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ORIGINAL ARTICLE

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

Abeer Hashem [a,b,*], E.F. Abd_Allah [c], A.A. Alqarawi [c], Asma A. Al Huqail [a], D. Egamberdieva [d], S. Wirth [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, 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*

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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.

* Corresponding author at: Botany and Microbiology Department, Faculty of Science, King Saud University, P.O. Box. 2460, Riyadh 11451, Saudi Arabia.
E-mail address: habeer@ksu.edu.sa (A. Hashem).
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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

and W, weight of tissue. $A^{car} = OD_{480} + 0.114(OD_{663}) - 0.638(OD_{645})$ and Em = 2500.

2.3. Estimation of hydrogen peroxide

Fresh leaf tissue (0.5 g) was ground in a prechilled mortar with 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was then centrifuged at 12,000*g* for 15 min. To 0.5 mL of the supernatant, 0.5 mL of potassium phosphate buffer (pH 7.0) and 1 mL of KI were added, and after vortexing the mixture, the absorbance was read at 390 nm (Velikova et al., 2000).

2.4. Estimation of lipid peroxidation (malondialdehyde)

Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid reaction as described by Heath and Packer (1968). The absorbance was recorded at 532 and 600 nm (1% thiobarbituric acid in 20% TCA was used as blank). The concentration of MDA was calculated using an extinction coefficient of 155 mM cm^{-1}: $MDA = \Delta (OD532 - OD600)/1.56 \times 10^5$.

2.5. Estimation of total phenolics

Total phenolics were extracted with 80% acetone and estimated using 20% (w/v) sodium carbonate (Na_2CO_3) and Folin-Ciocalteau's phenol reagent following Julkunen-Tiitto (1985). The absorbance of the mixture was assessed at 750 nm.

2.6. Estimation of proline

The proline was extracted from leaf samples (0.5 g) using sulfosalicylic acid solution (3%; w/v) and estimated by acid ninhydrin solution according to Bates et al. (1973). The optical density of the filtrate was measured at 520 nm against toluene.

2.7. Membrane stability index

Membrane stability index (MSI) was determined according to the method of Sairam et al. (1997). Fresh leaf samples (0.1 g) were placed in test tubes in two sets and 10 mL of double distilled water was added to each. One set was placed at 40 °C for 30 min in a water bath and the electrical conductivity of the solution was recorded using an electric conductivity meter Adawa-260, Germany (C_1). The second set was kept in a boiling water bath (100 °C) for 10 min and its conductivity was also recorded (C_2). A calculation for MSI was performed using the following formula: $MSI = [1 - (C_1/C_2)] \times 100$.

2.8. Antioxidant enzyme estimation

Five grams of fresh leaves was homogenized with 50 volumes of 100 mM Tris-HCl (pH 7.5) containing 5 mM DTT, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM magnesium acetate and 1.5% Polyvinylpyrrolidone (PVP, average molecular weight 40,000). After filtration in cheesecloth, the homogenate was centrifuged at 10,000*g* for 15 min. The supernatant collected was used as the source of enzymes. Serine and cysteine proteinase inhibitor (1 mM PMSF + 1 μg/mL aproptinin) was also added to the extraction buffer. The extraction buffer for ascorbate peroxidase (APX) was supplemented with 2.0 mM ascorbate in addition to other ingredients. The soluble protein content was determined by Bradford (1976) with standard curves prepared using bovine serum albumin.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by the method of Van Rossun et al. (1997) by following the photoreduction of nitroblue tetrazolium (NTB). The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM NBT, 2 μM riboflavin and 100 μL of crude enzyme. The reaction was initiated by placing the tubes under two 15 W fluorescent lamps. The reaction was terminated after 10 min by removing the reaction tubes from under the light source. Non-illuminated reaction mixtures served as blanks. The absorbance of the reaction products was measured at 560 nm. SOD activity was expressed as units mg^{-1} protein. One unit of SOD was defined as the amount of protein causing a 50% decrease of the SOD-inhibitable NBT reduction. The Luck (1974) method was employed for the catalase (CAT, EC 1.11.1.6) assay. Enzyme extract (50 μl) was added to 3 mL of 20 mM hydrogen peroxide (H_2O_2) and 50 mM phosphate buffer (pH 7.0) solution. The decrease in absorbance was measured at 240 nm. Enzyme activity was calculated using the extinction coefficient of 36×10^3 mM^{-1} cm^{-1} and expressed as units mg^{-1} protein. Ascorbate peroxidase (APX, EC1.11.1.11) was spectrophotometrically assayed following Nakano and Asada (1981). Three mL of assay mixture contained 0.1 mM EDTA, 0.5 mM ascorbate and 0.1 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7.0) with 0.1 mL of the enzyme extract. The H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm. APX activity was expressed as units mg^{-1} protein. Glutathione reductase (GR, EC 1.6.4.2) activity was determined according to Carlberg and Mannervik (1985). The assay mixture (1 mL) contained 0.75 μL potassium phosphate buffer (pH 7) with 2 mM EDTA, 75 μM NADPH (2 mM), and 75 μL GSSG (20 mM). The reaction was initiated by adding 0.1 mL of enzyme extract to the mixture and the decrease in absorbance was measured at 340 nm for 2 min. GR activity was calculated with the extinction coefficient of NADPH of 6.2 mM^{-1} cm^{-1} and expressed as μmol NADPH oxidized min^{-1} (units mg^{-1} protein).

