



Alleviation of allelopathic stress of benzoic acid by indole acetic acid in *Solanum lycopersicum*



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ABSTRACT

The present study investigates the alleviation of allelochemical stress induced by benzoic acid (BA), an autotoxic compound, by exogenous indole acetic acid (IAA) in tomato seedlings. The experiment was conducted in hydroponic culture in glass house conditions. BA was applied 0.5 and 1.0 mM concentrations with and without IAA (1.0 mM). Root and shoot length, fresh and dry weight, pigment, protein, sugar content and nitrate reductase activity decreased in the seedlings treated with BA, while they increased in BA combined with IAA. Lipid peroxidation, electrolyte leakage and proline level enhanced in the seedlings under BA stress, but these parameters decreased in combined BA + IAA treatment. IAA protected the seedlings against oxidative stress caused by reactive oxygen species (ROS). IAA buttressed defense system of tomato plant against BA toxicity which was evident from increased activities of antioxidant enzymes. IAA has potential to enhance tolerance to stress in the tomato seedlings under allelochemical toxicity.

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1. Introduction

Autotoxicity is a common phenomenon in the natural and agro-ecosystems (Liu et al., 2007; Zhang et al., 2010). A process in which a plant species or its decomposing plant parts release phytotoxins into the surroundings to inhibit the growth and development of the same species is generally known as autotoxicity or autointoxication (Miller, 1996; Sannigrahi and Chakraborty, 2005; Cruz-Ortega et al., 2008). Autotoxicity prevalent in monocropping system decreases production and yield equally in agriculture and forestry (Chou and Lin, 1976; Baziramakenga et al., 1995; Cao and Luo, 1996; Batish et al., 2001; Bonanomi et al., 2007; Zhang et al., 2010). The various physiological processes of economical important crops are adversely affected in monoculture due to autotoxicity. In natural conditions plants are rarely subjected to a single stress factor, rather a group of factors. Allelochemicals are low molecular weight secondary metabolites which interfere with various metabolic processes in plants. Allelochemicals influence a number of physiological processes (Blum, 1995; Inderjit and Duke, 2003; Singh et al., 2010).

Tomato is major commercial vegetable crop grown worldwide. Continuous cropping of tomato for long period in the same field may lead to soil sickness (Zheng et al., 2004; Enping et al., 2015).

Accumulation of allelopathins in monocropping decrease plant growth and yield (Yu et al., 1993; Yu and Matsui, 1997). Autotoxicity related soil problems in tomato are commonly reported (Yu and Matsui, 1997; Wu et al., 1997). Aqueous extract of tomato plant and hydroponic medium from tomato culture are autotoxic and decreased plant height and biomass (Zhou et al., 1997; Singh et al., 2008). Exudates from different plant parts of tomato are allelopathic to lettuce (Kim and Kil, 1989). The presence of di-iso-octyl phthalate, di-iso-butyl phthalate, tannic acid and salicylic acid in tomato plants are reported by Zhou et al. (1998). Allelochemicals are released into soil through leaching, volatilization, root exudation, microbial decomposition and microbial phytotoxins. Allelochemicals released as a mixture of compounds. The effects of individual allelochemicals are often different from their mixture. Tomato leaf extract has inhibitory effects on growth and biomass of the seedlings (Bonanomi, 2007). Roots are in close contact with allelopathic compounds released in growth medium. Phenolics compounds viz. salicylic acid, vanillic acid and genilic acid, benzoic acid, palmitic acid, sinapic acid, para-hydroxybenzoic acid, ferulic acid, caffeic acid and synaptic acid are reported in tomato plants (Yu and Matsui, 1997; Mizutani, 1984).

Benzoic acid (BA) is one of the common secondary metabolites with allelopathic potential which affects growth and metabolism of plants. Soil sickness caused by autotoxicity has significant impact on tomato productivity. BA derivatives extracted from soil suppress growth and development of plants (Vaughan and Ord, 1991). Allelochemicals cause oxidative stress which impose injurious effects

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on membrane stability via lipid peroxidation (Lin et al., 2000), DNA damage, protein oxidation and ultimately cell death (Cruz-Ortega et al., 2002). Plants develop several defence mechanisms to cope with oxidative stress (Singh et al., 2010). Production of reactive oxygen species (ROS) scavengers is an important device to increase tolerance against oxidative stress (Sairam et al., 1998). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) are antioxidant enzymes which detoxify ROS (Rubio et al., 2002; Ünyayar et al., 2005) and protect crop plants in oxidative stress. Yadav and Singh (2013) reported that BA at 0.5, 1.0 and 1.5 mM concentrations exhibited toxic effects on crop plants.

IAA was used to ameliorate environmental stresses like salt stress (Torres-Garcia et al., 2009; Javid et al., 2011; Kaya et al., 2013), water stress (Torres-Garcia et al., 2009), stress tolerance and adaptation (Egamberdieva, 2009), metal stress (Gangwar et al., 2011). IAA is the only natural auxin responsible for cell elongation and lateral root formation which increased absorption and accumulation of minerals causing increased growth of crops plants (Egamberdieva, 2009). Exogenous IAA can promote the plant growth and alleviate the harmful effects of biotic and abiotic stresses in plants (Gangwar et al., 2011; Javid et al., 2011; Kaya et al., 2013). Stresses declined endogenous level of IAA (Wang et al., 2001). IAA actively participates in mobilization and accumulation of carbohydrates in seeds (Kato and Takeda, 1993). Exogenous auxin alleviates the adverse effects of stress and thus improves germination, growth, development and yields and quality of crops (Khan et al., 2004; Egamberdieva, 2009).

