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Full Length Research Paper

Effect of root-knot nematode and two species of crown gall on antioxidant activity of grape leaves

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***Agrobacterium tumefaciens* and *Agrobacterium vitis* significantly reduced the entire criteria (galls, embedded stages, final population, Pf/Pi and egg production) of the root-knot nematode, *Meloidogyne incognita*, when they were in concomitance with grape (*Vitis vinifera* var) superior roots. Greater suppression in such criteria was observed with high inoculum levels (5X10^7 cfu/pot) than with lower (2.5X10^7 cfu/pot) ones. All treatments resulted in the increase of leaves contents of H_2O_2 and lipid peroxidation (TBARS) dramatically, which were considered the most damaging stresses in plant cells. The contents of AsA, GSH, TPH and PAL specific activity increased as a strongly antioxidant defense compound against induced oxidative damage. In addition, the increase in the activity of various antioxidant defense specific enzymes (SOD, APX, CAT and GST) represented the protective activity used to counteract the oxidative injury promoted by nematode and nematode-bacteria infections. The rate of the chemical increase was significantly higher in interaction treatments than in the leaves of plants treated singly with *M. incognita*. Moreover, higher inoculum levels resulted in higher values of the measured chemicals. Symptoms appearance at low levels of nematode and bacterial treatments were significantly preceded by significant induction of AsA, GSH and TPH contents, and APX, CAT, SOD, GST and PAL activities in grape leaves. However, under high levels of nematode and bacteria, an obvious depletion at all non-antioxidants enzymes' levels and antioxidants enzymes' activities was observed. It is supposed that stimulated antioxidative processes contributed to the suppression of necrotic symptom development in grape leaves depending on the level of pathogen inoculum.**

Key words: *Meloidogyne incognita*, grape, crown gall, *Agrobacterium tumefaciens*, *Agrobacterium vitis*, antioxidant activity.

INTRODUCTION

The crown gall bacteria, *Agrobacterium tumefaciens* and *Agrobacterium vitis*, enhanced gall production of the root-knot nematode, *Meloidogyne incognita*, on host plant roots. Damage and interactions of these pathogens was reported by El-Sherif and El-Wakil (1991) on *Lycopersicon*; Sule et al. (1995), Al-Sayed et al. (1999), Anwar et al. (2000), Anwar and Mckenry (2001) and Eastwall et al. (2006) on *Vitis* varities; Karimi et al. (2000) on *Arabidopsis*; Giri et al. (2001) on *Artemisia*; and Rubio-Cabetas et al. (2001) on *Prunus*.

Exposure of plants to certain environmental stresses leads to the generation of reactive oxygen species (ROS), including superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Injury caused by ROS, known as oxidative stress, is one of the major damaging factors in plants exposed to environmental stresses such as pathogens (Mehdy, 1994). The rapid production and accumulation of reactive oxygen species (ROS), the oxidative burst, has been shown to occur in a variety of plant/bacteria or nematode (Low and Merida, 1996). ROS are cytotoxic and can seriously disrupt normal metabolism through oxidative damage to lipids (Fridovich, 1986; McKersie and Leshem, 1994), nucleic acids and protein (Imlay and Linn, 1988; Davis, 1987), resulting

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Abbreviations: TBARS, AsA, GSH, TPH, PAL, SOD, APX, CAT, GST, etc.

in mutation, protein destruction and peroxidation of membrane lipids, respectively.

Plants have evolved a wide range of enzymatic and non-enzymatic mechanisms to scavenge ROS. Different non-enzymatic [ascorbate (AsA), glutathione (GSH), polyamines, α-tocopherol, and carotenoids] and enzymatic [superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione-S-transferase (GST), glutathione reductase (GR), and catalase (CAT)] molecules are involved in scavenging excess ROS in plant cells (Yoshimura et al., 2004). Among all the antioxidative enzymes, superoxide dismutase (SOD) and ascorbate peroxidase (APX) play key roles in ROS detoxification in cells. Some GSTs presumably function to protect the cell from oxidative damage by quenching reactive molecules with the addition of GSH (Mc Gonigle et al., 2000). Certain plant GSTs have secondary activities as glutathione peroxidase (GPx) and are purported to be able to protect cells from cytotoxicity by reducing organic hydroperoxides to their corresponding less toxic alcohols (Cummins et al., 1999). These enzymes play a crucial role in the protection of the plant cell from oxidative damage at the sites of enhanced ROS generation (Soylu et al., 2003; Kuzniak and Sklodowska, 2001). Phenolic compounds have been shown to protect the plant cell wall during times of UV, salt, or pathogenic stress (Shetty et al., 1996). Plant stress response involves the synthesis of several secondary metabolites of the phenylpropanoid pathway. Phenolics are intermediates in the phenylpropanoid pathway and they play important roles in flavanoid production and lignin biosynthesis (Shetty et al., 1996; Lewis et al., 1998). Phenolic alcohols are cross-linked into the cell wall matrix by the activity of peroxidase enzymes (Brunow et al., 1998). Peroxidases are free radical scavengers that utilize hydrogen peroxide as a substrate during the cross-linking of mono- and dilignols. Peroxidase activity can be induced under stress to accommodate lignin biosynthesis and other stress response pathways (McDougall, 1991; Ebermann and Pichorner, 1989); thus, some indicators of stress response can be measured using biochemical assays for total phenolics and peroxidase enzyme activity. The activation of PAL activity is a common response of plant cells to biotic and abiotic stresses and may also function as antioxidants because of their free-radical trapping properties (Haslam, 1998).

The aim of this study was to investigate the interaction between root-knot nematode, *Meloidogyne incoginta* and *A. tumefaciens* or *A. vitis*, on grape and their effect on antioxidants and enzyme activity in plant leaves as defense mechanisms against pathogens.

MATERIALS AND METHODS

Source of nematode

Pure culture of the root-knot nematode, *M. incognita*, was obtained from isolates belonging to the Nematology Research Center, Faculty of Agriculture, Cairo University. *M. incognita* was propagated on eggplant cv. Classic plants grown in 20 cm diameter clay pots filled with sterilized loamy soil. The culture was examined and periodically renewed in order to ensure continuous supplies of inocula for the experimental work.