2.9. Lipid extraction and separation

Total lipids were extracted according to the method of Fölch et al., 1957 modified by Bligh and Dyer (1959) using chloroform: methanol (2:1 v/v) in which 0.05% L^{-1} butylated hydroxytoluene (BHT) was added to all solvents as an antioxidant to prevent lipid peroxidation (Cachorro et al., 1993). The activity of phospholipases in plant tissues was stopped by adding tubes to boiling water for 5 min (Douce, 1964).

Total lipids were separated into glycolipids (GL), neutral lipids (NL) and PL on silica gel TLC plates (G 60, Merck), using the following solvent systems: $CHCl_3$-Me_2CO-MeOH-HOAc-H_2O [10:4:2:2:1] (Tremolieres and Lepage, 1971); petrol-Et_2O-HOAc [70:30:0.4] (Metcalfe et al., 1966) and chloroform:acetone:methanol:acetic acid:water [50:20:10:10:5, v/v/v/v] (Lepage, 1967). To visualize lipid bands, the plates were divided into two groups, one sprayed with iodine (I_2) vapor and the other sprayed with Rhodamine 6G (0.1%, w/v

in ethanol). Lipid classes were identified by comparison with lipid standards and by specific stains for PL and galactolipids with the help of R_f values of each class.

Phospholipid classes were separated by two-dimensional chromatography with $CHCl_3$-MeOH-28% (w/v) NH_4OH (13:5:1, v/v/v) for the first dimension and CHC_3-Me_2 CO-MeOH-HOAc-H_2O (6:8:2:2:1, v/v/v/v) for the second dimension (Rouser et al., 1970). Identification was made by comparing R_f values to those of pure standards and by a specific staining reaction using molybdenum reagent (Dittmer and Lester, 1964). Spots were outlined with a pencil, scraped off of the plates, and total PL were determined (Dittmer and Wells, 1969).

Fatty acid methyl esters (FAME) were prepared according to the method described by Metcalfe et al. (1966). FAME were separated and quantified with by gas liquid chromatography (GLC) [Perkin-Elmer Model 910, Perkin Elmer, Shelton, CT, USA] equipped with a flame ionization detector (Johnson and Stocks, 1971). A dual-open recorder and a computing integrator (Perkin-Elmer Model M1) were attached to GLC for recording. Both the injector and detector were maintained at 230 and 250 °C, respectively. Nitrogen was used as the carrier gas at 1 mL/min with split injector system (split ratio 1:100). The separation and quantitation of FAME peaks were identified by comparing their retention times with those of an authentic methyl ester standard (Sigma Co., St. Louis, USA).

2.10. Statistical analysis

All experiments were repeated three times. Treatment means were statistically analyzed using Least Significant Difference (LSD) analysis of variance for a completely randomized design.

