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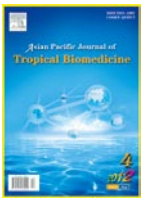

Document heading

Biochemical changes in grape rootstocks resulted from humic acid treatments in relation to nematode infection

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ABSTRACT

Objective: To investigate the effect of humic acid on nematode infected, resistant and susceptible grapes in relation to lipid peroxidation and antioxidant mechanisms on selected biochemical parameters known as proactive substances. **Methods:** The grape rootstocks, superior, superior/freedom and freedom were reacted differently to *Meloidogyne incognita* and *Rotylenchulus reniformis* according to rootstock progenitor. Two weeks after inoculation, two commercial products of humic acid were applied at the rate of (2, 4 mL or grams/plant) as soil drench. After 4 months, nematode soil populations were extracted and counted. A subsample of roots from each plant was stained and gall numbers, embedded stages per root were calculated, final population, nematode build up (Pf/Pi), average of eggs/eggmass were estimated. Subsamples of fresh root of each treatment were chemically analyzed. **Results:** Freedom reduced significantly the nematode criteria and build up. Humic acid granules appeared to be more suppressive to nematode build up on superior and the higher dose on superior/freedom than liquid treatments. On freedom, all treatments reduced significantly the nematode build up regardless to the material nature. The higher dose was more effective than the lower one. As a result of humic acid applications, the malondialdehyde (MDA) and H_2O_2 contents were significantly reduced after humic acid treatments while the antioxidant compounds glutathione (GSH), ascorbic acid (ASA) and total phenol contents were significantly increased when compared with check. Antioxidant defense enzymes ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and polyphenol oxidase (PPO) showed significant increase in their specific activities in treated plants compared with nematode treated check. **Conclusions:** Humic acid treatments improve the yield of grape by increasing the contents of antioxidant compounds and the specific activities of antioxidant enzymes.

1. Introduction

Damage of the root-knot and reniform nematode species to *Vitis vinifera* (*V. vinifera*) varieties has been extensively reported[1-3].

Plant endoparasitic nematodes, including the potato cyst nematode (*Globodera rostochiensis*), spend a major part of their life cycles being embedded in the roots of a host plant and are therefore exposed to a variety of host defense responses[4]. These responses may include generation of damaging reactive oxygen species (ROS). ROS such as superoxide anion (O_2^-), singlet oxygen, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are produced continuously as byproducts of various metabolic pathways that are localized in different cellular compartments[5-8]. However, under stressful conditions, their formation might increase to exceed the antioxidant scavenging capacity, thus creating oxidative stress by reaction and damage to all biomolecules[9]. Compared to animal parasitic nematodes, little is known about the defence proteins employed by plant parasitic nematodes. Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities have been detected in some endoparasitic nematodes but little is known about the roles of these proteins in the host-parasite interaction and none of these proteins has been characterized in detail. There is no information regarding peroxiredoxins in plant parasitic nematodes[10]. The incompatible resistant interactions to nematode infection may include many physiological defense actions, production of H_2O_2, jasmonic acids, the formation of ROS[11-13], different enzymatic and non-enzymatic glutathione and ascorbate[14],

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increase in peroxidase and polyphenol oxidase level[15], antioxidant properties, and polyphenols[16].

Crops, especially those having *Vitis champinii* as a progenitor (freedom) have been reported as resistant to different root-knot and reniform nematode species[17,18]. Growing plants supplemented with fertilizers containing humic acid improved their resistant to nematode infection[19]. Growing resistant grape varieties supplemented with humic acid were supposed to result better nematode management.

The present study aims to investigate the effect of humic acid on nematode infected, resistant and susceptible grapes in relation to lipid peroxidation and oxidant mechanisms on selected biochemical parameters known as proactive substances.