Source of bacteria

A. tumefacien*s* and *A.* vitis, used as crown gall pathogens, were isolated from a viticulture farm located at 60 km Cairo-Alexandria desert road and identified at Plant Pathology Department, Faculty of Agriculture, Cairo University. The inocula of each species were prepared separately from a sterilized suspension of a single colony where it was placed in a number of Erlenmeyer flasks containing nutrient glycerol medium and was shaken at 50 rpm for 24 h at 27°C, after which the contents of the flasks were centrifuged at 10,000× *g*. The supernatant was discarded and the pellets were suspended in sterilized water. Concentrations of 2.5 x 10^7 and 5 x 10^7 CFU/pot of each bacterial species were prepared and used as inocula.

Glasshouse experiments

One year old seedlings of grape cultivar (Superior) with uniform size were obtained from Grape Department, Horticulture Research Institute, Agriculture Research Center and were cultivated singly in 20 cm diameter clay pots filled with steam sterilized sandy loam soil (1:1, v/v). After adaptation, seedlings were divided into two groups. The first (25 seedlings) was inoculated with 2000 J_2 of *M. incognita*/pot (M1) and the second (25 seedlings) was inoculated with 4000 J_2 of *M. incognita*/pot (M2) by pipetting the nematode water suspension into 4 holes around the root system which was immediately covered with soil.

One week after inoculation, two species of *Agrobacterium* [*tumefaciens* (A) and *vitis* (B)] were applied separately at two levels (A1 or B1 = 2.5 x 10^7 and A2 or B2 = 5 x 10^7 CFU/pot) by pipetting the inocula suspension into four holes around the root system which was immediately covered with soil. Every single treatment was replicated five times, while five seedlings were kept without any treatment as healthy check. All treatments were arranged in a fully randomized design on a clean bench in the glasshouse at 32±5°C and received similar horticultural treatments. After one year, the soil population was extracted as described by Hooper et al. (2005) and was counted using a Hawksley counting slide, under a binocular microscope. A subsample (5 g) of roots from each plant was stained and the gall numbers and embedded stages (developmental stages + eggmasses) per root were calculated, after which the final population (embedded stages + nematodes in soil), nematode build up (Pf/Pi) and average of eggs/eggmass were estimated.

Plant chemical analysis

Samples of fresh leaves of each treatment were chemically analyzed as follows:

Preparation of enzyme extracts

Samples of grape leaves (1 g) were homogenized in 3 ml of 50 mM phosphate buffer pH 7.0 containing 0.1 N NaCl, 1% PVP (Sigma) and 1 mM ascorbate (Sigma) at 4°C. After centrifugation at 15,000 × *g* for 15 min, the supernatant was collected.

Assay of protein content

Protein was determined by the method of Bradford (1976) with standard curves prepared using bovine serum albumin.

Determination of oxidative burst

Lipid peroxidation (TBARS contents)

Lipid peroxidation products were estimated by the formation of thiobarbaturic acid reactive substances (TBARS) and quantified in terms of malondialdhyde (MDA) as described by Haraguchi et al. (1995), after which 0.5 g ground leaves were homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA), followed by centrifugation at 12,000 × *g* for 20 min. The supernatant (1 ml) obtained was mixed with an equal volume of TCA (10%) containing 0.5% (w/v) TBARS or no TBARS as blank, and heated at 95 1°C for 30 min and then cooled in ice. The reaction product was centrifuged at 12,000 × g for 15 min and the supernatant absorbance was measured at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA concentration was determined by its molar extinction coefficient of 155 mM^{-1} cm^{-1} and the results were expressed as µ mol/g f.w.

Assay of hydrogen peroxide concentration

Hydrogen peroxide was measured by the method described by Capaldi and Taylor (1983) with a slight modification. The ground leaves in 5% TCA (2.5 ml per 0.5 g powder) with 50 mg active charcoal at 0°C were centrifuged for 10 min at 15,000 × *g*. The supernatant was collected, neutralized with 4 N KOH to pH 3.6 and used for H_2O_2 assay. The reaction mixture contained 200 µl of leaf extract and 100 µl of 3.4 mM 3-methylbenzothiazoline hydrazone (MBTH). It was initiated by adding 500 µl of horseradish peroxidase solution (90 U per 100 ml) in 0.2 M sodium acetate (pH 3.6). 2 min later, 1400 µl of 1 N HCl was added and the absorbance was read at 630 nm after 15 min.

Determination of total glutathione

The ground leaves (0.5 g) were added to 2 ml ice-cold 5% (w/v) sulphosalicylic acid solution and the mixture was centrifuged for 30 min at 10,000 × *g*, after which the supernatants were collected and immediately assayed. Subsequently, glutathione was measured with Ellman's reagent (Silber et al., 1992) and 300 ml of the supernatant was mixed with 1.2 ml of 0.1 M phosphate buffer solution (pH 7.6). After a stable absorbance, a reading of 412 nm was obtained, while 25 mM 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added and the increase in absorbance at 412 nm was monitored ($\Sigma 412$ = 13.6 mM^{-1} cm^{-1}). However, GSH was used as a standard.

Total ascorbate (AsA) determination

Levels of AsA followed the procedure described by Singh et al. (2006) with few modifications. A fresh leaf sample of known weight (1 g) was briefly extracted with 3 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 18,000 × *g* for 15 min. AsA was determined in a reaction mixture consisting of 0.2 ml of the supernatant, 0.5 ml of 150 mM phosphate buffer (pH 7.4, containing 5 mM EDTA) and 0.2 ml of deionized water. Colour was developed in the reaction mixtures with the addition of 0.4 ml of 10% (w/v) TCA, 0.4 ml of 44% (v/v) phosphoric acid, 0.4 ml of α,α- dipyridyl in 70% (v/v) ethanol and 0.2 ml of 3% (w/v) $FeCl_3$. The reaction mixtures were incubated at 40°C for 40 min, and the absorbance was read at 532 nm.

Assay of total phenols (TPH)

The total phenols assay was conducted as per the method of Zieslin and Ben-zaken (1993). The samples were homogenized at the rate of 0.1 g per 1 ml of 80% methanol and the methanolic extract was kept in a water bath at 70°C for 15 min with frequent agitation. 1 ml of methanolic extract was added to 5 ml of distilled water and 250 ml of Folin-Ciocalteau reagent (1 N) was added and the solution was kept at 25°C for 30 min. Finally, 1 ml of saturated solution of Na_2CO_3 and 1 ml of distilled water was added and the reaction mixture was incubated for 1 h at 25°C. After the blue color development, the absorbance was recorded at 725 nm. Total phenols were determined with the use of an external standard curve and expressed as mg gallic acid/g fresh weight of samples.

