presented in graphical form as $\triangle OD470 \cdot min^{-1} \cdot g^{-1}$ FW.

The activity of CHT was tested as follows. Leaf samples (0.2 g) were mixed with 5 mL of 0.1 mol·L^{-1} acetic acid

Table 1. Overview of the JA-related genes used in this study, including the GenBank accession numbers and the primer pairs used for qRT-PCR

Gene	Accession No.	Forward Primer (5'–3')	Reverse Primer (5'–3')
LePrs	M84800.1	TTGTTGTATTATGTCAGATG	CCGATAGATTCACCATTA
LeSR160	AY112661	TGGAATTGGACTTGTCATAC	ATCAACAGGCAACTTACC
LeLoxA	U09026	CTATCCATACGCTGTTGA	GCTTGTAGTTCATTGTCTT
LeLoxC	U37839	CTATCCATACGCTGTTGA	CGCTTGTAGTTCATTGTC
LeLoxD	U37840	TCCATAACTTAATTCCATCTC	TGATAGTGCTAACAACCT
LeAos1	AJ271093	CATCATCATCGTCATCAC	GAAGTAATCAAGTCTGTCTT
LeAos2	AF230371	TTCCGATTACCTCCGATT	TAGCGAAGAGAAGATTATGAC
LeAos3	AF454634	CCTCTACCTTACATCCTT	CCATACGAATTGAATCCT
LeAoc	NM-001247090	CTATCTTCTGCCTTCCAA	TGTTAGTTGAATCTGTTGAG
LeOpr3	AW034958	CTATTCCATAACCGACTCT	GTGAGAAGAACAGTTGTATT
LeDes	AF317515	ATGTCCTATGGTCTAATG	GGTGAATGTATCGTATCT
LeJA3	AF011557	AATCCTCCTCAGCAACAA	TCCATTCCTATTACTACTTCCAT
LeCOI1	AY423550	TCTCCTCCATCTTCTTCAA	AGTTCCGTTCCTCCATTA
LebZIP	AF176641	GCTCCTTGTATCCTCTT	GCTACTAACTCACATTGC

buffer solution. After grinding on ice, the mixtures were centrifuged at 10,000 g. A 0.4 mL aliquot of enzyme extraction solution was added to 40 μL of 1% snailase and allowed to react for 30 min at 37°C. Subsequently, 0.2 mL of saturated borax was added to the mixture and incubated for 7 min in a boiling water bath. After cooling, 2 mL of glacial acetic acid and 1 mL of 1% of dimethyl amine benzaldehyde (DMAB) were added and incubated for 15 min at 37°C. Finally, optical density was measured at 470 nm.

The activity of GLU was tested as follows. The enzyme solutions were incubated in a boiling water bath for 10 min. Enzyme solution alone served as the control, and laminarin served as the reaction substrate. The enzyme activity unit (U) for laminarin is defined as the amount of enzyme required to produce 1 μg of glucose per gram of fresh tissue per minute.

Determining the expression levels of genes related to the JA pathway

At 0 and 12 hai, leaves were collected for RNA extraction and qRT-PCR. qRT-PCR was carried out with SYBR Green PCR Master Mix (Applied Biosystems) using first-strand cDNA as a template. The reactions were performed using a sequence detection system (ABI Prism 7500 fast real-time PCR system, Applied Biosystems). All primers (Table 1) were designed based on cDNA sequences, and their specificity was previously assessed by NCBI Blast and PCR amplification of a tomato cDNA library.

Root-knot observation after inoculation of LA0655 with RKNs for 6 h, 12 h and 30 days

At 6 hai, 12 hai and 30 day after infection (dai), the roots of tomato plants were stained with NaClO-acid fuchsin-glycerin, and the number of root knots was counted. Root tissue staining was conducted using the method described by Byrd et al. (1983). Roots were washed and placed in a 150 mL beaker with 50 mL of tap water. The root tissues were allowed to remain in 1.5% NaOCl solution for 4 min with occasional agitation. Following the NaOCl treatment, the root segments were rinsed in running water for 1 min and soaked in tap water for 15 min to remove residual NaOCl. The material was then stained and transferred to a beaker containing 30 mL of water with 1 mL of stain (3.5 g of acid fuchsin, 250 mL of acetic acid, and 750 mL of distilled water). Next, the solution was heated to boiling for approximately 45 s. After cooling to room temperature, excess stain was removed by rinsing in running water. The root material was subsequently placed in glycerin acidified with a few drops of 5 mol·L^{-1} HCl, heated to a boil, and then cooled. The fading roots were observed directly in a Petri dish containing deionized water. Images were captured with a camera.