2. Materials and methods

2.1. Nematode species stock cultures

Pure cultures of the root-knot nematode, *Meloidogyne incognita* (*M. incognita*) and the reniform nematode, *Rotylenchulus reniformis* (*R. reniformis*) were obtained from isolates belonging to the Nematology Research Center, Faculty of Agriculture, Cairo University. Nematode species have been propagated separately, *i.e. M. incognita* on eggplant cv. Classic and *R. reniformis* on pigeon pea. The plants were grown in 20 cm diameter clay pots filled with sterilized loamy soil. To avoid contamination, cultures of each species were arranged separately, examined and periodically renewed in order to ensure continuous supplies of inocula for the experimental work.

2.2. Glasshouse experiments

One year old seedlings of grape rootstocks, superior, superior/freedom and freedom with uniform size were obtained from Grape Department, Horticulture Research Institute, Agriculture Research Center and cultivated singly in 20 cm diameter clay pots filled with steam sterilized sandy loam soil (1:1, v/v). One month later, 5 seedlings of each rootstock were inoculated separately with either 5 000 infective stages of *M. incognita* or *R. reniformis* by pipetting the nematode water suspension into 4 holes around the root system which was immediately covered with soil. Pots were labeled and arranged randomly on a glasshouse clean bench, receiving similar horticulture treatments. Seedlings were left out after 4 months from inoculation. Soil population was extracted by means and counted[20]. The nematode embedded stages of both species were also counted.

For testing the effect of humic acid on the root-knot nematode development and reproduction, another 5 seedlings of each rootstock were inoculated with 5 000 J_2 of *M. incognita*/pot. Two weeks after inoculation, two commercial products of humic acid (liquid and granules) were applied at the rate of (2, 4 mL or grams/plant) as soil drench. All treatments were arranged in a fully randomized design on a clean bench in the glasshouse at (32±5) ℃ receiving similar horticultural treatments. After 4 months, nematode soil populations were extracted and counted using a Hawksley counting slide, under a binocular microscope. A subsample (5 g) of roots from each plant was stained and gall numbers, embedded stages (developmental stages + eggmasses) per root were calculated, final population (embedded stages + nematodes in soil), nematode build up (Pf/Pi), average of eggs/eggmass were estimated.

2.3. Plant chemical analysis

Subsamples of fresh root of each treatment were chemically analyzed as follows.

2.3.1. Preparation of enzyme extracts

Samples of 0.25 g were homogenized in 5 mL of 50 mM phosphate buffer (pH 7.0) containing 1.0 N NaCl, 1% PVP (Sigma) M.W. 40 000, 1 mM ascorbate (Sigma) at 4 ℃. After centrifugation at 15 000 × g for 15 min the supernatant was collected.

2.3.2. Assay of protein content

Soluble proteins were measured by the Bio-Rad micro assay according to the method of Bradford with some modifications[21] using crystalline bovine serum albumin as a reference.

2.4. Determination of oxidative burst

2.4.1. Lipid peroxidation

About 0.5 g ground roots was 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) TBA or no TBA as the blank, and heated at 95 ℃ 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 400, 532 and 600 nm. The malondialdehyde (MDA) equivalent was derived from the absorbance[22].

2.4.2. Assay of hydrogen peroxide concentration

Hydrogen peroxide was measured by the method described by Capaldi and Taylor[23], with a slight modification. The ground roots was homogenized in 5% TCA (2.5 mL per 0.5 g powder) with 50 mg active charcoal at 0 ℃, and centrifuged for 10 min at 15 000×g. 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, 100 μL of 3.4 mM 3-methylbenzothiazoline hydrazone (MBTH). The reaction 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 1 400 μL of 1 N HCl was added. Absorbance was read at 630 nm after 15 min.

2.4.3. Determination of total glutathione (GSH)

The level of total acid-soluble SH compound (GSH) was determined with Ellman's reagent[24]. The buffer was mixed with 630 μL of 0.5 M K_2HPO_4 and 25 μL of mM 5, 5′-dithiobis (2-nitrobenzoic acid) (final pH 7). The absorbance at 412 nm was read after 2 min. GSH was used as a standard.

2.4.4. Ascorbic acid (ASA) determination

Levels of ASA followed the procedure as described by Singh *et al* with few modifications[25]. Briefly, fresh leaf sample of a known weight (1 g) was extracted with 3 mL of