Determination of specific activities of the antioxidant defense enzymes

Assay of SOD specific activity

The specific activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of Beauchamp and Fridovich (1971). The 3 ml reaction mixture contained 50 mM phosphate buffer pH 7.8, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 1.0 mM EDTA and 20 µl enzyme extract. Riboflavin was added last to the mixture and the reaction was initiated by placing the tubes 30 cm below 15 W fluorescent lamps. The reaction was started by switching on the light and was allowed to run for 10 min. Switching off the light stopped the reaction and the tubes were covered with black cloth. Moreover, non-illuminated tubes served as control and the absorbance at 560 nm was read. The volume of enzyme extract corresponding to 50% inhibition of the reaction was considered as one enzyme unit.

Assay of ascorbate peroxidase (APX) specific activity

The specific activity of APX was measured by estimating the rate of ascorbate oxidation (extinction coefficient; 2.8 mM^{-1} cm^{-1}). The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable aliquot of the enzyme extract. The change in absorbance was monitored at 290 nm (Nakano and Asada, 1981) and the enzyme specific activity was expressed as units min^{-1} mg^{-1} protein.

Assay of catalase specific activity

For measurement of the catalase (CAT) specific activity, the method of Aebi (1983) was used. The 3 ml reaction mixture comprised 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and a suitable aliquot of enzyme. Decrease in the absorbance was taken at 240 nm (the molar extinction coefficient of H_2O_2 is 0.04 mM^{-1} cm^{-1}). Enzyme specific activity was expressed as units min^{-1} mg^{-1} protein.

Assay of glutathione-s-transferase specific activity

Glutathione-S-transferase (GST) was assayed by using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate in absorbance at 334 nm

according to Li et al. (1995). The reaction mixture contained 68 mM potassium phosphate buffer at pH 7.0, freshly prepared 10 mM 1-chloro-2,4-dinitrobenzene (CDNB), freshly prepared 10 mM glutathione (GSH) and enzyme extract. The enzyme activity was expressed by the amount of enzyme that catalyses the formation of 1 µmol of S-2,4-dinitrophenylglutathione min^{-1} and was expressed as units min^{-1} mg^{-1} protein.

Assay of phenylalanine ammonia lyase (PAL) specific activity

Phenylalanine ammonia lyase specific activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm as described by Dickerson et al. (1984). A known weight of samples was homogenized in 5 ml of 0.1 M sodium borate buffer, at pH 7.0 containing 0.1 g of insoluble polyvinyl pyrolidone (PVP). The homogenate was centrifuged at 15000 *g* for 20 min, while the supernatant was used as the enzyme source for assay. Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer at pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The reaction was held by adding 0.5 ml of 1 M trichloroacetic acid and incubated at 37°C for 5 min. The blank contained 0.4 ml of crude enzyme extract and 2.7 ml of 0.1 M borate buffer (pH 8.8), and the absorbance was measured at 290 nm using an extinction coefficient of 9630 per min cm 71 for trans-cinnamic acid in 0.1 M borate buffer (pH 8.8). The absorbance of 9630 was equal to 1 mol/1 min or the absorbance itself was 0.963, and the product formed was 100 nmol/ml/min. The enzyme activity was expressed based on the amount of the fresh weight of trans-cinnamic acid. However, enzyme specific activity was expressed as units min^{-1} mg^{-1} protein.

Statistical analysis

Data were compared by Duncan's Multiple Range Test (DMRT) at the 5% level of probability using MSTAT version 4 (1987).

RESULTS

Effect of *A. tumefaciens* or *A. vitis* treatments on *M. incognita* reproductivity and plant response

The data in Table 1 indicated that all nematode criteria (gall formation, embedded stages, final population, nematode build up, and egg production) were significantly reduced in all nematode-bacteria co-inhabited treatments when compared with the control (nematode only). High levels of both bacterial species were more suppressive to nematode development and reproduction. Moreover, *A. tumefaciens* was more effective and significantly reduced by *M. incognita* galling, maturity and reproductivity than *A. vitis* either at low or high levels of bacterial inoculations.

Visual examination of roots for xylem sap demonstrated that the bacteria became systemic in plant vessels. Most of the feeder roots were found dead at points of secondary root branching, while dark lesions were also observed in all nematode-bacteria co-inhabitant treatments. In many cases, the infection stopped the growth of the main or secondary roots.

Effect of *M. incognita* and *A. tumefaciens* or *A. vitis* treatments on grape leaves contents of lipid peroxidation (TBARS) and H_2O_2

Results showed that the contents of both lipid peroxidation (TBARS) and H_2O_2 were significantly increased in all treatments against healthy plants. It is known that infection leads to the generation of superoxide radicals and H_2O_2 in plant tissues which generate oxidative burst. H_2O_2 content showed marked induction after the treatments by *M. incognita* (low and high levels) or double treatments by *M. incognita* and *Agrobacterium* species (*tumefaciens* or *vitis*) at lower or higher levels (Table 2). In addition, significant accumulation of TBARS was observed in infected plants by different levels of both *M. incognita* and *Agrobacterium* species. These results clearly indicated that production of TBARS correlated with the generation of ROS caused by pathogen infections or other abiotic stresses.

Effect of *M. incognita* and *A. tumefaciens* or *A. vitis* treatments on grape leaves contents of GSH, TAA and TPH

Data in Table 2 indicated that *M. incognita* at two levels (M1 and M2), that is, low levels of *M. incognita* and two *Agrobacterium* species (M1A1 and M1B1), significantly enhanced the contents of non-enzymatic antioxidant [total glutathione (GSH), ascorbate (AsA) and total phenols (TPH)] while M1A2, M2A1, M1B2 and M2B1 showed an obvious increase in GSH, AsA and TPH as compared to the healthy plants; they showed low levels than nematode infections only, but the low levels of nematode coincided with the two *Agrobacterium* species infections. In contrast, high levels of nematode and both bacterial species (M2A2 and M2B2) caused dramatic inhibition in non-enzymatic antioxidant contents.