3. Results

Results related to the various growth parameters like length and fresh/dry weight are depicted in Table 1. Cadmium stress reduced shoot and root length by 55% and 20.6%, respectively. A marked reduction in fresh and dry weight of roots and shoots was observed as a result of Cd treatment. Cadmium stress reduced shoot and root fresh weight by 64% and 61.8%, respectively compared to the control. However, in AMF-inoculated cadmium stressed plants, the reduction in shoot and root fresh weight was only 29.3% and 42.0%, respectively. Relative to control plants, AMF-inoculated plants showed a 15% and 14.9% increase in the length of shoots and roots.

Cadmium stress reduced chl *a*, chl *b* and total chlorophyll content in tomato by 50.11%, 31.7% and 43.75%, respectively. Inoculation of AMF enhanced the content of chlorophyll *a*, chlorophyll *b* and total chlorophyll by 34.18%, 36.6% and 35%, respectively. AMF inoculation caused a substantial increase in chlorophyll content under Cd stress (Table 2).

Cadmium stress caused a drastic decline (43.75%) in MSI relative to the control. AMF ameliorated Cd stress-induced changes in MSI. AMF alone increased MSI by 4.3% (Table 3). Tomato plants subjected to Cd stress showed an increase in the accumulation of deleterious H_2O_2 and inoculation of AMF reduced H_2O_2 under Cd stress (Table 3). A non-significant increase in H_2O_2 was observed in AMF-inoculated tomato plants. Increased lipid peroxidation (i.e., higher MDA content) was observed in Cd-stressed plants and AMF colonization resulted in a considerable reduction in MDA content. Under Cd stress, AMF inoculation also reduced the extent of lipid peroxidation. Compared to the control, the increase in MDA

Table 1 Effect of cadmium (50 μM) in the presence and absence of AMF on length (cm), fresh weight (g), and dry weight (g) of root and shoot of *S. lycopersicum* seedlings.

Treatments		Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Control (non-stressed)	AMF −	27.16	2.435	0.634	10.12	0.542	0.105
	AMF +	31.24	3.157	0.952	11.63	0.612	0.213
Cadmium (50 μM)	AMF −	12.03	0.875	0.217	8.03	0.207	0.041
	AMF +	25.16	1.721	0.486	9.12	0.314	0.093
LSD at: 0.05		1.47	0.51	0.18	0.67	0.04	0.01

Table 2 Effect of cadmium (50 μM) in the presence and absence of AMF on chlorophyll (chlorophyll *a*, chlorophyll *b*) content (mg/g fresh weight) of *S. lycopersicum* seedlings.

Treatments		Chlorophyll content (mg/g fresh weight)			
		Chlorophyll *a*	Chlorophyll *b*	*a*/*b*	Total chlorophyll
Control (non-stressed)	AMF −	1.752	0.924	1.89	2.676
	AMF +	2.351	1.263	1.86	3.614
Cadmium (50 μM)	AMF −	0.874	0631	1.38	1.505
	AMF +	1.623	0.975	1.66	2.598
LSD at: 0.05		0.07	0.04	0.02	0.06

Table 3 Effect of cadmium (50 μM) in the presence and absence of AMF on MSI, H_2O_2, MDA, proline and total phenols of *S. lycopersicum* seedlings.

Treatments		Acquired resistance criteria				
		MSI	H_2O_2	MDA	Proline	Total phenols
Control (non-stressed)	AMF −	93.14	12.34	4.12	0.54	2.87
	AMF +	97.23	13.75	3.46	0.78	2.93
Cadmium (50 μM)	AMF −	52.37	27.15	7.85	1.24	4.27
	AMF +	78.93	18.46	5.74	2.17	3.12
LSD at: 0.05		3.01	0.79	0.54	0.16	0.04

Membrane stability index [MSI]; Hydrogen peroxide [**H_2O_2**]; Malondialdehyde [**MDA**].

content under Cd stress was 90.5% while AMF inoculation reduced MDA by 16%.

Proline content increased with Cd stress and inoculation of AMF under Cd stress further enhanced the accumulation of proline (Table 3). Under Cd stress alone and AMF inoculation and Cd stress (50 μM + AMF), proline increased by 129.6% and 301.8%, respectively. AMF alone enhanced proline content 44.44% more than control uninoculated plants. Total phenol content was enhanced in AMF-colonized plants as well as in Cd-stressed plants. Exposure to Cd stress enhanced phenol content by 48.78% while AMF-inoculated Cd-stressed plants showed an 8.7% increase over the control (Table 3).