Statistical and data analyses

All the tests were performed in three independent replicates. The data were analyzed using OriginPro (OriginLab Corporation, Northampton, United States) and DPS (Refine Information Tech, Hangzhou, Zhejiang, China) software.

Results

The Mi-1 gene confirmation

Use of the SCAR marker yielded a single band of approximately 750 bp (Fig. 1 A). After digestion with TaqI, two fragments were visualized that were 570 bp and 180 bp

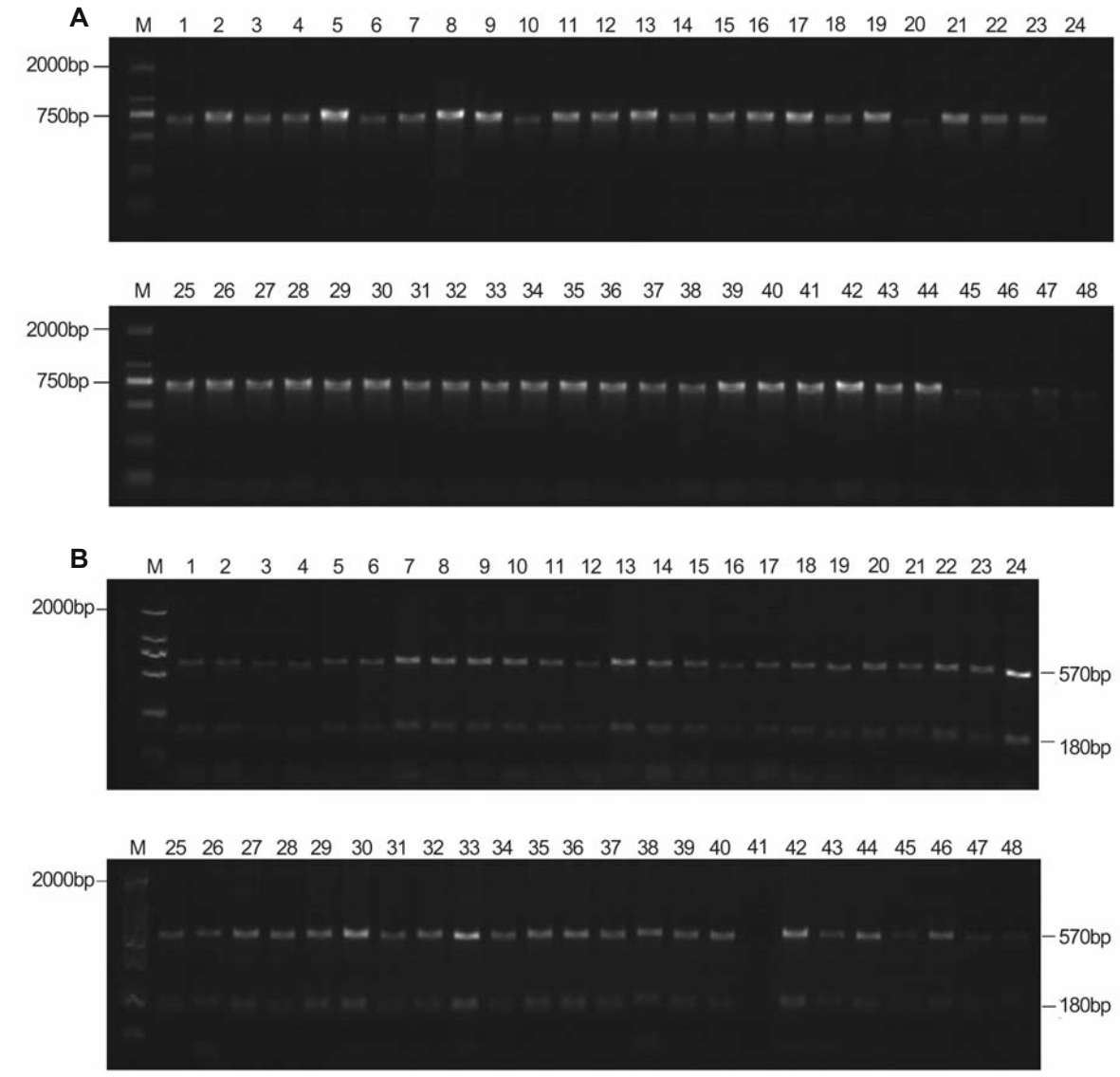

Fig. 1. Products of PCR amplification and digestion of SCAR with *Taq*I. M, DL2000 DNA Marker; A, products of PCR amplification; B, PCR products after digestion with *Taq*I.

in size (Fig. 1 B). The numbers from 1 to 48 represent 48 tomato seedlings used in the experiment (Fig. 1). This result was in agreement with that of Wang et al. (2009) and confirmed the presence of the *Mi-1* gene in LA0655.