Effect of *M. incognita* and *A. tumefaciens* or *A. vitis* treatments on the specific activities of antioxidant enzymes (APX, SOD and CAT), GST and PAL in infected grape leaves

M. *i*ncognita at two levels (M1 and M2), and at low levels of nematode and bacteria (M1A1 and M1B1) significantly elevated the activities of antioxidant enzymes (APX, SOD and CAT), GST and PAL (Table 3). Other treatments of M1A2, M2A1, M1B2 and M2B1 resulted in variable induction of enzymes activities against healthy plants, while the high levels of M2A2 and M2B2 led to significant inhibition in all enzymes' specific activities.

DISCUSSION

The results of the study indicate that *A. tumefaciens* and

Table 1. Effect of *A. tumefaciens* or *A. vitis* on *M. incognita* reproductivity of grape roots.

Treatment	Dose/pot	Gall	Embedded Stage (D.S .+ Eggmasse)	Final population (E.S. + In soil)	Pf/Pi	Egg/eggmass
M. incoginta (M1)	2000 J_2	522^{C}	1101^{b}	10501	5.25^{b}	174^{b}
M. incoginta (M2)	4000 J_2	648^{a}	1445^{a}	32115	8.03^{a}	216^{a}
M. incoginta + *A. tumefaciens* (M1A1)	2000 J_2 + 2.5 x 10^7cfu	313^{h}	551^{h}	6181	3.09^{e}	105^{f}
M. incoginta + *A. tumefaciens* (M1A2)	2000 J_2 + 5 x 10^7cfu	209^{j}	301^{j}	4061	2.03^{h}	78^{h}
M. incoginta + *A. tumefaciens* (M2A1)	4000 J_2 + 2.5 x 10^7cfu	389^{f}	716^{f}	7716	1.93^{i}	129^{c}
M. incoginta + *A. tumefaciens* (M2A2)	4000 J_2 + 5 x 10^7cfu	259^{i}	453^{i}	5118	1.28^{j}	97^{g}
M. incoginta + *A. vitis* (M1B1)	2000 J_2 + 2.5 x 10^7cfu	470^{d}	776^{e}	9236	4.62^{c}	122^{d}
M. incoginta + *A. vitis* (M1B2)	2000 J_2 + 5 x 10^7cfu	365^{g}	628^{g}	7198	3.60^{d}	94^{g}
M. incoginta + *A. vitis* (M2B1)	4000 J_2 + 2.5 x 10^7cfu	583^{b}	974^{c}	11464	2.87^{f}	114^{e}
M. incoginta + *A. vitis* (M2B2)	4000 J_2 + 5 x 10^7cfu	454^{e}	787^{d}	8957	2.24^{g}	97^{g}

Means followed by the same letter(s) within a column in each block are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.
D.S. = Developmental stages; E.S. = embedded stages.

Table 2. Effect of *M. incognita* and *A. tumefaciens* or *A. vitis* on grape leaves contents of lipid peroxidation (TBARS), hydrogen peroxide (H_2O_2), total glutathione (GSH), total ascorbate (ASA) and total phenols (TPH).

Treatment	Dose/pot	TBARS (µ mol/g FW)	H_2O_2 (µ mol/g FW)	GSH (µ mol/g FW)	AsA (mg/g FW)	TPH (mg/g FW)
Healthy	-	1.55^{k}	62.12^{k}	2.48^{i}	3.43^{g}	1.43^{i}
M. incoginta (M1)	2000 J_2	4.46^{j}	125.35^{j}	6.22^{f}	5.89^{f}	3.63^{h}
M. incoginta (M2)	4000 J_2	6.32^{i}	154.06^{i}	8.78^{e}	8.75^{e}	4.78^{f}
M. incoginta + *A. tumefaciens* (M1A1)	2000 J_2 + 2.5 x 10^7 cfu	10.78^{g}	190.23^{g}	15.34^{a}	14.26^{a}	7.82^{b}
M. incoginta + *A. tumefaciens* (M1A2)	2000 J_2 + 5 x 10^7 cfu	15.53^{c}	228.13^{c}	10.82^{d}	11.87^{c}	6.11^{d}
M. incoginta + *A. tumefaciens* (M2A1)	4000 J_2 + 2.5 x 10^7 cfu	12.28^{e}	201.43^{e}	11.36^{c}	12.47^{b}	8.24^{a}
M. incoginta + *A. tumefaciens* (M2A2)	4000 J_2 + 5 x 10^7 cfu	22.67^{a}	250.71^{a}	3.09^{h}	3.05^{h}	4.08^{g}
M. incoginta + *A. vitis* (M1B1)	2000 J_2 + 2.5 x 10^7 cfu	9.88^{h}	187.36^{h}	14.94^{b}	12.89^{b}	6.94^{c}
M. incoginta + *A. vitis* (M1B2)	2000 J_2 + 5 x 10^7 cfu	14.21^{d}	219.04^{d}	10.64^{d}	10.04^{d}	5.76^{e}
M. incoginta + *A. vitis* (M2B1)	4000 J_2 + 2.5 x 10^7 cfu	11.58^{f}	197.31^{f}	11.85^{c}	12.27^{b}	7.65^{b}
M. incoginta + *A. vitis* (M2B2)	4000 J_2 + 5 x 10^7 cfu	20.32^{b}	243.52^{b}	4.49^{g}	3.28^{g}	4.13^{g}

Means followed by the same letter(s) within a column in each block are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Table 3. Effect of *M. incognita* and *A. tumefaciens* or *A. vitis* on the specific activities of Ascorbate peroxidase (APX), Superoxide dismutase (SOD), Catalase (CAT), Glutathione-s-transferase (GST) and Phenylalanine ammonia lyase (PAL) in infected grape leaves.