The activities of antioxidant enzymes studied showed a considerable increase under Cd stress (Fig. 1a–e), specifically 45.3%, 66.7%, 48.8%, 36.7% and 50.7% increase in SOD, POD, APX, CAT and GR activity, respectively. AMF alone reduced SOD and GR activity non-significantly but enhanced the activities of POD, APX and CAT by 5.3%, 1.6% and 9.8%, respectively. However, in combination with Cd, AMF further increased the activities of all antioxidants studied: 82% for SOD, 94.9% for POD, 65.3% for APX, 16.7% for CAT and 76.2% for GR.

In our study a marked reduction in total lipids was observed in Cd-stressed plants. However, inoculation of AMF increased lipids and also ameliorated the Cd-induced reduction to some extent (Table 4). Different GL studied were markedly reduced by Cd stress: 57.8% for monogalactosyldiacylglycerol (MGDG), 28% for digalactosyldiacylglycerol (DGDG), and 30.2% for sulfolipids (SL) thereby reducing the total GL content by 53.5% compared to control plants. Colonization of AMF caused a marked increase in GL under normal as well as Cd stress conditions. Total GL content was enhanced 20.5% by AMF (Table 4).

Tomato plants subjected to Cd stress showed a slight increase in neutral lipid content. AMF-inoculated plants showed a substantial increase in neutral lipid content and the impact of AMF in combination with Cd was not so obvious (Table 5). Among various neutral lipids estimated, only triacylglycerol and sterol contents were enhanced by 20.6% and 16.3%, respectively following AMF inoculation while others showed a decline.

Results related to the impact of AMF and Cd on PL are depicted in Table 6. Tomato plants subjected to Cd stress showed a 14.6% increase in PL content. Phosphatidyl choline, phosphatidyl ethanol amine and phosphatidylglycerol increased by 21.5%, 100.7% and 34.7%, respectively under Cd stress and AMF-inoculated plants showed only a slight reduction in these three PL. The fatty acid profiles are depicted in Table 7. AMF alone caused a 54.1% increase in total unsaturated fatty acids. Tomato plants subjected to Cd stress showed a 53.6% reduction in total unsaturated fatty acids while AMF caused a considerable mitigation of Cd-induced deleterious effects.

4. Discussion

Tomato plants subjected to Cd stress showed reduced growth in terms of length and weight. Cadmium alters growth, triggers leaf necrosis and impedes cell division and elongation (Liu et al., 2004) and reduced growth attributes in tomato (Jing et al., 2005), *Brassica juncea* (Ahmad et al., 2011); *Hordeum vulgare* (Juknys et al., 2012) and *Helianthus annuus* (Abd_Allah et al., 2015). AMF alone caused a significant increase in the growth of tomato plants. Inoculation of AMF mitigated the Cd stress-induced reduction of all growth-related attributes studied. AMF enhances the resilience of crop plants through its active participation in nutrient uptake and maintaining cell water content (Ling-Zhi et al., 2011). The increased growth in AMF-colonized plants in our study was also observed by Tang et al. (2009) for maize and Ling-Zhi et al. (2011) for *Tagetes erecta*. In *T. erecta*, Ling-Zhi et al. (2011) demonstrated that AMF mitigated the deleterious impact of Cd. The reduced growth of plants under Cd stress and subsequent amelioration by AMF were also observed by Rivera-Becerril et al. (2005) for *Pisum sativum*, and Ling-Zhi et al. (2011) for *T. erecta*. In most cases, inhibited growth due to Cd is ascribed to reduced photosynthesis. In our study, Cd stress reduced the chlorophyll pigment content which was also observed by Mangal et al. (2013) for *Abelmoschus esculentus* L. and *Cyamopsis tetragonoloba* L., Liu et al. (2014) for cotton and Abd_Allah et al. (2015) for *H. annuus*. Liu et al. (2014) demonstrated a drastic decline in chlorophyll content due to Cd stress. Cadmium impedes the biosynthesis of chlorophyll (Hsu and Kao, 2003). Moreover, heavy metal stress exposure enhances chlorophylase activity and mediates increased degradation of chlorophyll (Singh and Jain, 1981). In this study in tomato, Cd stress reduced chlorophyll content, a negative impact that was mitigated by AMF. Enhanced chlorophyll content in AMF-inoculated plants and subsequent recovery of Cd-stressed plants may be due to the effect of AMF on magnesium uptake. Sheng et al.