The *Mi-1* gene expression level responds to RKNs at soil temperatures of 25°C and 32°C

As shown in Fig. 2, *Mi-1* mRNA level peaked at a normal soil temperature of 25°C at 6 hai. However, at a higher soil temperature of 32°C, *Mi-1* mRNA level peaked at 12 hai, which was nearly twice as long as the time required to peak at 25°C. This result indicates that although the *Mi-1* mRNA level increased at a soil temperature of 32°C, the transcriptional peak was delayed until 12 hai.

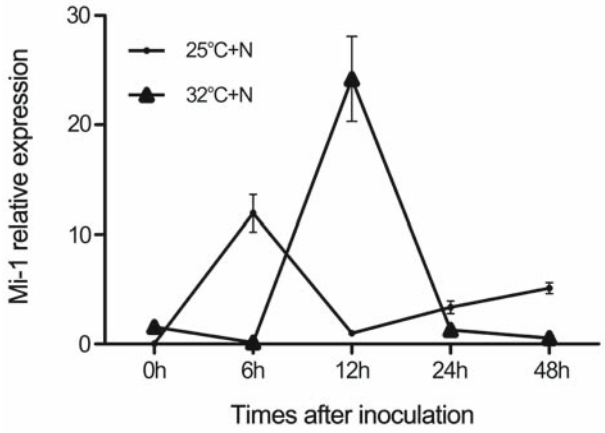

Fig. 2. Effects of high soil temperature on *Mi-1* mRNA levels. 25°C + N, inoculated with RKNs at 25°C; 32°C + N, inoculated with RKNs at 32°C; bars, SE.

Activities of SOD, POD, CHT and GLU at high soil temperatures in the presence of RKNs

As shown in Fig. 3 a, SOD activity was higher at 25°C than at 32°C. The activity of SOD was lowest at 12 hai at soil temperatures of 25°C and 32°C, and its activity at 25°C was 13.5% higher than that at 32°C (Fig. 3 A).

Before 72 hai, POD activity at 32°C was higher than that at 25°C; after 72 hai, POD activity was higher at 25°C than at 32°C. At later time points, POD activity at 32°C was lower than that at 25°C. The average POD activity at 25°C was 10.6% higher than that at 32°C (Fig. 3 B).

Before 24 hai, CHT activity at 32°C was higher than that at 25°C. After 24 hai, CHT activity was higher at 25°C than at 32°C. The average CHT activity at 25°C was 6% higher than that at 32°C (Fig. 3 C).

After RKN infection, GLU activity increased at both 25°C and 32°C; however, the GLU activity at 25°C was consistently higher than that at 32°C. At 15 dai, the activity reached a peak, and GLU activity at 25°C was two times higher than that at 32°C (Fig. 3 D).

Transcriptional levels of genes related to the JA pathway

At 0 h, genes related to the JA pathway could be divided into four categories based on their expression levels. The expression levels of genes in the first category, including *LePrs*, *LeSR160*, *LeLoxC*, and *LeAos3*, decreased by more than 15-fold at a soil temperature of 32°C compared with those observed at a soil temperature of 25°C. The expression levels of genes in the second category, including *LeLoxA* and *LeDes*, decreased by 10- to 15-fold at 32°C. Genes in the third category, including *LeLoxD* and *LeCOI1*, showed expression decreases of 5- to 10-fold at 32°C. Finally, genes in the fourth category, including *LeAos1* and *LeJA3*, showed expression decreases of under 5-fold at 32°C compared with those observed at 25°C (Fig. 4). The rest of the four genes, *LeAoc*, *LeOpr3*, *LebZIP*, and *LeAos2,* genes' expression increased at 32°C compared with at 2°C.

Fig. 4 shows that significant changes in the expression of most genes were observed at 12 hai. At 0 h, the expression levels of genes such as *LePrs*, *LeSR160*, *LeLoxA*, *LeLoxC*, *LeLoxD*, *LeAos1*, *LeAos3*, *LeCOI1* and *LeJA3* were higher