Treatment	Dose/pot	APX (unit/mg protein)	SOD (unit/mg protein)	CAT (unit/mg protein)	GST (unit/mg protein)	PAL (unit/mg protein)
Healthy	-	6.21h	85.04^{i}	18.37^{i}	0.52^{j}	4.39^{h}
M. incoginta (M1)	2000 J_2	12.64^{d}	115.08^{d}	33.73^{f}	2.16^{i}	6.38^{g}
M. incoginta (M2)	4000 J_2	18.04^{c}	146.19^{c}	54.09^{c}	4.74^{f}	11.56^{c}
M. incoginta + *A. tumefaciens* (M1A1)	2000 J_2 + 2.5 x 10^7 cfu	25.43^{a}	167.07^{a}	68.16^{a}	6.10^{e}	12.92^{a}
M. incoginta + *A. tumefaciens* (M1A2)	2000 J_2 + 5 x 10^7 cfu	10.31^{g}	100.15^{h}	27.51^{h}	8.89^{a}	6.24^{g}
M. incoginta + *A. tumefaciens* (M2A1)	4000 J_2 + 2.5 x 10^7 cfu	12.41^{e}	111.03^{f}	36.16^{d}	8.06^{c}	7.41^{d}
M. incoginta + *A. tumefaciens* (M2A2)	4000 J_2 + 5 x 10^7 cfu	4.06^{j}	69.16^{k}	14.63^{k}	2.68^{h}	3.05^{j}
M. incoginta + *A. vitis* (M1B1)	2000 J_2 + 2.5 x 10^7 cfu	23.18^{b}	160.17^{b}	64.12^{b}	7.55^{d}	12.59^{b}
M. incoginta + *A. vitis* (M1B2)	2000 J_2 + 5 x 10^7 cfu	12.42^{e}	113.11^{e}	29.41^{g}	8.72^{b}	6.61^{f}
M. incoginta + *A. vitis* (M2B1)	4000 J_2 + 2.5 x 10^7 cfu	11.81^{f}	108.06^{g}	35.09^{e}	8.67^{b}	7.04^{e}
M. incoginta + *A. vitis* (M2B2)	4000 J_2 + 5 x 10^7 cfu	4.73^{i}	70.45^{j}	15.02^{j}	2.99^{g}	3.60^{i}

Means followed by the same letter(s) within a column in each block are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

A. vitis significantly precluded *M. incognita* development and reproduction. Such preclusion was more pronounced with *A. tumefaciens* treatments than *A. vitis* and with high inocula than the lower ones. Many soil bacteria produce toxic metabolites to nematodes (Zavalenta, 1985). Other factors such as levels of bacterial inoculation, bacterial species and competitive conditions for space and feeding sites may also be involved (Sule et al., 1995; Karimi et al., 2000; Rubio-Cabetas et al., 2001; Eastwell et al., 2006). Our results are inconsistent with those of El-Sherif and El-Wakil (1991) who found that *A. tumefaciens* enhanced *M. incognita* gall formation, final population and build up. Such contradiction may be due to many factors, that is, pathogen strain or inoculation level, host type or susceptibility, method of application, soil type or other environmental factors. Generally, crown gall bacteria can live for years within the vascular system of infected vines without any outward expression of disease (Eastwell et al., 2006).

The toxic effects of many environmental stresses such as salt stress, Fe deficiency, cadmium stress, lead toxicity, ionizing radiation insecticide, organisms and micro-organisms (El-Beltagi et al., 2008, 2010, 2011; Salama et al., 2009; Mohamed et al., 2009; Shehab et al., 2010; El-Beltagi and Mohamed, 2010; Aly and El-Beltagi, 2010; Afify and El-Beltagi, 2011; Afify et al., 2011) are partially due to generation of ROS which is considered as the most damaging stress in plants. It attacks cellular macromolecules, causes membrane damage, and affects protein synthesis and stability. Plants respond to pathogen attack by inducing oxidative burst and increasing ROS production, which gives rise to an oxidative environment in the cells (Foyer et al., 1994). Thus, H_2O_2 plays a key role in the orchestration of a localized hypersensitive response during the expression of plant disease resistance (Levine et al., 1994). The results show higher induction in H_2O_2 content after the different treatments of nematode and *Agrobacterium* sp. These results are similar with those of Waetzig et al. (1999) and Parrott et al. (2002) who found that *Agrobacterium* and nematode treatments increased the production of H_2O_2 in cells of *Arabidopsis thaliana* and wheat which resulted in plant cell death.

Lipid peroxidation can be assessed by monitoring the formation of TBARS and the decomposition product of polyunsaturated fatty acids (PUFAs), which are the main components of membrane lipids. TBARS formation in plants exposed to adverse environmental conditions is a reliable indicator of cellular free-radical generation. TBARS measurements have also been used routinely in stressed plant samples as an indicator of lipid peroxidation caused by oxidative stress (Ahsan et al., 2007; El-Beltagi et al., 2008). Lipid peroxidation induced by ROS may be one of the mechanisms accounting for cell death (Jabs, 1999). In this study, cellular TBARS concentration was measured as one of the first consequences of oxidative stress, and also as an indicator of

cellular damage. Thus, the results of this study are in agreement with these facts, which show that when comparing healthy plants, nematode and *Agrobacterium* sp. infections enhanced the content of lipid peroxidation and (TBARS) in grape leaves. In this study, infection with nematode only at both levels of inoculation (M1 and M2) and at lower levels of both nematode *Agrobacterium* species (M1A1 and M1B1) resulted in increasing the entire measured leaf parameters of antioxidants and antioxidative enzymes to maximum concentrations. However, other nematode and *Agrobacterium* infections (M1A2, M2A1, M1B2 and M2B1) showed less induction against healthy plants.

Plant leaves detoxify ROS by a combination of antioxidants, such as ascorbate (AsA), glutathione (GSH), phenols and α-tocopherol as well as antioxidative enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). Antioxidative enzymes involved in the ascorbate-glutathione (AsA–GSH) pathway, mainly monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR), are considered to be of paramount importance in plant antioxidant defense mechanism (Potters et al., 2002).

The induction of the contents of GSH and AsA may be due to its important role in scavenging different types of ROS at AsA–GSH pathway (Foyer et al., 1994). GSH serves as the main storage form of reduced sulfur and plays an important role in the control of the thiol-disulfide status of the cell, in the detoxification of xenobiotics and finally in plant responses to abiotic stress and pathogens (Jimenez et al., 1997). AsA is a major antioxidant reacting directly with hydroxyl radicals, superoxide anion, and singlet oxygen (Noctor and Foyer, 1998). In addition to AsA importance as an antioxidant, it is also a cofactor of many enzymes (Smirnoff and Wheeler, 2000), a regulator of cell division and growth and a molecule for signal transduction in plants. On the other hand, the significant increase in total soluble phenols (TPH) contents may strongly affect the antioxidant natural products induced under oxidative stress condition in controlling the oxidative damage after pathogen infection (Bolwell et al., 1985). It is synthesized during secondary metabolism and has many functions in plant stress response. The activation of phenolic metabolism is known to be an important phenomenon involved in restricting pathogen growth and enhancing plant host cell survival under stress conditions (Benhamou and Nicole, 1999).

