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Research Paper

Exogenously applied putrescine improves the physiological responses of tomato plant during nematode pathogenesis

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ABSTRACT

Polyamines are ubiquitous organic polyvalent compounds involved in regulating the plant growth and development. The present study deals with the investigation of their role in plant physiology during nematode pathogenesis. Tomato seeds var. Pusa Ruby (susceptible to nematodes) were treated with different concentrations (0.5, 0.7 and 0.9 mM) of putrescine and allowed to germinate in earthen pots. Seedlings were inoculated with second stage juveniles (J2s) of *Meloidogyne incognita* with 130 J2s per seedling. After 45 days of nematode inoculation, various growth parameters, stress indices, photosynthetic pigments, enzymatic and non enzymatic biochemical parameters were studied in control (C), nematode inoculated (NI) and nematode inoculated plus treated plants (NI + 0.5, NI + 0.7 and 0.9 mM). Percentage germination, number of leaves, shoot/root length, fresh shoot/root weight, and dry shoot/root weight enhanced with putrescine application whereas the number of galls, average gall index, number of egg masses and root knot nematode severity level decreased. Stress indices like H_2O_2 and MDA content decreased whereas proline content increased with putrescine treatment. Further, reduced specific activities of enzymes in putrescine treated infected plants and enhanced levels of non-enzymatic antioxidants depicted improved response of tomato plant during nematode stress. Thus, the polyamines were found to improve the physiological responses of tomato plant even during nematode pathogenesis.

1. Introduction

Polyamines (PAs) are positively charged, low molecular weight, ubiquitous organic molecules, occurring in both free as well as conjugated forms. At physiological pH, they are positively charged ions and this property contributes to their biological role in plants and animals. As a result of their cationic nature they are able to interact with macromolecules in the cell such as DNA, RNA, proteins and phospholipids which carries negative charge on them. In this way, PAs are involved in the regulation of physical and chemical properties of membranes, nucleic acids structure and function and modulation of enzyme activities (Aloisi et al., 2016). The common PAs in plants are spermidine (Spd), spermine (Spm), and their diamine precursor, putrescine (Put) (Jiménez-Bremont et al., 2014). Along with their regulatory role in plant development and physiological processes, PAs play pivotal role against various abiotic and biotic stresses. For instance, they maintain the integrity of cell membranes and reduces the alleviation of MDA content due to lipid peroxidation (Tang and Newton, 2005; Zhang et al., 2009), reduces the growth inhibition during oxidative stress induced by flooding (Yiu et al., 2009), minimizes the production of superoxide radicals and H_2O_2 quantity (Yiu et al., 2009) and alleviates chilling injury (Liu et al., 2016; Wang et al., 2016). Tobacco plants that express a Spm-responsive gene called ZFT1 were found resistant to Tobacco Mosaic Virus (TMV) (Uehara et al., 2005). So, they are assumed to act as stress messenger during plant responses to a variety of stress signals (Yamakawa et al., 1998). In 2012, Zhang et al. (2011) investigated the up-regulation of polyamines in methyl-salicylate treated cherry tomato during chilling stress. Enhancement in polyamines occurred in response to arginine catabolism which provided stress tolerance to these tomatoes. In another report, MeJA induced arginine catabolism enhanced tolerance to chilling stress in cherry tomato. It was also found that free putrescine content in treated plants helped them to withstand chilling stress (Zhang et al., 2012). Recently, Liu et al. (2017) has also increased drought tolerance in centipedegrass after exogenous application of Put.

Root knot nematodes (RKNs) are obligate biotropic, economically important plant parasitic nematodes that causes huge lose in many crop plants (Chitwood, 2003). In worldwide scenario, economic loss of about 100 billion USD occur annually due to these nematodes (Sikora and Fernàndez, 2005). In India, infestation of root knot nematode has been reported in various horticultural crops such as tomato (Azam et al., 2011; Singh and Kumar, 2015), okra (Hussain et al., 2012), cowpea (Singh et al., 2010) etc. Root knot nematodes are sedentary,

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microscopic endoparasites that interrupt the uptake of minerals and water in plant due to which plant growth and various physiological processes are negatively affected (Jaouannet et al., 2012). In addition, these cause oxidative stress in plants. This stress in plants is relieved by various enzymatic and non-enzymatic antioxidants in plants. This system of defense involves a wide array of physiological processes in plants and provides protection from oxidative stress. Although, protective role of putrescine has been reported in plants under abiotic stress (Groppa and Benavides, 2008) but, little investigation is done regarding their defensive role in plants during pathogenic infection (Montilla-Bascon et al., 2016). So, the present study was designed accordingly to evaluate the effect of exogenously applied putrescine (Putrescine dihydrochloride) on physiological response of tomato plant during nematode pathogenesis.

2. Material and methods

2.1. Plant material and treatments

Surface sterilized tomato (*Solanum lycopersicum* L.) var. Pusa Ruby (susceptible to nematodes) seeds were soaked for 4 h in different concentrations (0.5, 0.7 and 0.9 mM) of putrescine (Himedia). The seeds were allowed to germinate in 7.62 × 10.16 cm of earthen pots in the department glasshouse with naturally prevailing conditions (temperature 28 ± 5 °C and humidity 50% ± 10%). The seedlings were inoculated with second stage juveniles of *Meloidogyne incognita* @ 130 juveniles (J2s) per seedling at true-leaf stage. Nine replicates were made for each treatment; nematode inoculated and untreated (NI) as well as control (C). Seeds dipped in distilled water served as control.

