Saudi Journal of Biological Sciences (2016) **23**, 39–47

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Bioremediation of adverse impact of cadmium toxicity on *Cassia italica* Mill by arbuscular mycorrhizal fungi

Abeer Hashem [a,b], E.F. Abd_Allah [c,*], A.A. Alqarawi [c], Dilfuza Egamberdieva [d]

[a] *Botany and Microbiology Department, Faculty of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia*
[b] *Mycology and Plant Disease Survey Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt*
[c] *Department of Plant Production, Faculty of Food & Agricultural Sciences, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia*
[d] *Institute for Landscape Biogeochemistry, Leibniz Centre for Agricultural Landscape Research (ZALF), 15374 Müncheberg, Germany*

Received 7 October 2015; revised 4 November 2015; accepted 11 November 2015
Available online 27 November 2015

KEYWORDS

Cassia italic;
AMF;
Antioxidant enzymes;
Proline;
Phenol;
Lipid peroxidation

Abstract *Cassia italica* Mill is an important medicinal plant within the family Fabaceae. Pot experiment was conducted to evaluate cadmium stress induced changes in physiological and biochemical attributes in *C. italica* with and without arbuscular mycorrhizal fungi (AMF). Cadmium stressed plant showed reduced chlorophyll pigment and protein content while AMF inoculation enhanced the chlorophyll and protein content considerably. AMF also ameliorated the cadmium stress induced reduction in total chlorophyll and protein contents by 19.30% and 38.29%, respectively. Cadmium stress enhanced lipid peroxidation while AMF inoculation reduced lipid peroxidation considerably. Increase in proline and phenol content was observed due to cadmium stress and AMF inoculation caused a further increase in proline and phenol content ensuring better growth under stressed conditions. AMF alone also enhanced proline and phenol content. Activity of antioxidant enzymes enhanced under cadmium treatment and AMF inoculation further enhanced their activity thereby strengthening the antioxidant system. Enhanced activities of antioxidants and increased accumulation of osmolytes help plants to avoid damaging impact of oxidative damage. The research has shown that AMF inoculation mitigated the negative impact of stress by reducing the lipid peroxidation and enhancing the antioxidant activity. The present study strongly supports employing AMF as the biological mean for enhancing the cadmium stress tolerance of *C. italica*.

* Corresponding author.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

Cadmium stress is among the potential toxic heavy metals present in soil in low concentrations. Cadmium is highly mobile between soil–plant systems and therefore is quickly absorbed

http://dx.doi.org/10.1016/j.sjbs.2015.11.007
1319-562X

by the plants and hence transported to upper parts causing toxicity (Irfan et al., 2014). Processes including weathering of cadmium rich rocks, mining, smelting, over supplementation of phosphate fertilizers to agricultural soils and applying sewage sludge and metal polluted water for crop irrigation contribute to aggravate the situation by increasing the levels of cadmium (Zoffoli et al., 2013). Cadmium being a non essential metal is absorbed rapidly by plant roots and alters growth as well as developmental processes (Pagani et al., 2012). Cadmium accumulation promotes necrosis and chlorophyll destruction, perturbs nutrient uptake, carbon assimilation and enzyme activity (Singh and Prasad, 2014; Abd_Allah et al., 2015). Cadmium induced perturbation in enzyme activity is due to its high affinity toward the sulfhydryl group of enzymes (Wada et al., 2014).

Production and accumulation of reactive oxygen species (ROS) including O_2^-, H_2O_2 and OH^- is increased manifolds on exposure to stresses (Wu et al., 2014; Abd_Allah et al., 2015). Although being a non-redox metal, cadmium initiates the over-production of ROS by interfering with the enzymes that are involved in the maintenance of redox homeostasis (Yan et al., 2013). Stress triggered enhancement in levels of toxic ROS leads to peroxidation of the membrane lipid thereby causing oxidative damage (Wu et al., 2014). Increased membrane lipid peroxidation leads to loss of their integrity resulting in membrane leakage. In addition, ROS also affects important cellular components like nucleic acids, proteins and chlorophylls through oxidation and ultimately results in perturbed cell functioning (Alqarawi et al., 2014; Abd_Allah et al., 2015; Ahmad et al., 2015). Plants have several indigenous defence mechanisms which are actively involved in mitigating the damage induced by stresses like heavy metals. Increase in synthesis and accumulation of osmotic constituents (Alqarawi et al., 2014), increased activity of antioxidant enzymes (Irfan et al., 2014; Abd_Allah et al., 2015) and efficient compartmentation of toxic metal ions into less sensitive cellular compartments like vacuoles (Liu et al., 2014) contribute to enhance stress tolerance. Moreover increased production of protective compounds, like metallothioneins and phytochelatins, which mediate chelation of toxic metals and metalloids therefore help in averting the stress effects (Sylwia et al., 2010). Moreover, enzymatic antioxidant system including superoxide dismutase [SOD], peroxidases [POD], catalase [CAT] and glutathione reductase [GR] also mediate the scavenging of toxic ROS hence help in preventing oxidative stress (Wu et al., 2014; Alqarawi et al., 2014; Abd_Allah et al., 2015).