The significant increase of antioxidant enzyme activities such as APX, SOD and CAT, GST and PAL may be driven by enhancing TBARS, ROS and H_2O_2 formation in either single nematode or low and moderate nematode and *Agrobacterium* infections. Oxidative burst has been shown to induce the expression of a variety of defense genes (Levine et al., 1994; Baker and Orlandi, 1995). To evade the potential damaging effects of ROS, cells have evolved with protection mechanisms, including antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), and low-molecular-weight antioxidants such as ascorbate (AsA), glutathione (GSH) and total phenols (TPH) (Foyer et al., 1994). SOD catalyses the dismutation of superoxide to hydrogen peroxide and oxygen. However, hydrogen peroxide is also toxic to cells and has to be further detoxified by CAT and/or APX to water and oxygen. The antioxidant enzymes' activities play an important role in scavenging ROS and therefore their improvement could increase the ability of plants to tolerate ROS stress and delay its senescence (Alscher et al., 2002). These findings suggest that genes encoding antioxidative enzymes can be activated by the increased production of active oxygen derivatives in nematode and *Agrobacterium*-infected plants. It is possible that these antioxidative processes protect plant tissues from lipid peroxidation processes around the infection sites, thereby protecting them from the spread of necrotic lesions (Levine et al., 1994).

Obviously, the activation of antioxidative defense systems in plants by abiotic and biotic stresses is a general phenomenon and probably contributes to increased resistance against a subsequent stress. Our results are in agreement with Perl et al. (1996) who observed that elevated levels of antioxidant enzymes' specific activity in grape tissues correlated with *Agrobacterium*-induced necrosis in the host tissues during *Agrobacterium*-mediated transformation, and with those of Zheng et al. (2004), Wegener and Olsen (2004) and Wuyts et al. (2006a) who observed an obvious induction PAL activity in grape, potatoes' cells and banana roots after *Agrobacterium* and nematode infections. It was shown that significant enhancement in PAL activity in *Arabidopsis* and tobacco plants improved resistance against fungal and nematode infection (Howels et al., 1996; Shadel et al., 2003; Wuyts et al., 2006b). Therefore, it was used as indicators of plant resistance to pathogen infection.

In contrast, the higher infection levels with nematode and *Agrobacterium* (M2A2 and M2B2) led to significant inhibition of the contents of non-enzymatic antioxidants and antioxidant enzyme activities. Such results are in agreement with Parrott et al. (2002) who reported that after *Agrobacterium* infection, wheat cells rapidly produced hydrogen peroxide (H_2O_2), displayed altered cell wall composition and resulted in higher levels of cellular necrosis and subsequent cell death. Increased superoxide concentration was also associated with higher lipid peroxidation and membrane permeability (Dalal and Khanna-Chopra, 1999), which were the common symptoms of oxidative stress. The accumulation of superoxide anion preceding necrotic lesion formation during hypersensitive response and induction of tumor formation by wild type *A. tumefaciens* was observed by Jabs et al. (1996) and Jia et al. (1996). The decline in CAT activity

could be an important factor in the establishment of necrosis in tobacco leaves. CAT was essential for the protection of ascorbate and glutathione pool from oxidetion in maintaining the redox balance in cells (Willekens et al., 1997; Dalal and Khanna-Chopra, 2001). Thus, availability of ascorbate and reduced glutathione may become a rate-limiting step during antioxidant defense (Foyer et al., 1994).

Conclusions

A. tumefaciens and *A*. vitis significantly reduced the entire criteria (galls, embedded stages, final population, Pf/Pi and egg production) of the root-knot nematode, *M. incognita*, when they were in concomitance with grape, *Vitis vinifera* var. superior roots. All treatments resulted in increasing the H_2O_2 and lipid peroxidation contents in leaves (TBARS) dramatically, which were considered the most damaging stresses in plant cells. The contents of AsA, GSH, TPH and PAL specific activity increased as strong antioxidant defense compounds against induced oxidative damage. In addition, the increase in the specific activity of various antioxidant defense enzymes (SOD, APX, CAT and GST) represents the protective activity to counteract the oxidative injury promoted by nematode and nematode-bacterial infections. The rate of the chemical increase was significantly higher in interaction treatments than in leaves of plants treated singly with *M. incognita*. Moreover, higher inoculum levels resulted in higher values of the measured chemicals.

REFERENCES

Afify AMR, El-Beltagi HS (2011). Effect of the insecticide cyanophos on liver function in adult male rats. Fresen. Environ. Bull. 20(4): 1084-1088.

Afify AMR, El-Beltagi HS, Fayed SA, Shalaby EA (2011). Acaricidal activity of successive extracts from *Syzygium cumini* L. Skeels (Pomposia) against *Tetranychus urticae* Koch. Asian Pac. J. Trop. Biomed. 1(5): 359-364.

Ahsan N, Lee DG, Lee SH, Kang KY, Lee JJ, Kim PJ (2007). Excess copper induced physiological and proteomic changes in germinating rice seeds. Chemosphere, 67: 1182-1193.

Aly AA, El-Beltagi HS (2010). Influence of ionizing irradiation on the antioxidant enzymes of *Vicia faba* L. Grasas y Aceites, 61(3): 288-294.

Al-Sayed AA, Kheir AM, El-Naggar HI, Kesba HH (1999). Varietal reaction of some grape cultivars to the infection with three plant parasitic nematode species. J. Agric. Sci. Mans. Univ. 24: 7707–7718.

Alscher RG, Erturk N, Heath LS (2002). Role of superoxide dismutases in controlling oxidative stress in plants. J. Exp. Bot. 53(372): 1331–1341.

Anwar SA, Mckenry MV (2001). Susceptible of genetically transformed Freedom grape rootstocks to *Meloidogyne arenaria* Pt. Freedom. Inter. J. Nematol. 11: 1–7.

Anwar SA, Mckenry MV, Faddoul J (2000). Productivity field populations of *Meloidogyne* spp. on grape rootstocks. J. Nematol. 32: 265–270.

Baker CJ, Orlandi EW (1995). Active oxygen in plant pathogenesis. Annu. Rev. Phytopathol. 33: 299–321.

Beauchamp C, Fridovich I (1971). Superoxide dismutase improved assays and assay applicable to acrylamide gels. Anal. Biochem. 44: 276–287.

