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INTERACTION OF ROOT-KNOT NEMATODE (*MELOIDOGYNE JAVANICA*) AND TOMATO AS AFFECTED BY HYDROGEN PEROXIDE

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Abstract: The effect of hydrogen peroxide (H_2O_2) on root-knot nematode (RKN, *Meloidogyne javanica*) in tomato was investigated. Soil drenching with exogenous H_2O_2 was done using seven H_2O_2 concentrations (1, 10, 100, 250, 500, 750 and 1000 mM) at different application times (24 hours before the time of plant inoculation with the RKN (T1), at the time of inoculation (T2), and 24 hours after the inoculation time (T3). The nematode reproduction rate (eggs/g fresh root) was significantly reduced in all H_2O_2 treatments compared with the untreated control. The lowest reduction in nematode reproduction occurred at 10 mM H_2O_2. The application times T1 and T2 were significantly higher in reducing the reproduction rate than T3 at 250, 750 and 1000 mM H_2O_2. The content of endogenous H_2O_2 in the treated plants was significantly higher than in the non-treated plants. Some phytotoxicity was apparent at the higher concentrations of H_2O_2 (≥ 500 mM) in the treated plants due to the accumulation of the endogenous H_2O_2. The treatments with 1 and 10 mM H_2O_2 did not differ from the untreated control in plant chlorophyll content while the content was significantly reduced at the higher concentrations. Exogenous application of H_2O_2 may have a direct effect on the nematode reproduction and an indirect effect on the treated tomato plants that can be elicited by H_2O_2 to resist the nematode infection.

Key words: Host resistance, nematode reproduction, host-pathogen interaction.

INTRODUCTION

Root-knot nematodes (RKNs, *Meloidogyne* spp.) attack a wide range of crop species. Annually, about 5% of the world crop production is destroyed by *Meloidogyne* species (Sasser *et al*. 1983; Barker *et al*. 1985; Sasser 1987).

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Three *Meloidogyne* species (*M. javanica* (Teurb) Chitwood, *M. incognita* (Kofoid and White) Chitwood, race 1 and 2, and *M. arenaria* (Neal) Chitwood, race 2) were reported to occur in Jordan, with a predominance of *M. javanica* (Abu-Gharbieh 1982; Atieh 1986; Karajeh *et al*. 2005). The average annual losses of irrigated vegetable crops cultivated in the Jordan Valley due to RKNs has been estimated for nearly 15% (Abu-Gharbieh 1994)

Hydrogen peroxide (H_2O_2) functions as a stress signal in plants, mediating adaptive responses to various stresses. Exposure to various abiotic and biotic stresses results in the accumulation of H_2O_2 (Desikan *et al*. 2001). It is already known that H_2O_2 can induce the expression of genes involved in antioxidant defense (Levine *et al*. 1994; Karpinski *et al*. 1999; Morita *et al*. 1999; Lopez-Huertas *et al*. 2000; Jaiti *et al*. 2004). Many studies have reported the killing capacity of H_2O_2 produced by some bacteria or applied exogenously on nematodes e.g. *Caenorhabditis elegans* (Gustin *et al*. 2002; Jansen *et al*. 2002; Bolm *et al*. 2004).

To our knowledge, no previous studies were conducted to explore the effect of H_2O_2 on plant-parasitic nematodes except for its use for clearing cyst walls and for increasing egg hatching when it is applied with irrigation to increase soil aeration (Goodey 1963). Hydrogen peroxide could affect the nematodes directly by its toxicity and/or indirectly as an elicitor triggering the host-plant defense. Therefore, this study was done to investigate the influence of H_2O_2 applied exogenously on *M. javanica* and its interaction with tomato.

MATERIALS AND METHODS

Source of RKN Inoculum

Inoculum of *M. javanica* was obtained from a population that was naturally infecting eggplant plants in the central part of Jordan Valley. Galled root samples were washed thoroughly under tap water for 5 minutes. Small-galled roots were excised and examined under a dissecting microscope for finding developmental stages of the RKNs. Identification of *M. javanica* was done by observing female perineal patterns and measuring length of second-stage juveniles (Barker *et al*. 1985) and confirmed by species-specific SCAR-PCR (Karajeh 2004). The nematode eggs were extracted from galled roots by using 0.5% sodium hypochlorite (Hussey and Barker 1973).