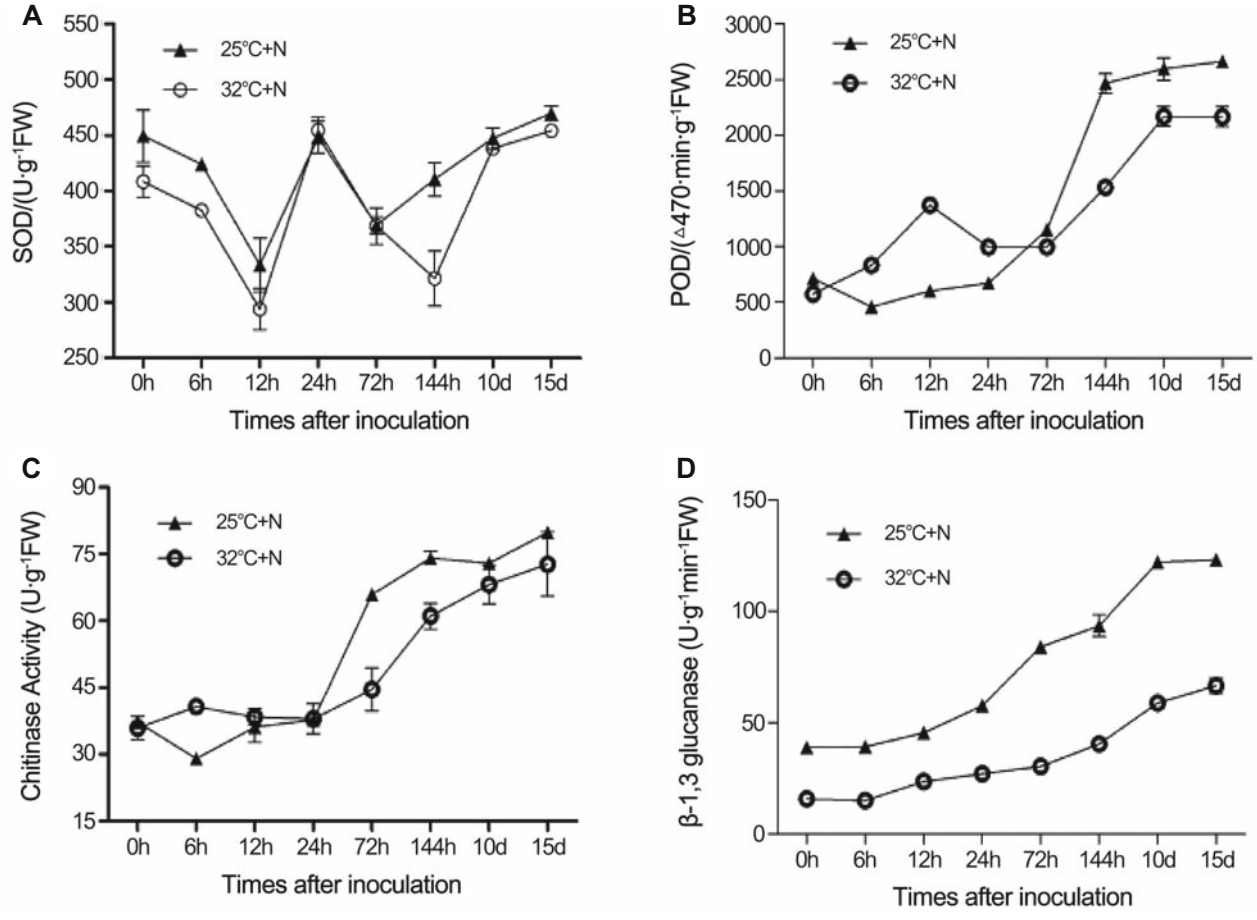

Fig. 3. Effects of high soil temperature on the activities of SOD, POD, CHT and GLU in tomato leaves inoculated with RKNs. (A, activity of SOD; B, activity of POD; C, activity of CHT; D, activity of GLU; bars, SE.