Benhamou N, Nicole M (1999). Cell biology of plant immunization against microbial infection: the potential of induced resistance in controlling plant disease. Plant Physiol. Biochem. 37: 703–719.

Bolwell G, Robins M, Dixon R (1985). Metabolic changes in elicitor treated bean cells. Enzymic responses associated with rapid changes in wall components. Eur. J. Biochem. 148: 571-578.

Bradford MM (1976). A Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

Brunow G, Kilpelainen I, Sipila J, Syrjanen K, Karunen P, Setala H, Rummakko P (1998). Oxidative coupling of phenols and the biosynthesis of lignin. In: Lignin and Lignin Biosynthesis. Oxford: Lewis and Sarkanen. pp. 131–147.

Capaldi DJ, Taylor KE (1983). A new peroxidase color reaction: oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with its formaldehyde azine application to glucose and choline oxidases. Anal. Biochem. 129: 329–336.

Cummins I, Cole DJ, Edwards R (1999). A role for glutathione transferases functioning as glutathione peroxidases in resistance to multiple herbicides in black-grass. Plant J. 18: 285–292.

Dalal M, Khanna-Chopra R (1999). Lipid peroxidation is an early event in necrosis of wheat hybrid. Biochem. Biophys. Res. Commun. 262: 109–112.

Dalal M, Khanna-Chopra R (2001). Differential response of antioxidant enzymes in leaves of necrotic wheat hybrids and their parents. Physiol. Plant. 111: 297–304.

Davis KJA (1987). Protein damage and degradation by oxygen radicals I. General aspects, J. Biol. Chem. 262: 9895–9901.

Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL (1984). Phenylalanine ammonia-lyase and hydroxyl cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiol. Mol. Plant Pathol. 25: 111–123.

Eastwell KC, Sholberg PL, Sayler RJ (2006). Characterizing potential bacterial agents for suppression of *Rhizobium vitis*, causal agent of crown gall disease in grapevines. Crop Prot. 25: 1191-1200.

Ebermann R, Pichorner H (1989). Detection of peroxidase catalyzed phenol polymerization induced by enzymatically reduced paraquat. Phytochemistry, 28: 711–714.

El-Beltagi HS, Salama ZA, El-Hariri DM (2008). Some Biochemical Markers for Evaluation of Flax Cultivars under Salt Stress conditions. J. Nat. Fibers, 5(4): 316-330.

El-Beltagi HS, Mohamed AA, Rashed MM (2010). Response of antioxidative enzymes to cadmium stress in leaves and roots of Radish. Not. Sci. Biol. 2(4): 76-82.

El-Beltagi HS, Mohamed AA (2010). Changes in non protein thiols, some antioxidant enzymes activities and ultrastructural alterations in Radish plants (*Raphanus Sativus L*.) grown under lead toxicity. Not. Bot. Hort. Agrobot. Cluj 38(3): 76-85.

El-Beltagi HS, Ahmed OK, El-Desouky W (2011). Effect of low doses γ-irradiation on oxidative stress and secondary metabolites production of Rosemary (*Rosmarinus officinalis* L.) callus culture. Radiat. Phys. Chem. 80(9): 968-976.

El-Sherif AG, El-Wakil MA (1991). Interaction between *Meloidogyne incognita* and *Agrobacterium tumefaciens* or *Fusarium oxysporum* f. sp. *lycopersici* on Tomato. J. Nematol. 23: 239-242.

Foyer CH, Descourvieres P, Kunert KJ (1994). Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Environ. 17: 507–523.

Fridovich I (1986). Biological effects of the superoxide radical. Arch. Biochem. Biophys. 247: 1–11.

Giri A, Ravindra ST, Dhingra V, Narasu ML (2001). Influence of different strains of *Agrobacterium rhizogenes* on induction of hairy roots and artemisinin production in *Artemisia annua*. Curr. Sci. 81: 378-382.

Haraguchi H, Saito T, Okamura N, Yagi A (1995). Inhibition of lipid peroxidation and superoxide generation by diterpenoids from rosmarinus offcinalis. Planta Med. 61: 333–336.

Haslam E (1998). Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action. Cambridge University Press, Cambridge.