Plant Material and Treatments

Seeds of tomato cultivar Speedy were sown in nursery polystyrene trays filled with a pasteurized mixture of peatmoss, perlite and clay soil (1:2:1) in the greenhouse. The tomato plants were transplanted to pots and were transferred to a growth chamber (25 ± 3°C air temperature and 16 h day). Twenty-nine treatments were arranged in a completely randomized design with 10 replicates. A technical grade of H_2O_2 was used. The treatments were soil drenching with H_2O_2 at 7 concentrations (1, 10, 100, 250, 500, 750 and 1 000 mM) in plant rhizosphere at three different application times: 24 hours before the time of plant inoculation with the RKN (T1), at the time of inoculation (T2), and 24 hours after the inoculation (T3). Treatments were repeated on un-inoculated plants (NI) and some inoculated plants were not treated with H_2O_2, used as controls. The seedlings were inoculated with 3 000 eggs of *M. javanica* per pot. The experiment was ended after sixty days. The nematode eggs were re-extracted

from treated plants by using 0.5% NaOCl. The number of eggs/g root fresh weight was then determined.

Analysis of data

Data were statistically analyzed using the general linear model (GLM) procedure of the system of analytical statistics (SAS). Least significant difference (LSD) test was used for mean separation at 0.05 probability (Steel and Torrie 1986).

Determination of H_2O_2 Content

Plant content of endogenous hydrogen peroxide was determined five days after the exogenous application according to Velikova *et al.* (2000). Briefly, plant material (500 mg fresh weight) was homogenized in 2 ml trichloroacetic acid (TCA) solution (1 g/l). After centrifugation at 12000Xg for 15 minutes, 0.5 ml of the supernatant was added to the reaction mixture containing 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 mM KI. Absorbance was determined at 390 nm using a UV spectrophotometer (Helios Alfa, Thermo Electron Corporation, USA) and the amount of H_2O_2 was evaluated using a standard curve under the same conditions.

Determination of Chlorophyll Content

Chlorophyll content was determined at the end of the experiment according to Seśtak *et al.* (1971). Samples (30 mg of fresh leaves) were immersed in 5 ml of 96% ethanol at 80°C for 10 min to extract the pigments. The absorbance of extracts was measured at 663 and 647 nm using the UV spectrophotometer. The amount of chlorophyll (chlorophyll a + chlorophyll b) was evaluated using a standard curve determined under the same conditions.

RESULTS AND DISCUSSION

Compared with the untreated control, the RKN reproduction, expressed by the number of eggs/g fresh root was significantly reduced in all H_2O_2 treatments. The lowest reduction in RKN reproduction occurred at 10 mM H_2O_2 concentration used (Fig. 1). Regarding the application time of exogenous H_2O_2, T1 and T2 were definitely significantly higher in reducing the nematode reproduction than T3 at 250, 750 and 1000 mM H_2O_2. The reduction in the RKN reproduction rate could be correlated with the direct killing effect of H_2O_2 of the nematode eggs and hatched juveniles. Hydrogen peroxide vapor was reported to have the ability to kill the eggs of *C. elegans in vitro* (Gustin *et al*. 2002). Streptococcus bacteria are able to kill *C. elegans* and this killing is only mediated by hydrogen peroxide. Streptococci can produce sufficient amounts of hydrogen peroxide to kill *C. elegans*, with killing kinetics similar to those of equimolar concentrations of pure hydrogen peroxide (Jansen *et al*. 2002; Bolm *et al*. 2004).

Content of endogenous H_2O_2 in plants increased at higher concentrations of exogenous H_2O_2 (≥ 500 mM) in treated plants compared with the control plants regardless the time of application (Fig. 2). There were significant differences between the treatments with exogenous H_2O_2 used and the untreated control of uninoculated plants in the content of endogenous H_2O_2 (Fig. 2). In general, chlorophyll content of treated plants was reduced with the increase in the concentration of exogenous H_2O_2 used except for 1 and 10 mM where there was no difference (Fig. 3). At the higher concen-

tration of exogenous H_2O_2, treated plants were yellowish (low in chlorophyll content); some plants were stunted with relatively small root systems and some plants died before the end of the experiment. No significant differences were found among T1, T2 and T3 in plant contents of endogenous H_2O_2 and chlorophyll (Fig. 2 and 3).

