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ORIGINAL ARTICLE

Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance

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Abstract Experiments were conducted to evaluate cadmium (Cd) stress-induced changes in growth, antioxidants and lipid composition of *Solanum lycopersicum* with and without arbuscular mycorrhizal fungi (AMF). Cadmium stress (50 μM) caused significant changes in the growth and physio-biochemical attributes studied. AMF mitigated the deleterious impact of Cd on the parameters studied. Cadmium stress increased malonaldehyde and hydrogen peroxide production but AMF reduced these parameters by mitigating oxidative stress. The activity of antioxidant enzymes enhanced under Cd treatment and AMF inoculation further enhanced their activity, thus strengthening the plant's defense system. Proline and phenol content increased in Cd-treated as well as AMF-inoculated plants providing efficient protection against Cd stress. Cadmium treatment resulted in great alterations in the main lipid classes leading to a marked change in their composition. Cadmium stress caused a significant reduction in polyunsaturated fatty acids resulting in enhanced membrane leakage. The present study supports the use of AMF as a biological means to ameliorate Cd stress-induced changes in tomato.

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1. Introduction

Heavy metal stress is one of the most severe difficulties that crop plants are often confronted with, resulting in yield losses, delayed development, or decreased quality. Cadmium (Cd), a non-redox reactive toxic metal is present in low concentrations in most/several soils. Moreover, cadmium is continuously

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accumulated to soil through natural as well as anthropogenic means such as mining, smelting, weathering of Cd-rich rocks, excessive use of phosphate fertilizers, or the application of sewage sludge and metal-polluted water for crop irrigation (Zoffoli et al., 2013). Cadmium, being a non-essential metal, is absorbed rapidly by plant roots (Pagani et al., 2012). Higher solubility of Cd and its mobility within the soil-plant system contributes to its toxicity (Groppa et al., 2012) affecting growth, promoting necrosis and chlorophyll destruction, altering nutrient uptake, and carbon assimilation (Ahmad et al., 2011; Singh and Prasad, 2014; Abd_Allah et al., 2015). Furthermore, cadmium perturbs enzyme activity because of its higher affinity toward the sulfhydryl group of enzymes (Mendoza-Cozatl et al., 2005).

Exposure to stresses enhances the production and accumulation of reactive oxygen species (ROS) including O_2^-, H_2O_2 and OH^- (Mittler, 2002). Cadmium mediates the production of ROS by interfering with the enzymes involved in maintaining redox homeostasis (Wu et al., 2014). Excessive production of ROS leads to peroxidation of membrane lipid and hence causes oxidative damage (Shah et al., 2001). Plants have evolved indigenous defense mechanisms that are actively involved in averting the ROS induced oxidative stress damage (Wu et al., 2014). Enhanced synthesis and accumulation of organic osmolytes (El-Beltagi and Mohamed, 2013) increase the activity of antioxidant enzymes (Bhaduri and Fulekar, 2012; Morsy et al., 2012), and compartmentation of toxic metal ions into less sensitive cellular compartments like vacuoles (Liu et al., 2014) contributes to enhance the acclimation of plants to stress. Moreover, improved production of cysteine-rich thiol peptides like metallothioneins and phytochelatins, which mediate chelation of toxic metals and metalloids, avert metal stress (Wojas et al., 2010). Superoxide dismutase (SOD), peroxidases (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) are among the key antioxidants involved in scavenging toxic ROS (Mittler, 2002; Wu et al., 2014).

The main component of all biological membranes is lipids, which are responsible for membrane permeability. Lipids have an irreplaceable role in several physiological activities such as photosynthesis, respiration and transport (Janicka et al., 2008). Membrane lipids have an important role in signaling. Isoforms of membrane phospholipids (PL) and their associated enzymes, kinases and phosphatases, show crosstalk so that a coordinated response is elicited during stress so as to promote maintained plant growth (Valluru and den Ende, 2011). Heavy metal stress alters membrane structure by triggering the conversion of unsaturated fatty acids into small fragments of hydrocarbons like malondialdehyde (MDA). Environmental variations cause alterations in the composition of membranes by enhancing the ratio of saturated/unsaturated fatty acids and hence affecting their permeability (Morsy et al., 2012).