Hooper DJ, Hallmann J, Subbotin SA (2005). Methods for extraction,

processing and detection of plant and soil nematodes: Plant parasitic nematodes in subtropical and tropical agriculture. In: Luc M, Sikora RA, Bridge J (Eds.), Wallingford: UK, CABI Publishing. pp. 53-86.
Howles PA, Sewalt VJH, Paiva NL, Elkind Y, Bate NJ, Lamb CJ, Dixon RA (1996). Overexpression of L-phenylalanine ammonia-lyase in transgenic tobacco plants reveals control points for flux into phenylpropanoid biosynthesis. Plant Physiol. 112: 1617–1624.
Imlay JA, Linn S (1988). DNA damage and oxygen radical toxicity. Science, 240: 1302–1309.
Jabs T (1999). Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. Biochem. Pharmacol. 57: 231–245.
Jabs T, Dietrich RA, Dangl JL (1996). Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. Science, 273: 1853–1856.
Jia SR, Kumar PP, Kush A (1996). Oxidative stress in *Agrobacterium*-induced tumors on *Kalanchoe* plants. Plant J. 10: 545–551.
Jimenez A, Hernandez JA, Del Rio LA, Sevilla F (1997). Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol. 114: 275–284.
Karimi M, Van Montagu M, Gheysen G (2000). Nematodes as vectors to introduce *Agrobacterium* into plant roots. Mol. Plant Pathol. 1: 383–387.
Kuzniak E, Sklodowska M (2001). Ascorbate, glutathione and related enzymes in chloroplasts of tomato leaves infected by *Botrytis cinerea*. Plant Sci. 160: 723–731.
Levine A, Tenhaken R, Dixon R, Lamb C (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell, 79: 583–593.
Lewis NL, Davin LB, Sarkanen S (1998). Lignin and lignan biosynthesis: distinctions and reconciliations. In: Lignin and Lignin Biosynthesis. Oxford: Lewis and Sarkanen. pp. 1–27.
Li ZS, Zhen RG, Rea PA (1995). 1-chloro-2,4-dinitrobenzene elicited increase in vacuolar glutathione-S-conjugate transport activity. Plant Physiol. 109: 177–185.
Low PS, Merida JR (1996). The oxidative burst in plant defense: Function and signal transduction. Physiol. Plant. 96(3): 533–542.
McDougall GJ (1991). Cell-wall-associated peroxidase and lignification during growth of flax fibers. J. Plant Physiol. 139: 182–186.
McGonigle B, Keeler SJ, Lau SMC, Koeppe MK, O'Keefe DP (2000). A genomics approach to the comprehensive analysis of the glutathione S-transferase gene family in soybean and maize. Plant Physiol. 124: 1105–1120.
McKersie BD, Leshem Y (1994). Stress and stress coping in cultivated plants. Kluwer Academic Publishers.
Mehdy MC (1994). Active oxygen species in plant defense against pathogens. Plant Physiol. 105: 467–72.
Mohamed AA, El-Beltagi HS, Rashed MM (2009). Cadmium stress induced change in some hydrolytic enzymes, free radical formation and ultrastructural disorders in Radish plant. Electronic J. Environ, Agric. Food Chem. 8(10): 969–983.
Mstat VERSION 4 (1987). Software program for the design and analysis of agronomic research experiments. Michigan, USA, Michigan State University.
Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22: 867-880.
Noctor G, Foyer CH (1998). Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49: 249-279.
Parrott DL, Anderson AJ, Carmam JG (2002). *Agrobacterium* induces plant cell death in wheat (*Tritiicum aestivum* L.). Physiol. Mol. Plant Pathol. 60: 59-69.
Perl A, Lotan O, Abu-Abied M, Holland D (1996). Establishment of an *Agrobacterium*-mediated transformation system for grape (*Vitus vinifera* L.): the role of antioxidant during grape-*Agrobacterium* interactions. Nat. Biotechnol. 14: 624-628.
Potters G, De Gara L, Asard H, Horemans N (2002). Ascorbate and glutathione guardians of the cell cycle partners in crime. Plant Physiol. Biochem. 40: 537-548.
Rubio-Cabetas MJ, Minot JC, Voisin R, Esmenjaud D (2001). Interaction of root-knot nematodes (RKN) and the bacterium, *Agrobacterium tumefaciens* in roots of *Prunus cerasifera*: Evidence of the protective effect of the MARKN resistance genes against expression of crown gall symptoms. Eur. J. Plant Pathol. 107: 433-441.
Salama ZA, El-Beltagi HS, El-Hariri DM (2009). Effect of Fe deficiency on antioxidant system in leaves of three flax cultivars. Not. Bot. Hort. Agrobot. Cluj, 37(1): 122-128.
Shadle GL, Wesley SV, Korth KL, Chen F, Lamb C, Dixon RA (2003). Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of l-phenylalanine ammonia-lyase. Phytochemistry, 64: 153–161.
Shehab GG, Kansowa OA, El-Beltagi HS (2010). Effects of various chemical agents for alleviation of drought stress in Rice plants (*Oryza sativa* L.). Not. Bot. Hort. Agrobot. Cluj, 38(1): 139-148.
Shetty K, Carpenter TL, Kwok D, Curtis OF, Potter TL (1996). Selection of high phenolic containing clones of thyme (*Thymus ulgaris* L.) using *Pseudomonas* Spp. J Agric. Food Chem. 44: 3408–3411.
Silber R, Farber M, Papopoulos E, Nervla D, Liebes L, Bruch M, Bron R (1992). Glutathione depletion in chronic lymphocytic leukemia β-lymphocytes. Blood. 80: 2038–2040.
Singh N, Ma LQ, Srivastava M, Rathinasabapathi B (2006). Metabolic adaptations to arsenic-induced oxidative stress in *Pteris vittata* L. and *Pteris ensiformis* L. Plant Sci. 170(2): 274-282.
Smirnoff N, Wheeler GL (2000). Ascorbic acid in plants: biosynthesis and function. Critical Rev. Plant Sci. 19: 267–290.
Soylu EM, Soylu S, Baysal Ö (2003). Induction of disease resistance and antioxidant enzymes by Acibenzolaar-s-methyl against bacterial canker (*Clavibacter michiganensis* SUBSP. *michiganensis*) in tomato. J. Plant Pathol. 85(3): 175–181.
Sule S, Lehoczky J, Jenser G, Nagy P, Burr TJ (1995). Infection of grapevine roots by *Agrobacterium vitis* and *Meloidogyne hapla*. J. Phytopathol. 143: 169–171.
Waetzig GH, Sobczak M, Grundler FMW (1999). Localization of hydrogen peroxide during the defence response of *Arabidopsis thaliana* against the plant parasitic nematode *Heterodera glycines*. Nematology, 1: 681–686.
Wegener CB, Olsen O (2004). Heterologous pectate lyase isoenzymes are not different in their effects on soft rot resistance in transgenic potatoes. Physiol. Mol. Plant. Pathol. 65: 59–66.
Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, Van Montagu M, Inze D, Van Camp W (1997). Catalase is a sink for H_2O_2 and is indispensable for stress defense in C3 plants. Plant J. 16: 4806–4816.
Wuyts N, Dirk DW, Rony S (2006a). Activity of phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase in roots of banana (*Musa acuminata* AAA, cvs Grande Naine and Yangambi km5) before and after infection with *Radopholus similis*. Nematology, 8: 201–209.
Wuyts N, Lognay G, Swennen R, De Waele D (2006b). Nematode infection and reproduction in transgenic and mutant Arabidopsis and tobacco with an altered phenylpropanoid metabolism. J. Exp. Bot. 57: 2825–2835.
Yoshimura K, Miyao K, Gaber A, Takeda T, Kanaboshi H, Miyasaka H, Shigeoka S (2004). Enhancement of stress tolerance in transgenic tobacco plants overexpressing *Chlamydomonas glutathione* peroxidase in chloroplasts or cytosol. Plant J. 37: 21–33.
Zavalenta ME, Gundy SV, Van GS (1985). Volatile toxicity of *Serratia marcescens* Bizio and other bacteria on the root knot nematode *Meloidogyne incognita* (Kofoid and White) Chit wood. Revesta Mexicana de fitopatologia, 7: 188–194.
Zheng ZY, Li JY, Wang JH, Wang HM (2004). Effects of E26 agent on the expressions of defense genes *pal* and *sts* of grape suspension cells. J. Agric. Biotechnol. 12: 698–703.
Zieslin N, Ben-Zaken R (1993). Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Physiol. Mol. Plant Pathol. 31: 333–340.