The use of exogenous H_2O_2 at low concentrations (1–100 mM) did not affect plant growth. As a result of the application of exogenous H_2O_2, there were elevated levels of endogenous H_2O_2 in the treated plants. Hydrogen peroxide is known as mobile and reactive compound in plants and in fact, tomato plants are known to accumulate relatively high levels of H_2O_2 (Orozco-Cańdenas and Ryan 1999). Hydrogen peroxide has

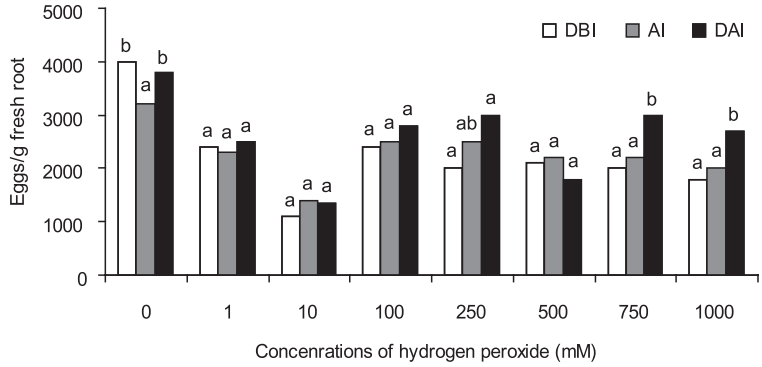

Fig. 1. Effect of soil drenching with hydrogen peroxide on the reproduction of *Meloidogyne javanica* at three application times (DBI (T1): one day (24 hours) before the time of plant inoculation with the RKN, AI (T2): at the time of inoculation, and DAI (T3): one day (24 hours) after the inoculation). Columns followed by the same letters are not significantly different according to least significant (LSD) test at 0.05 probability level.

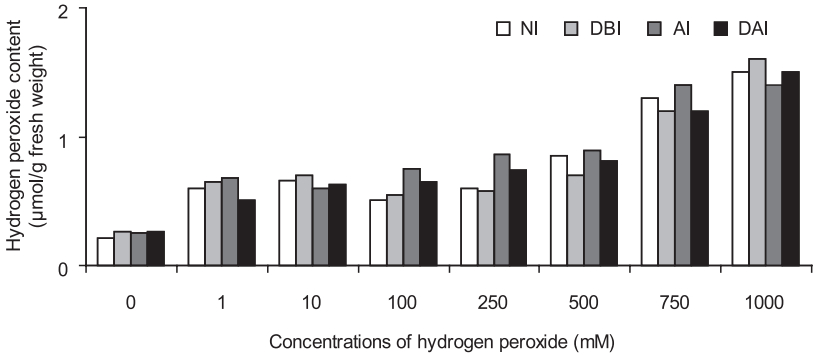

Fig. 2. Plant content of hydrogen peroxide five days after treatment with exogenous hydrogen peroxide at different concentrations in plants uninoculated (NI) or inoculated with *Meloidogyne javanica* (DBI (T1): one day (24 hours) before the time of plant inoculation with the RKN, AI (T2): at the time of inoculation, and DAI (T3): one day (24 hours) after the inoculation). H_2O_2 content means ($LSD_{0.05}$ = 0.25) are not significant at 1, 10, 100 and 250 mM H_2O_2 concentrations.