2.2. Morphological parameters

After 45 days of inoculation (DAI) various morphological parameters were studied i.e. percentage germination, number of leaves, shoot and root length, fresh shoot and root weight, dry shoot and root weight, number of galls, number of egg masses, average gall index (Sasser et al., 1984) and RKN infection rating (Bridge and Page, 1980).

2.3. H_2O_2 concentration

The endogenous hydrogen peroxide (H_2O_2) produced in plant was determined after 45 days of inoculation (Velikova et al., 2000). Fresh plant leaves (0.5 g) were homogenized in 3 ml of 1% trichloro acetic acid (TCA) and centrifuged at 12,000 × *g* for 15 min at 4 °C. For estimation, 0.5 ml of extract was mixed with 0.5 ml of potassium phosphate buffer (PPB) (10 mM, pH 7.0) and 1 ml of potassium iodide (KI, 1 M). The absorbance was recorded at 390 nm (Thermo Scientific Genesys 10 S UV–vis spectrophotometer). A standard curve of H_2O_2 was used for evaluation with same conditions.

2.4. Photosynthetic pigments

Extraction of photosynthetic pigments from leaves was done with 80% acetone. One gram of fresh plant tissue was homogenized in 4 ml of 80% acetone and subjected to centrifugation at 10,000 rpm at 4 °C. The absorbance (A) of supernatant was recorded at 480, 510, 645 and 663 nm (Arnon, 1949).

2.5. Proline content

Proline content was measured according to the method given by Bates et al. (1973). About 250 mg of fresh leaves sample was homogenized in 5 ml of 3% aqueous salfosalicylic acid and filtered through Whatman Sheet No. 2. For estimation, filtrate, acid-ninhydrin and glacial acetic acid was taken in the ratio 1:1:1 (v/v/v ml) in test tubes and kept at 100 °C for 1 h. The reaction was terminated in ice-bath. Now 2 ml of toluene was added to reaction mixture and vigorously mixed for 10–20 s. The intensity of chromophore layer containing toluene at the top was measured at 520 nm.

2.6. Malondialdehyde (MDA) content

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content by the method given by Heath and Packer (1968). Fresh plant seedlings were homogenized with 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 5000 rpm. Supernatant was used for further analysis. The mixture containing 1 ml of supernatant, 6 ml of 20% (w/v) TCA containing 0.5% (w/v) TBA was heated at 95 °C for 30 min and immediately cooled on ice. The optical density of the supernatant was taken at 532 nm. Correction of non-specific absorbance was done by subtraction of absorbance taken at 600 nm. The level of MDA formed was determined by using extinction coefficient 155 mM cm^{-1}.

2.7. Antioxidative enzymes

Fresh plant sample (seedlings) was homogenized in 0.1 M PPB (pH 7.0) in liquid nitrogen (−196 °C) for all enzymes, except phenylalanine lyase (PAL). Homogenates were then subjected to centrifugation at 13,000 rpm for 20 min at 4 °C. Sample for PAL was crushed in sodium borate buffer (0.1 M, pH 8.8) and centrifugation was carried at 12,000 × *g* for 15 min at 4 °C. The activities of various antioxidative enzymes were determined by standard protocols reported by Kono (1978) for superoxide dismutase (SOD) (E.C.1.15.1.1), Aebi (1983) for catalase (CAT) (E.C.1.11.1.6), Nakano and Asada (1981) for ascorbate peroxidase (APOX) (E.C.1.11.11.1), Putter (1974) for guaiacol peroxidase (GuPOX) (E.C. 1.11.1.7), Esterbauer et al. (1977) for polyphenol oxidase (PPO) (E.C.1.10.3.1) and Kar and Mishra (1976) for pyrogallol peroxidase (POX) (E.C.1.11.1.6) The specific activity of POX was calculated using extinction coefficient 2.47 mM^{-1} cm^{-1} for purpurogallin (Haddadchi and Gerivani, 2009) and the protocol of Kovacik and Klejudus (2012) was used for estimation of phenylalanine lyase (PAL) (E.C.4.3.1.5). Protein content was determined using Follin Ciocalteu phenol reagent (Lowry et al., 1951) and Bovine Serum Albumin was used as a standard for its evaluation.

2.8. Non-enzymatic antioxidants

For estimation of non-enzymatic antioxidants, seedlings were weighed and crushed in pre-chilled pestle and mortar using ice-cold 80% methanol. Extracts were collected and centrifuged at 10,000 rpm for 20 min at 4 °C. Supernatant was used for estimation of phenols (Malik and Singh, 1998) and flavonoid (Lamaison and Carnet, 1990) content with slight modification. For ascorbic acid content, method of Chinoy (1962) and for estimation of total glutathione content, method of formation and utilization of Sedlak and Lindsay (1968) was followed.

2.9. Confocal microscopy

Monochlorobimane (MCB) dye (Sigma Aldrich, Bangaluru, India) was used for estimation of Glutathione content in plants under nematode stress using the methodology of Stevenson et al. (2002) with slight modifications. 45-DAI, plant roots tips of 1 cm were cut from each treatment and dipped in the 100 µM solution of MCB dye. After staining, slides were prepared and observed under confocal microscope (Model Nikon A1R with resonance scanner). Multiline argon gas laser is used for MCB to excite the electrons at the wavelength of 405 nm.

2.10. Statistical analysis

One way ANOVA and Tukey's multiple comparison test were performed by using ASSISTAT version 7.7 beta at $P < 0.01$ and $P < 0.05$ and self coded softwares in Microsoft Office Excel.