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RESEARCH ARTICLE

Increased resistance of drought by *Trichoderma harzianum* fungal treatment correlates with increased secondary metabolites and proline content

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Abstract

Plant secondary metabolites play vital role in plant stress response. In this study we investigated whether root colonization of tomato (*Solanum lycopersicum*) infected by *Trichoderma harzianum* leads to alterations in the biosynthesis of secondary plant metabolites including phytohormones and osmolyte proline under drought stress. Exposure of tomato to drought caused a drastic decline in plant growth and physiological parameters. Tomato inoculated with *T. harzianum* showed increased root and shoot growth and chlorophyll pigments as compared to uninoculated controls as well as drought stressed plants. Proline and total soluble protein content was increased in plants inoculated with *T. harzianum* under both normal as well as drought conditions. An obvious increase in phenol and flavonoid content was observed due to *T. harzianum*. In addition, *T. harzianum* inoculated plants maintained higher levels of growth regulators indole acetic acid, indole butyric acid, and gibberellic acid under drought stress. Improved secondary metabolites which play an important role in plant stress tolerance by *T. harzianum* may have coordinately worked for bringing the growth regulation by protecting membranes from reactive oxygen species (ROS) and enhance plant growth through accessing more nutrients by root system.

Keywords: antioxidants, proline, polyphenols, tomato, drought, *Trichoderma harzianum*

1. Introduction

Drought is an abiotic stress resulting in devastation of agricultural crops and causing considerable losses in yield (de Vries *et al*. 2012). Though the general effects of drought stress on plant growth are known, the different tolerance mechanisms involved vary from one plant species to another (Farooq *et al*. 2009). Plants show a range of variations in drought tolerance potential and the mechanisms involved may include up- and down-regulation of several physiological and biochemical traits (Ahmad *et al*. 2010; Hameed *et al*. 2014). Plants have potential mechanisms to withstand stress, the induced oxidative damage through the antioxidant defense systems (Ahanger *et al*. 2014a; Hashem *et al*. 2016). In addition to this, accumulation of osmotic

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constituents like proline, and sugars during stress enable plants to maintain the osmoregulation and recover from the oxidative stress quickly (Jatav *et al*. 2012; Ahmad *et al*. 2015). Changes in the endogenous levels of phytohormones are another typical response of plants counteracted by stress (Hashem *et al*. 2016). The ability of plants to tolerate abiotic stress also depends on their association with microbes, such as mycorrhizal fungi, rhizobial and plant-growth-promoting rhizobacteria, endophytic fungi, which play a vital role in modulating their physiological processes (Ali *et al*. 2014; Egamberdieva *et al*. 2016; Hashem *et al*. 2016). Among microbes, *Trichoderma* species have the potential to induce host plant tolerance to several biotic and abiotic stresses including salinity and drought, by its involvement in root growth promotion, maintenance of nutritional uptake and in addition triggers protective mechanisms to avert the oxidative damage (Ahmad *et al*. 2015). Gusain *et al*. (2014) have observed enhanced drought tolerance in rice due to *Trichoderma harzianum* T35 colonisation and they evidenced that *T*. *harzianum* promoted activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and thereby preventing oxidative damage to rice through quick elimination of reactive oxygen species (ROS). Mastouri *et al*. (2012) observed that the enhanced tolerance of tomato to water stress by *T*. *harzianum* T22 was due to the ability of plants to remove damaging ROS, which was accompanied by an increase in the activity of antioxidant enzymes. Since plant secondary metabolites play vital role in plant stress response, we studied whether root colonization by *T. harzianum* leads to alterations in the biosynthesis of secondary plant metabolites under drought stress.

2. Materials and methods

2.1. Fungi, seeds and pot experiments

The endophytic mold, *T. harzianum* Rifai (TH) was isolated previously from the rhizosphere of tomato plants grown in Ismailia, Egypt, using *Trichoderma* selective medium (Williams *et al*. 2003). The identification was carried out up to species level according to Domch *et al*. (1993). Inoculum of *T*. *harzianum* was produced on potato dextrose agar (PDA) medium (Booth 1977) in Petri plates, at continuous light at 24°C for 10 d to produce abundant conidia (Resende *et al*. 2014). The conidia were collected in 0.05% (w/v) carboxymethylcellulose (CMC) as sticker, and then adjusted to 5.0×10^9 conidia mL^{-1}.

Seeds of tomato (*Solanum lycopersicum* L., *var*. Rio Grande) (Heirloom & Perennial Ltd., Turkey) were used for pot experiments. The seeds of tomato were sown in nursery plastic plates containing sand, perlite and peat (1:1:1, v/v/v) under control growth chamber conditions. The mean temperature was 28.0°C and photoperiod 16 and 8 h, for day and night (200 μE m^{-2} s^{-1} light intensity), respectively, with average relative humidity (RH) (65.0±2)%. In the 2nd true leaf stage, the root of seedlings were immersed in conidial suspension (5.0×10^9 conidia mL^{-1}) of TH for 1 h and transplanted to plastic pots (2 kg volume) containing sand, perlite and peat (1:1:1, v/v/v). Plants were grown under greenhouse climate conditions described above for more than 10 wk after transplantation. The experimental designed as completely randomized design with five replicates per each treatment. The drought stress was achieved by dissolving polyethylene glycol (PEG-8000; Sigma Chemical Co., St. Louis, MO, USA) in Hoagland Solution (Hoagland and Arnon 1950) to get water potential of –1.2 MPa according to Plaut and Federman (1985). Plants were bottom irrigated (100 mL per pot) every 3 d. Control pots were used as reference for each treatment. At end of the experiment (after 10 wk), the plant was removed very carefully from the pots and the length and depth of shoot and root system were measured, respectively. Leaf samples were collected by excising the leaf at the petiole from five replicates for biological analysis. The shoot and root systems were dried at 110°C for 24 h to measure plant biomass.

2.2. Photosynthetic pigments

For estimation of photosynthetic pigments, the uppermost fresh leaf samples (100 mg) were extracted in acetone and the absorbance of supernatant was recorded at 622, 664, and 440 nm using spectrophotometer (Lichtenthaler and Wellburn 1983).

2.3. Estimation of total soluble phenolic and flavonoid compounds

Total phenolics were estimated in the fresh uppermost leaf samples following Slinkard and Singleton (1977). After extraction with ethanol (80%, v/v), the supernatant was reacted with Folin and Ciocalteau's reagent (Julkunen-Tiitto 1985) and optical density of the mixture was read at 750 nm. Standard of pyrogallol was used for calculation. Content of flavonoid was estimated according to Zhishen *et al*. (1999) using catechin as standard. Samples were extracted in methanol and the absorbance was recorded at 510 nm and content of flavonoid was expressed as mg g^{-1} fresh weight (FW).

2.4. Determination of proline

Fresh uppermost leaf sample (0.5 g) was extracted in 3% sulphosalicylic acid and centrifuged for 10 min at 10 000×g. 2 mL of supernatant was incubated with equal