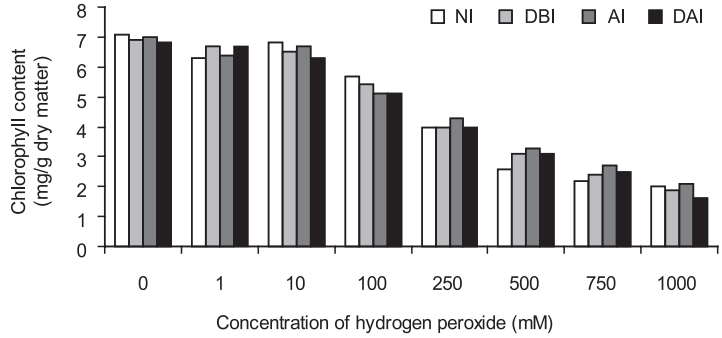

Fig. 3. Chlorophyll content of hydrogen peroxide five days after treatment with exogenous hydrogen peroxide at different concentrations in plants uninoculated (NI) or inoculated with *Meloidogyne javanica* (DBI (T1): one day (24 hours) before the time of plant inoculation with the RKN, AI (T2): at the time of inoculation, and DAI (T3): one day (24 hours) after the inoculation). Chlorophyll content means ($LSD_{0.05}$ = 0.65) are not significant at 1 and 10 mM H_2O_2 concentrations.

been reported to play an important role in the development of disease resistance in plant species (Grant *et al*. 2000). Its accumulation in specific tissues, and in the appropriate quantities, is of benefit to plants and can mediate cross-tolerance toward other stresses (Bolwell 1999). The exogenous application of H_2O_2 and ethephon protected tomato seedlings from chilling injury (Al-Haddad *et al*. 2002). The response to H_2O_2 in tomato plant may be associated with the defense of plants against both herbivores and pathogens (Orozco-Cardenas and Ryan 1999). Hydrogen peroxide in plant cells has been shown to regulate the hypersensitive response and cell death as a defense response against pathogen attack (Klessig *et al*. 2000). At the higher concentrations of H_2O_2 (≥ 500 mM), the apparent toxicity in the treated plants might be due to the direct exposure of plant roots to the exogenous H_2O_2 within few days after the application or due to the extreme accumulation of the endogenous H_2O_2 in plant leaf tissues.

As a conclusion, the reduction of RKN reproduction may be attributed to the direct effect of the exogenous H_2O_2 on the nematode eggs and hatched juveniles and indirectly to the H_2O_2 effect on the treated tomato plants that could be elicited by H_2O_2 to resist the nematode infection.

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POLISH SUMMARY

WSPÓŁDZIAŁANIE MATWIKA KORZENIOWEGO *MELOIDOGYNE JAVANICA* I ROŚLIN POMIDORA WYWOŁANE NADTLENKIEM WODORU

Badano wpływ nadtlenku wodoru (H_2O_2) na porażenie korzeni pomidora mątwikiem *Meloidogyne javanica.* W tym celu nasączono ziemię roztworami nadtlenku wodoru o zróżnicowanym stężeniu (1, 10, 100, 250, 500, 750 i 1 000 mM H_2O_2) w trzech terminach: T1 – 24 h przed inokulacją patogenem; T2 – w czasie inokulacji; T3 – 24 h po inokulacji. We wszystkich traktowanych kombinacjach uzyskano istotne zmniejszenie reprodukcji mątwika (jaja/g świeżej masy korzeni) w porównaniu do nietraktowanej kombinacji kontrolnej. Najniższy stopień zmniejszenia reprodukcji nicienia stwierdzono przy użyciu stężenia 10 mM H_2O_2. Przy stosowaniu stężeń 250, 750 i 1 000 mM H_2O_2 w terminach T1 i T2 wystąpiło istotnie wyższe ograniczenie tempa reprodukcji szkodnika, niż w terminie T3. Zawartość endogennego H_2O_2 w traktowanych roślinach była istotnie niższa niż w roślinach nietraktowanych. W przypadku wyższych stężeń (≥ 500 mM H_2O_2) stwierdzono na traktowanych roślinach fitotoksyczność, co było wynikiem nagromadzenia endogennego H_2O_2. W kombinacjach za stężeniami 1 i 10 mM H_2O_2 rośliny nie różniły się pod względem zawartości chlorofilu od roślin w kombinacji kontrolnej, podczas gdy przy użyciu stężeń wyższych zawartość chlorofilu była istotnie niższa. Stwierdzono, że zewnętrzne zastosowanie H_2O_2 może wywierać bezpośrednie działanie na reprodukcję *M. javanica,* oraz pośrednie działanie na rośliny pomidora, które stają się mniej wrażliwe na zakażenie tym patogenem.