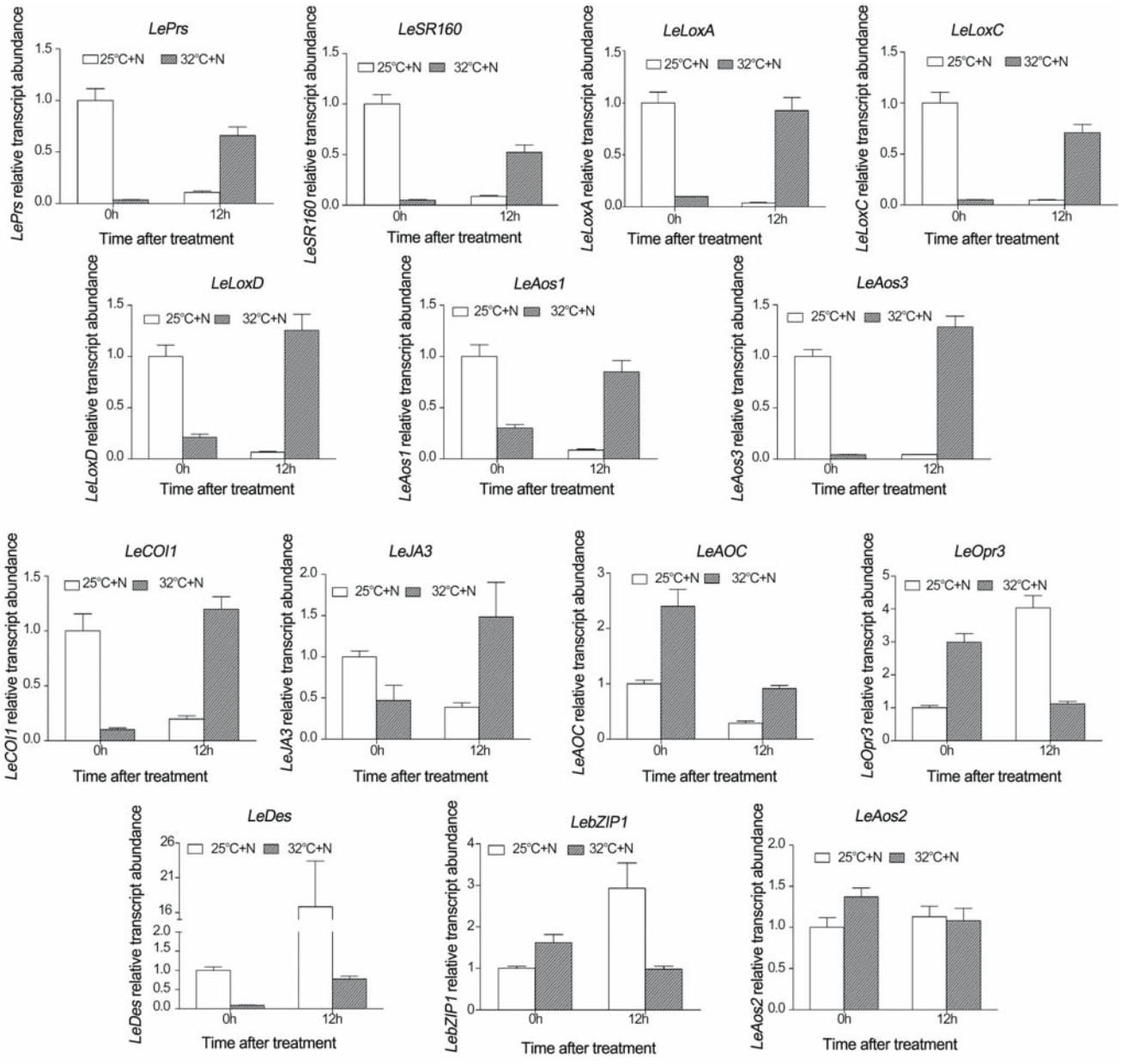

Fig. 4. Effects of high temperature on the transcription levels of genes related to the JA pathway; bars, SE.

at 25°C than at 32°C; however, at 12 hai, the expression levels of these genes were significantly higher at 32°C than at 25°C. *LeOpr3*, *LeDes*, and *LebZIP*, genes' expression levels were significantly lower at 32°C than at 25°C. And the expression level of *LeAos2* that remained higher at 25°C at 12 h.

Responses of plant roots to *M. incognita* at soil temperatures of 25°C and 3°C

Fig. 5 A, 5 B, 5 C, and 5 D show that at the high soil temperature of 32°C, RKNs invaded the root at 6 hai. More nematodes were observed at 12 hai than at 0 hai, 6 hai, and 30 dai. Additionally, a large number of root knots and egg masses were found in the roots (Fig. 5 E, 5 F, 5 G). At 25°C, no nematodes, root knots or egg masses were found in the roots (Fig. 5 G). These results indicate that at high soil temperatures, tomato plants expressing the *Mi-1* gene lose their resistance to RKNs.

Discussion

It is known that the *Mi-1* gene is effective at soil temperatures below 28°C; however, the resistance it confers breaks down irreversibly above 28°C (Dropkin, 1969). As expected, LA0655 expressing the *Mi-1* gene showed complete resistance to *M. incognita* at a soil temperature of 25°C.