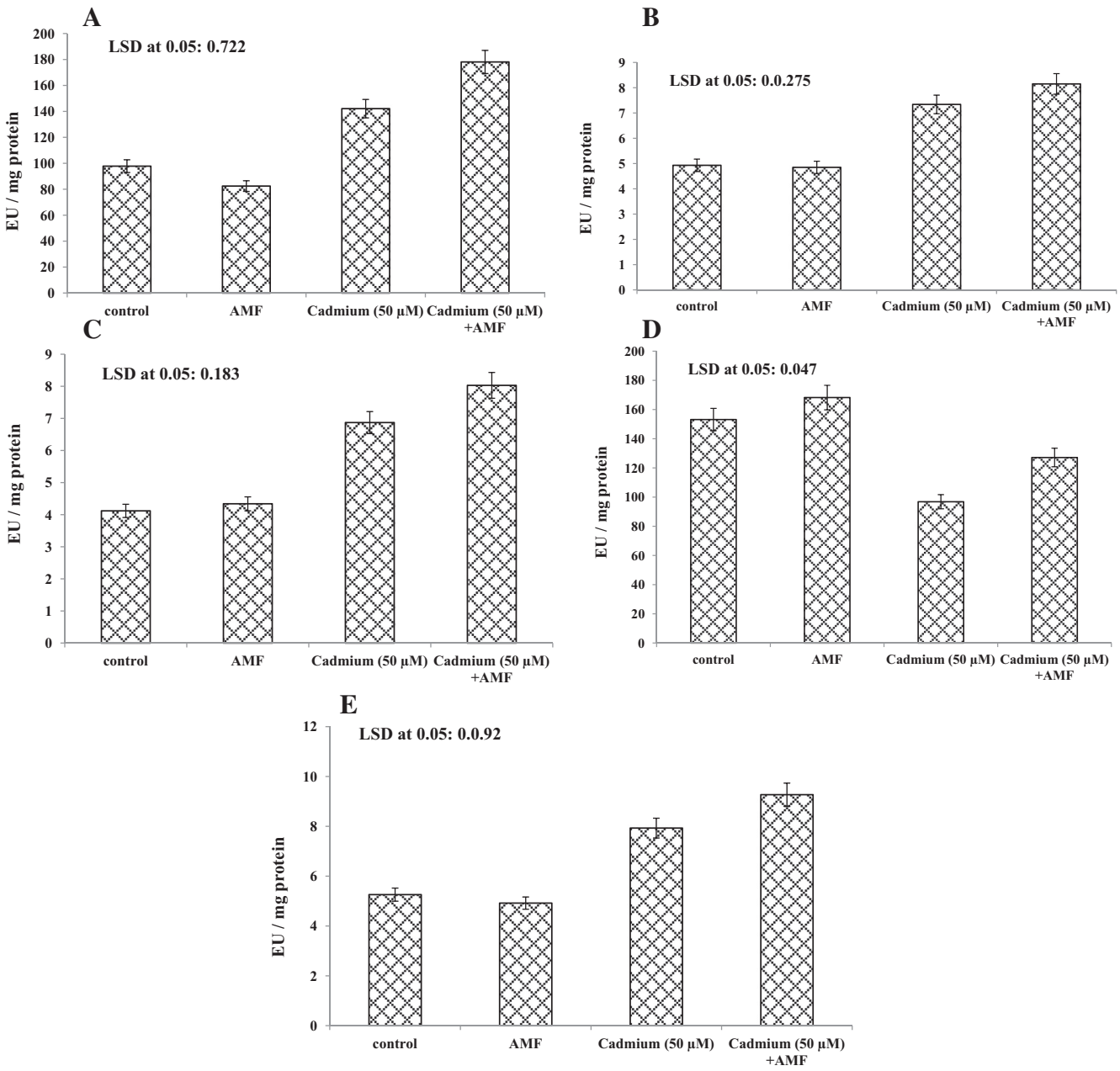

Figure 1 (A–E): Effect of cadmium (50 µM) in the presence and absence of AMF on (A) Superoxide dismutase (SOD); (B) peroxidases (POD); (C) ascorbate peroxidase (APX); (D) catalase (CAT) and (E) glutathione reductase (GR) of *S. lycopersicum* plants.