The two concentrations viz. 0.5 and 1.0 mM of BA were selected as a range of low doses with low toxic effects on the basis of lethal dose (LD50) to record the efficiency of IAA to mitigate the allelopathic stress caused by the allelochemical. It is reported that 0.5, 1.0 and 1.5 mM concentrations of BA exhibited toxic effects on crop plants (Yadav and Singh, 2013). Phytohormones are used to ameliorate various environmental stresses (Unal, 2013). However, the role of phytohormones under allelopathic stress is not well studied. The aim of the present study was to explore the interactive effect of IAA and BA on growth and metabolism of tomato crop grown under allelopathic stress in hydroponic culture.

2. Materials and methods

2.1. Seeds and chemicals

Seeds of tomato (*Solanum lycopersicum*) var. Pusa ruby were procured from seed agency in Allahabad, India. Benzoic acid (BA) was purchased from Loba Chemie Pvt., Ltd., Mumbai. Indole-3 acetic acid (IAA) (molecular weight: 175.19 g/mol) was purchased from CDH.

2.2. Hydroponic culture

The seeds were sown in October, 2014, in nursery beds (1 mX1 m) in the garden of the Department of Botany, University of Allahabad, Allahabad, India. The seed bed was irrigated as and when required. Twenty-one-days old seedlings of uniform size were uprooted and washed under tap water to remove soil adhering on roots. The seedlings were transferred in transparent plastic boxes (23 × 17 × 9 cm) filled with 2 L (L) half strength Hoagland solution (Hoagland and Arnon, 1950). Six seedlings in each box were planted at equal distance. One week after the establishment of the seedlings in hydroponic culture, the boxes were divided into five sets of three each. In one set, Hoagland solution was replaced by fresh Hoagland solution (2 L) and was taken as control. In the second and third sets, Hoagland solution was replaced by fresh Hoagland

solution (2 L) each containing two different concentrations viz. 0.5 and 1.0 mM of BA. In the fourth and fifth sets, Hoagland solution was replaced by Hoagland solution (2 L) each containing BA and IAA viz. BA₁ (0.5 mM)+IAA (1.0 mM) and BA₂ (1.0 mM)+IAA (1.0 mM). Stock solution (1.0 mM i.e. BA₂) of BA was prepared by dissolving requisite amount in 100 mL double distilled water (DDW). The stock solution (1.0 mM) was further diluted with DDW to get 0.5 mM (BA₁) concentration of BA. Requisite amount of IAA was dissolved in 100 mL DDW to obtained 1.0 mM concentration of IAA. The experiment was conducted in three replicates. The boxes were aerated for 12 h a day with the help of bubblers. The boxes were covered with black paper to avoid the algal growth in the medium. The sampling was done after one week of the treatment. The first fully expanded leaves of the seedlings were sampled for biochemical analyses. Root and shoot length and fresh and dry weight of the seedlings were recorded.

2.3. Pigment and protein contents

The leaves (10 mg) were homogenized with 10 mL of 80% acetone. Chlorophylls and carotenoids were extracted and quantified following the method of Lichtenthaler (1987). Protein content was determined according to the method of Lowry et al. (1951). The amount of protein was calculated with reference to the standard curve obtained from bovine serum albumin.

2.4. Sugar content

The estimation of total soluble sugars (TSS) was done following Hedge and Hofreiter (1962). About 0.1 g fresh leaf was homogenized in 5 mL 95% (v/v) ethanol. After centrifugation, 1 mL supernatant was mixed with 4 mL anthrone reagent and heated on boiling water bath for 10 min. Absorbance was recorded at 620 nm after cooling. The amount of sugar was determined by the standard curve prepared from glucose.

2.5. Nitrate reductase activity

Nitrate reductase (NR) activity was measured following the procedure of Jaworski (1971). Fresh leaf tissue (0.25 g) was incubated in 4.5 mL medium which contained 100 mM phosphate buffer (pH 7.5), 3% (w/v) KNO₃ and 3 N HCl and 0.02% (w/v) N-(1-naphthyl)ethylene diamine dihydrochloride. The absorbance was recorded at 540 nm. NR activity was measured with standard curve prepared from NaNO₂ and expressed as nmol NO₂ mg protein⁻¹h⁻¹.

2.6. Membrane leakage

Membrane integrity was assessed in terms of electrolyte leakage. Fresh leaf samples (0.1 g) were placed in a vial containing 10 mL of deionized water and allowed to stand in dark for 24 h at room temperature. Electrical conductivity (EC₁) of the bathing solution was measured at the end of incubation period. The tissue with bathing solution was then heated in water bath at 95 °C for 20 min and the electrical conductivity (EC₂) was again measured after cooling. Electrolyte leakage was calculated as percentage of EC₁/EC₂.

2.7. Lipid peroxidation

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by thiobarbituric acid reactive substance as described by Heath and Packer (1968). Fresh leaf (0.2 g) was ground in 0.1 w/v trichloroacetic acid (TCA) and centrifuged at 10,000g for 10 min. One milliliter supernatant was mixed with 4 mL

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