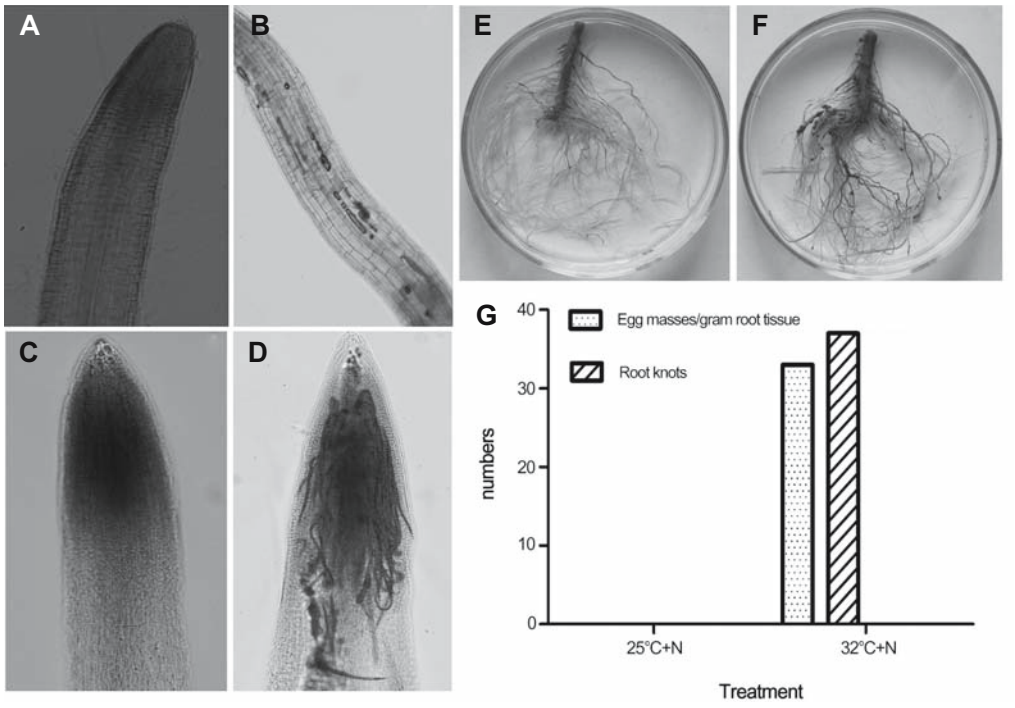

Fig. 5. The influence of high soil temperature on tomato RKN resistance. A, 6 hai at 25°C; B, 6hai at 32°C; C, 12 hai at 25°C; D, 12 hai at 32°C; E, 30 dai at 25°C; F, 30 dai at 32°C; G, RKN resistance of LA0655 tomato plants at different soil temperatures.

However, at 32°C, the plants lost resistance to *M. incognita* and exhibited several root knots and egg masses. At 6 hai and 12 hai, we observed that the nematodes had invaded the roots, as suggested by Liharska and Williamson (1997).

In this study, we found that the *Mi-1* gene expression varied at 25 and 32°C. At 25°C, the *Mi-1* gene expression increased between 0 and 12 hai, whereas at 32°C, the expression of the *Mi-1* gene only began to increase between 12 and 24 hai. At 6 hai, the *Mi-1* gene was active at 25°C and effectively prevented nematode invasion; therefore, no root knots were observed. At 32°C, however, the *Mi-1* gene expression was low between 0 and 6 hai. Nematodes invaded the roots during this time; therefore, although the *Mi-1* gene expression increased by 12 hai, RKNs had already invaded the root, and this invasion was irreversible. Thus, many root knots and egg masses were observed on the roots by 30 dai.

Previous studies have shown that during the early stages of nematode infection, SOD activity is initially reduced and later increases; our experimental results showed a similar trend (Jia, 2012). At a soil temperature of 32°C, SOD activity was lower than that at 25°C, and LA0655 lost its resistance to RKNs. SOD may, therefore, be positively correlated with resistance to RKNs. Previous studies have also shown that POD activity is positively correlated with resistance to RKNs (Ye et al., 2009). In the current study, when LA0655 was inoculated with RKNs, the average POD activity was lower at a soil temperature of 32°C compared with a soil temperature of 25°C. Thus, a high soil temperature causes LA0655 to lose its resistance to RKNs.

It is widely believed that CHT and GLU, which are important pathogenesis-related proteins, are related to plant resistance to pathogenic microorganisms (Wang et al., 2010; Zhang, 2010). Wu and Duan (2004) found that root CHT activity increased when plants were incubated with soybean cyst nematodes. In the current study, we found that the activity of CHT and GLU was enhanced, and the average activity at 25°C was significantly higher than that at 32°C. These results suggest that high soil temperatures decrease the activity of these enzymes, thus reducing the resistance to RKNs.