(2008) also showed a close relationship between chlorophyll content and magnesium uptake as it forms an important part of chlorophyll molecule.

Mahmood et al. (2007) also demonstrated that heavy metal stress (Cu, Zn, Pb) reduced membrane stability in crops, such as wheat (*Triticum aestivum*), barley (*H. vulgare*) and rice (*Oryza sativa*), hence reducing the stability of membranes leading to the leakage of essential ions. Increased MSI in AMF-treated plants may be due to enhanced uptake of essential mineral elements by AMF colonization resulting in sustained cell water content. Reduced MSI in Cd-stressed plants may also be as a result of increased peroxidation of membrane lipids. Increased lipid peroxidation as a result of Cd stress was also observed by John et al. (2009). Lipid peroxidation as measured by MDA content is accepted as an important criterion for assessing the magnitude of oxidative stress (Savoure et al., 1999). Reduced peroxidation of membranes in AMF-inoculated plants may be due to the possible role of AMF in phosphate uptake and antioxidant activity (Tang et al., 2009). Membrane lipids are very sensitive to toxic free radicals and are easily oxidized. Peroxidation of lipids is also ascribed to increased activity of lipoxygenase under stress (Djebali et al., 2005). Peroxidation of membrane lipids causes the loss of integrity and could also impart irreversible damage to cell functioning (Abd_Allah et al., 2015). Cadmium stress caused a marked increase in the production of H_2O_2. AMF-inoculated plants under Cd stress produced low levels of H_2O_2 and hence reduced oxidative stress. Cadmium induced

Table 4 Effect of cadmium (50 μM) in the presence and absence of AMF on total lipids (mg/g dry weight) and glycolipids (mg/g dry weight) of *S. lycopersicum* seedlings.

Treatments		Total lipids (mg/g dry weight)	Glycolipids (mg/g dry weight)			
			MGDG	DGDG	SL	Total
Control (non-stressed)	AMF −	10.34	4.12	1.27	1.39	6.78
	AMF +	12.75	7.06	1.39	1.53	9.80
Cadmium (50 μM)	AMF −	4.36	2.01	0.72	0.97	3.70
	AMF +	8.96	3.78	1.04	1.14	5.96
LSD at: 0.05		1.42	0.27	0.07	0.09	1.04

Monogalactosyldiacylglycerol [**MGDG**]; Digalactosyldiacylglycerol [**DGDG**]; Sulfolipids [**SL**].

Table 5 Effect of cadmium (50 μM) in the presence and absence of AMF on neutral lipids (μg/g dry weight) of *S. lycopersicum* seedlings.

Treatments		Neutral lipids (μg/g dry weight)					
		DG	TG	S	SE	FAA	Total
Control (non-stressed)	AMF −	0.613	1.034	0.814	0.912	0.413	3.786
	AMF +	0.572	1.248	0.973	0.834	0.376	4.004
Cadmium (50 μM)	AMF −	0.839	1.561	0.534	1.246	0.805	3.985
	AMF +	0.701	0.893	0.721	0.978	0.612	3.905
LSD at: 0.05		0.03	0.11	0.07	0.05	0.02	0.05

Diacylglycerol (**DG**); Triacylglycerol (**TG**); Sterol (**S**); Sterol Ester (**SE**) and Non-esterified fatty acids (**FAA**).

Table 6 Effect of cadmium (50 μM) in the presence and absence of AMF on phospholipid fractions (mg/g dry weight) of *S. lycopersicum* seedlings.