Arbuscular mycorrhizal fungi (AMF) and several plants form a symbiotic association. AMF possess the potential to improve soil structure and promote plant growth under normal as well as stressed environmental conditions (Smith et al., 2010). AMF act as essential bio-ameliorators of stress and help to mitigate stress-induced damage in plants (Hashem et al., 2014; Wu et al., 2014). Morpho-physiological and nutritional changes brought about by AMF colonization enhance the resistance of plants to abiotic stresses. Moreover, AMF also have a direct effect on plant growth and vigor (Evelin et al., 2009). Mycorrhizal inoculation affects root morphology as well as the physiological status of host plants. AMF-induced modifications in root architecture help roots to absorb sufficient water and nutrients (Aroca et al., 2013). AMF colonization enhances the uptake of essential mineral nutrients like nitrogen phosphorous and potassium (Hart and Forsythe, 2012).

Tomato (*Solanum lycopersicum* L.), an important vegetable crop plant within the Solanaceae, is the second largest commercially consumed vegetable after potato. Considerable work regarding the deleterious impact of Cd on the growth of plants has been performed but information regarding the ameliorative effect of AMF in Cd-stressed plants is rare. The present study was carried out with the hypothesis that AMF colonization can ameliorate the negative impact of Cd stress on tomato growth. The primary objective of the present work was to evaluate the growth, antioxidant activity, and lipid content of Cd-stressed tomato inoculated with AMF.

2. Material and methods

2.1. Experimental design and treatment

Seeds of tomato 'Edkawy' were obtained from the Agricultural Research Center, Giza, Egypt. The seeds were surface sterilized with sodium hypochlorite (0.5%) for 3 min, washed thoroughly with distilled water before germination on blotting paper. Healthy seedlings (two weeks after germination) were transferred to plastic pots (25 cm in diameter) containing peat, perlite, and sand (1:1:1, v/v/v). Plants were thinned to one plant per pot. Seedlings were allowed to grow at controlled growth chamber for eight weeks under constant temperature (25 ± 4 °C) under a 12-h photoperiod with a photosynthetic photon flux density of 1500 μmol m^{-2} s^{-1}. Pots were irrigated with Hoagland's solution (Hoagland and Arnon, 1950) supplemented with 50 μM $CdCl_2$ (Hayat et al., 2011). The rate of irrigation was 50 mL for each treatment every two days. The AMF used in the present study were *Funneliformis mosseae* (syn. *Glomus mosseae*), *Rhizophagus intraradices* (syn. *Glomus intraradices*) and *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*) which were previously isolated from salt march soil (Hashem et al., 2014; Alqarawi et al., 2014). The mycorrhizal inoculum was added to the experimental pots as 10 g of trap soil (approximately 100 spores/g trap soil, Mycelium = 80%). The control plants were kept free of AMF and were only supplied with normal Hoagland's solution. At the end of the pot experiment, plants were removed from pots very carefully and morphological parameters were measured. Fresh plant samples were dried at 70 °C and the dry mass was measured.

2.2. Photosynthetic pigments

Photosynthetic pigments were extracted from leaf samples (0.5 g) in 80% acetone as described by Arnon (1949). The absorbance of the supernatant was recorded at 480, 645 and 663 nm. Contents of chlorophylls and carotenoids were calculated using the following formulae:

Chl *a* (mg g f.wt.$^{-1}$) = [12.7(OD_{663}) − 2.69(OD_{645}) × V/1000 × W], Chl *b* (mg g f.wt.$^{-1}$) = [22.9(OD_{645}) − 4.68 (OD_{663}) × V/1000 × W], Carotenoids (mg g f.wt.$^{-1}$) = A^{car}/Em × 100, where, V represents volume of the aliquot