Previous reports have revealed that JA can reduce the damage caused by RKNs (Cooper et al., 2005; Fan et al., 2015; Fujimoto et al., 2011; Zhang et al., 2011). However, to date, the molecular mechanisms underlying JA signaling in plants expressing *Mi-1* at higher soil temperatures is unclear. We focused on examining the expression levels of several JA-related genes in plants expressing *Mi-1* at different soil temperatures (25 and 32°C).

It is well known that JA plays an important role in the response of plants to biotic stress (Mur et al., 2006; Sun et al., 2011). JA biosynthesis- and signal transduction-related genes, including *LOX*, *AOS*, *AOC*, and *OPR*, are induced defense genes, meaning that their expression can be induced by damage (Li, 2006). In this study, at 12 hai (Fig. 5 B), the expression levels of *LePrs*, *LeSR160*, *LeLoxA*, *LeLoxC*, *LeLoxD*, *LeAos1*, *LeAos3*, *LeCOI1* and *LeJA3* were higher

at 32°C than at 25°C; however, at 32°C, the nematodes had already penetrated the roots within 12 h. This finding indicates that although the expression levels of these genes were higher at 12 hai, this expression increase occurred much later than root penetration by RKNs. At 25°C, the nematodes could not invade the root by 12 hai, and the expression of genes related to the JA pathway was relatively low. Thus, by 30 dai, the plant roots exhibited a large number of root knots, and the plants lost their resistance to RKNs at 32°C.

Conclusion

In this study, the activities of POD, SOD, CHT and GLU were examined at different soil temperatures. The results showed that at a high soil temperature, tomato plants carrying the *Mi-1* gene lost their resistance to RKNs due to reductions in the activities of POD, SOD, CHT and GLU because of delayed *Mi-1* expression. Although the expression of genes related to the JA pathway was higher at high soil temperatures, this increased expression occurred at 12 hai, which was later than the time at which the nematodes invaded the roots. After 30 days, the plants exhibited a large number of root knots, and a small number of egg masses had been generated. These results show that tomato plants carrying *Mi-1* lose their resistance to nematodes at high soil temperatures.

Acknowledgment: We would like to thank the Great Wall of Scholars (CIT&TCD20130323) for supporting this study.

Literature Cited

Bhattarai, K.K., Q.G. Xie, S. Mantelin, U.Bishnoi, T.Girke, D.A. Navarre, and I. Kaloshian. 2008. Tomato susceptibility to root-knot nematodes requires an intact jasmonic acid signaling pathway. Mol. Plant-Microbe Interact. 21:1205-1214.

Boller, T., A. Gehre, F. Manch, and U. Vogrli. 1983. Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function. Planta 157:22-31.

Byrd, D.W., Jr., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. J. Nematol. 14:142-143.

Cooper, W.R., L.Jia, and L. Goggin. 2005. Effects of jasmonate-induced defenses on root-knot nematode infection of resistant and susceptible tomato cultivars. J Chem. Ecol. 31:1953-1967.

Cortada, L., F. J. Sorribas, O. César, M.F. Andrés, S. Verdejo-Lucas. 2009. Response of tomato rootstocks carrying the *Mi*-resistance gene to populations of *Meloidogynearenaria*, *M. incognita* and *M. javanica*. Eur. J. Plant Pathol. 124:337-343.

Devran, Z., M.A. Söğüt, and N. Mutlu. 2010. Response of tomato rootstocks with the *Mi* resistance gene to *Meloidogyne incognita* race 2 at different soil temperatures. Phytopathol. Mediterr. 49: 11-17.

Dropkin, V.H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: reversal by temperature. Phytopathology 59:1632-1637.

Fan, J.W., C.L. Hu, L.N. Zhang, Z.L. Li, F.K. Zhao, S.H. Wang. 2015. Jasmonic acid mediates tomato's response to root knot nematodes. J. Plant Growth Regul. 34:196-205.

Fujimoto, T., Y. Tomitakab, H. Abec, S. Tsuda, K. Futai, and T. Mizukubo. 2011. 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. 168:1084-1097.

Guo,Y.Y., X.F. Wang, K. Xu, and G.M. Zhang. 2005. Effect of *Meloidogyne incognita* on the physiological and chemical changes in ginger. Acta Phytopathol. Sin. 35:49-54.

Holtzman, O. 1965. Effects of soil temperature on resistance of tomato to root-knot nematode (*Meloidogyne incognita)*. Phytopathology 55:990-992.

Jia, S.S. 2012. Study on evaluation and mechanism of tomato rootstocks for resistance to *Meloidogyne incognita*. phD Diss., Shan Dong Agri. Univ., China.

Koenning, S.R., C. Overstreet, J.W. Noling, P.A. Donald, J.O. Becker, and B.A. Fortnum. 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J. Nematol. 13:587-618.