Treatments		Phospholipids (μg/g dry weight)						
		PA	PC	PE	PG	PI	PS	Total
Control (non-stressed)	AMF −	1.43	5.24	1.32	2.13	0.93	1.36	12.41
	AMF +	1.86	4.13	1.24	2.06	1.42	1.84	12.55
Cadmium (50 μM)	AMF −	0.83	6.37	2.65	2.87	0.75	0.73	14.20
	AMF +	1.24	5.07	1.68	2.35	1.16	1.12	12.62
LSD at: 0.05		0.11	0.14	0.05	0.06	0.09	0.09	0.11

Phosphatidic acid [**PA**]; Phosphatidyl choline[**PC**]; Phosphatidyl ethanol amine [**PE**]; Phosphatidyl glycerol [**PG**]; Phosphatidyl inositol [**PI**]; Phosphatidyl serine [**PS**].

Table 7 Effect of cadmium (50 μM) in the presence and absence of AMF on fatty acid profile (%) of *S. lycopersicum* seedlings.

Treatments		Fatty acids profile (%)													
		C_8	**C_{10}**	**C_{12}**	**C_{14}**	**C_{15}**	**$C_{15:1}$**	**C_{16}**	**$C_{16:1}$**	**C_{17}**	**C_{18}**	**$C_{18:1}$**	**$C_{18:3}$**	**C_{20}**	**TUS**
Control (non-stressed)	AMF −	1.67	2.34	0.27	0.84	1.51	4.36	15.51	3.87	7.16	0.67	37.02	7.89	16.89	48.78
	AMF +	0.47	1.03	0.04	0.21	0.72	1.04	8.44	8.37	5.12	0.42	52.63	14.17	7.34	75.17
Cadmium (50 μM)	AMF −	4.56	5.51	2.17	2.74	3.77	7.97	19.76	1.97	9.32	1.74	16.01	4.62	19.86	22.6
	AMF +	3.02	4.34	1.04	1.07	1.02	5.12	16.81	5.01	8.64	0.69	31.33	6.87	15.04	43.21
LSD at: 0.05		1.04	1.11	0.07	0.13	0.21	0.32	1.07	1.04	1.25	0.01	4.28	0.89	1.34	4.87

C_8: Caprylic; **C_{10}**: Capric; **C_{12}**: Lauric; **C_{14}**: Myristic; **C_{15}**: Pentadecanoic; **$C_{15:1}$**: Cis-10-Pentadecanoic acid; **C_{16}**: Palmitic; $C_{16:1}$: Palmitoleic; **C_{17}**: Heptadecanoic; **$C_{17:1}$**: cis-10-Heptadecenoic; **C_{18}**: Stearic; **$C_{18:1}$**: Oleic; **$C_{18:2}$**: Linolenic; **$C_{18:3}$**: Linolenic; **C_{20}**: Arachidi; **$C_{20:2}$**: Eicosadienoic; **C_{22}**: Behenic.

enhanced production of H_2O_2 was also observed by John et al. (2009) and Abd_Allah et al. (2015) in *B. juncea* and *H. annuus*, respectively. H_2O_2 is produced as a by-product of various physiological pathways like photosynthesis, and respiration. ROS mainly affect important molecules such as lipids and DNA (Tuteja et al., 2009).

The exposure of plants to adverse environmental condition leads to enhanced synthesis and accumulation of various organic osmolytes which have an important role in maintaining growth under such conditions (Jaleel et al., 2007). Proline and sugar accumulation help plants to maintain cellular water potential well below that of the soil solution. Increase in proline accumulation in Cd-stressed plants has earlier been demonstrated in tomato (Hayat et al., 2011). Enhanced proline content in our study supports the role of proline in the maintenance of growth under stress conditions. Further enhancement of proline in AMF-inoculated plants supports the potential role of AMF and proline in plants. In *Ocimum basilicum*, Shekoofeh et al. (2012) also reported AMF-induced enhancement of proline content and subsequent mitigation of salt stress. Under stress conditions, the activity of proline-synthesizing enzymes is upregulated and catabolizing enzymes are lowered (Jaleel et al., 2007). Phenols and other secondary metabolites have an important role in plant protection against various stresses (Alqarawi et al., 2014; Abd_Allah et al., 2015). AMF inoculation enhanced the content of total phenols in tomato. Our results showing increased phenol content in tomato plants due to AMF inoculation confirm the findings of Nell et al. (2009) in *Salvia officinalis* L. and Abohatem et al. (2011) in date palm. The increase in phenolic content of Cd-stressed plants corroborates the findings of Marquez-Garcia et al. (2012) for *Erica andevalensis*. Ahmad et al. (2015) also demonstrated a significant increase in phenol and proline content in *Cannabis sativa* subjected to Cd and salt stress.