Several plants form symbiotic associations with arbuscular mycorrhizal fungi (AMF). Studies have confirmed AMF as the best biological tool for improving plant growth under normal conditions and can also mitigate the adverse impacts of abiotic stresses on crop plants (Hashem et al., 2014; Alqarawi et al., 2014; Wu et al., 2014). AMF brings several morpho-physiological and biochemical changes in host plants that promote maintained plant growth and increased vigor. Modifications in root morphology induced by AMF colonization mediate increased water and mineral uptake (Ahanger et al., 2014; Wu et al., 2014). AMF has been reported to enhance the uptake of essential mineral nutrients like nitrogen phosphorous and potassium (Ahanger et al., 2014; Hashem et al., 2014; Alqarawi et al., 2014). The present study was carried to evaluate the impact of cadmium stress on growth and physio-biochemical parameters of *Casssia italica* Mill, and the role of AMF in ameliorating the adverse impact.

2. Materials and methods

2.1. Pot experiment setup and treatments

Healthy seeds were collected from mature pods of naturally grown *C. indica* Mill (Fig. 1 I-IV) found in the Arafat region, Holy Mecca, Saudi Arabia (Fig. 2). The seeds were geminated on blotter paper in petri dishes in a controlled growth chamber at 25 °C with a 16/8 h light/dark photoperiod and light intensity of 1500 μmol m^{-2} S^{-1}. The blotter papers were wetted with full strength Hoagland's solution for one week. After one week of germination, seedlings were transferred to pots containing peat and sand in the ratio of 1:1 (w/w) and were supplemented with Hoagland solution (100 mL pot^{-1}) after every two days. The experiment was laid down in a factorial completely randomized design having three replicates for each treatment. After eight weeks of normal growth, cadmium stress was induced by supplementing Hoagland's solution with 150 μM $CdCl_2$. Pots receiving only Hoagland's solution served as control. The arbuscular mycorrhizal fungi used in the present study contained a mixture of *Funneliformis mosseae* (syn. *Glomus mosseae*), *Rhizophagus intraradices* (syn. *Glomus intraradices*) and *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*) as described by Hashem et al. (2014). The mycorrhizal inoculum was added to the experimental pots as 10 g of trap soil (approximately 100 spores/g trap soil, $M = 80\%$). At the end of the incubation period, days plants were removed from the pots carefully and analyzed for several parameters.

2.2. Determination of Cadmium (Cd) concentrations in plant

Dry shoot and root materials (0.1 g) were digested in a mixture of nitric acid and perchloric acid (4:1) using the hot block digestion procedure (overnight at 60 °C) according to the method of Jackson (1962) and described by Burd et al. (2000). After cooling, 1.0 ml of hydrogen peroxide (30%, v/v) was added to the digested sample and incubated for 2 h. The cadmium concentration was measured by an atomic absorption spectrophotometer (Perkin Elmer AA700, USA).

2.3. Photosynthetic pigments

Leaf samples (0.5 g) were extracted in 80% acetone as described by Arnon (1949). The optical densities of the supernatant were recorded at 480, 645 and 663 nm against a blank containing acetone (80%).

2.4. Estimation of proline

Free proline was estimated according to the method of (Bates et al., 1973). Leaf samples (0.5 g) were extracted in sulfosalicylic acid followed by centrifugation at 3000 g for 30 min. 2.0 ml of supernatant was mixed with an equal volume of acid ninhydrin solution [1.25 g ninhydrin, with 30 ml glacial acetic acid, and 20 ml of 6 M phosphoric acid] and glacial acetic acid. The samples were incubated at 100 °C for 10 min and the reaction was terminated by keeping the tubes in a container filled