Lei, H., X. Qi, W. Chang, R. Yang, S.H. Wang. 2013. Jasmonic acid-mediated phloem transcriptome of tomato in response to root-knot nematode. J. Beijing Univ. Agric. 28:1-5.

Li, C.B. 2006. Tomato as a model system to study the molecular basis of plant defense responses to insect attact. Master thesis: Shan Dong Agri. Univ.

Liharska, T. B. and V.M. Williamson. 1997. Resistance to root-knot nematodes in tomato, p. 191-200. In: C. Fenoll, F.M.W. Grundler, S.A. Ohl (eds). Cellular and Molecular aspects of Plant-Nematode Interactions. Kluwer Acad. Publ. Netherland.

Liu, W.Z. 2000. Plant Pathogeny Nematode. Beijing: China Agric. Press.

Mur, L.A.J., P. Kenton, R. Atzorn, O. Miersch, and C. Wasternack. 2006. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140: 249-262.

Roberts, P.A. 1986. Variability in reproduction of isolates of *Meloidogyne incognita* and *M. javanica* on resistant tomato genotypes. Plant Dis. 70:547-551.

Sun, J.Q., H.L. Jiang, and C.Y. Li. 2011.Systemin/Jasmonate-mediated systemic defense signaling in tomato. Mol. Plant 4:607-615.

Tan, J.J. and J.R. Ye. 2003. Research advance in pathogenic mechanism of pine wood nematode disease. J. Huazhong Agric. Univ. 22:613-617.

Wang, M.X., C.S. Yan, H.K. Jiang, Y.X. Dong, L. Fang, and Q.A. Qi. 2009. Evaluation of molecule markers linked to root-knot nematode resistance gene (*Mi*) on tomato. China Veg. 18:21-24.

Wang, T.P., J.J. Ma, and F.Y. Zhao. 2010. Research advance of β -1,3-glucanase and chitinase in controlling insect pests of crops. J. Anhui Agric. Sci. 38:14417-14419.

Williamson, V.M. 1988. Root-knot nematode resistance genes in tomato and their potential for future use. Annu. Rev. Phytopathol. 36:277-293.

Williamson, V.M., J.Y. Ho, F.F. Wu, N. Miller, and I. Kaloshian. 1994. A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. Theor. Appl. Genet. 87:757-763.

Wu, H.Y. and Y.X. Duan. 2004. Interaction of chitinase isozyme and soybean resistant to *Heterodera glycines*. Acta Phytopathol. Sin. 34:555-557.

Ye, D.Y., C.T. Qian, Y.Y. Jia, Y.X. Zhang, and J.F. Chen. 2009. Cucumber and its related species for resistance to the Sourthern root-knot nematode *Meloidogyne incognita* and respond to changes ofenzyme. Acta Hortic. Sin. 36:1755-1760.

Yu, Y.T, Y.Y. Xie, L.L. Huang, Z.S. Kang. 2007. Effects of different

combinations of carbon and nitrogen sources in MS medium on activities of extracellular β-1,3-glucanase produced by take-all pathogen. J. Northwest A & F Univ. (Nat. Sci. Ed.) 35:110-114.

Zacheo, G. and T. Bleve-Zacheo. 1988. Involvement of superoxide dismutase and superoxide radicals in the susceptibility and resistance of tomato plants to *Meloidogyne incognita* attack. Physiol. Mol. Plant Pathol. 32:313-322.

Zhang, L.N., J.H. Cheng, R. Yang, Z.H. Sun, C.X. Wu, and S.H. Wang. 2011. Effects of JA synthesis-related genes Spr2 and LePrs on the resistance to root-knot nematodes in tomato. Sci. Agric. Sin. 44:4022-4028.

Zhang, S.Y., J.B. Wang, H.F. Wang, J.W. Shao, G.L. Li, and X.D. Li. 2005. The relationship between activities of chitinase and β-1,3-glucanase and resistance to rhizomania in sugar beet. J. plant physiol. mol. biol. 31:281-286.

Zhang, X.W. 2010. Effect of penicillin on β-1, 3-glucanase activity and chitinase activity of apple tree. Inn. Mong. Agric. Sci. Technol. 5:76-77.

Zhao, S.J., G.A. Shi, and X.C. Dong. 2000. Plant physiology experiment guidance. Beijing: China agricultural science and technology publishing house.

Zuo, Y.H., Z.S. Kang, C.P. Yang, H.Y. Rui, S.B. Lou, and X.R. Liu. 2009. Relationship between activities of β-1,3-glucanase and chitinase and resistance to phytophthora root rot in the soybean. Acta Phytopathol. Sin. 39:600-607.