In plants, the antioxidant defense system is comprised of both enzymatic as well as non-enzymatic components, protecting them from toxic ROS. Under stress conditions, ROS production is enhanced manifold and results in impeded growth and development. Enhanced activity of antioxidants mediates quick scavenging of ROS and hence protects cells from possible oxidative damage (Noctor and Foyer, 1998; Mittler, 2002). The present study demonstrated that SOD, CAT, POD, GR and APX increased in Cd-stressed tomato plants and that their activity was further enhanced by inoculation with AMF. This further increase in the activities of antioxidants suggests the role of AMF in mediating quick scavenging of ROS. SOD is involved in the scavenging of superoxide ions and marks the first line of defense. In mustard, John et al. (2009) also demonstrated a gradual increase in the activity of antioxidants as Cd concentration increased. Normal and sustained growth depends on regulated and controlled metabolic processes and any kind of disturbance in metabolism results in impeded growth. APX and GR are important enzymes of the ascorbate-glutathione pathway. APX is involved in scavenging H_2O_2 and GR carries out the NADPH-dependent conversion of GSSH into GSH so as to maintain a higher ratio of GSH/GSSH (Noctor and Foyer, 1998). Higher APX and GR activities suggest their role in preventing oxidative stress-induced changes. Under stress conditions, the activities of antioxidants increase due to AMF inoculation, as has also been reported in soybean (Ghorbanli et al., 2004) and *Ipomoea aquatica* (Bhaduri and Fulekar, 2012).

A reduction in lipids and GL in Cd-stressed plants was obvious and AMF enhanced these parameters and also ameliorated the effect of Cd stress to some extent. However Cd stress induced a slight increase in PL. In our results, among several PL estimated, only few PL such as phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) showed an increase in the presence of Cd stress. Altered fatty acid and lipid metabolism in Cd-stressed plants corroborate the findings of Djebali et al. (2005) in *Lycopersicon esculentum*, Nouairi et al. (2006) in *Brassica junc*ea and *Brassica napus*, Morsy et al. (2012) in *Zygophyllum* species and Elloumi et al. (2014) in *Prunus dulcis*. GL and PL are important constituents of membranes and any alteration in their content can directly affect membrane-related functions including signaling, energy transduction and transport (Morsy et al., 2012). Monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) lipids are among several important GL (Morsy et al., 2012) and exposure to environmental stresses alters the MGDG/DGDG ratio (Nouairi et al., 2006). Reduced lipid content in stressed plants may be due to enhanced lipoxygenase activity (Djebali et al., 2005). An increase in PC, PE and PG in our results supports the findings of Nouairi et al. (2006) and Elloumi et al. (2014), who also demonstrated an increase in PL due to Cd stress. Similar to our results, these authors also reported that polyunsaturated fatty acids are greatly affected by increasing Cd concentrations. AMF alone caused a 54.1% increase in total unsaturated fatty acids, however Cd stress caused significant reduction in total unsaturated fatty acids while AMF caused a considerable mitigation of Cd-induced deleterious effects. In the same context, Abd_Allah et al. (2015) reported that palmitoleic acid ($C_{16:1}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic acid ($C_{18:3}$) reduced in cadmium treated sunflower plants and the negative impact of cadmium was mitigated by AMF.

5. Conclusion

Cadmium stress affected tomato growth and metabolism. However, inoculation with AMF mitigated the Cd stress-induced deleterious changes. Increased lipid peroxidation and the activity of antioxidants under Cd stress were evident. However, Cd stress reduced MSI and affected lipid metabolism to a great extent and AMF could successfully overcome the stress-triggered changes in such attributes. In conclusion, we show that the AMF signal and antioxidative enzymes activity have important roles in the plant's response to cadmium stress in tomato. These findings may be used to biological researcher with enhanced resistance to Cd stress.

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