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Metal uptake, oxidative metabolism, and mycorrhization in pigeonpea and pea under arsenic and cadmium stress

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Abstract: Presence of arsenic (As) and cadmium (Cd) at elevated levels in the soils is threatening agricultural productivity. Arbuscular mycorrhizae (AM) enhance plant resistance to metal(loid)s by sequestering them into roots, thus restricting their translocation into leaves. The present study evaluated differential responses of AM-colonized *Cajanus cajan* and *Pisum sativum* plants to As and Cd uptake and oxidative metabolism under As and Cd stress (0, 30, and 60 mg kg^{-1}). Arsenic uptake was significantly higher than Cd uptake, which caused greater growth inhibitions and induced oxidative stress. Pea was more sensitive, with higher toxicity symptoms in roots than leaves. Mycorrhizae were tolerant to metal toxicity and formed stronger association with the roots of pigeonpea than pea. However, mycorrhization arrested metal(loid) uptake, reduced oxidative stress, and strengthened antioxidant enzyme activities. Stronger antioxidant enzyme activity and mycorrhizal symbiosis in pigeonpea when compared with pea could explain the differences in their metal(loid) tolerance.

Key words: Arsenic, cadmium, *Cajanus cajan*, mycorrhizal frequency, *Pisum sativum*

1. Introduction

Heavy metal (HM) pollution has become a major global problem as HMs tend to accumulate in agricultural soils in proportion to the pace of worldwide industrialization (Pant et al., 2011). Arsenic (As) and cadmium (Cd) pose serious environmental threats due to their significant toxicities (DalCorso et al., 2008; Bhattacharya et al., 2012) and their contamination in soils can be due to natural (disintegration of rocks and minerals and lixiviation) (Baker et al., 1990) and/or anthropogenic sources (Sun et al., 2008, 2009; Peralta-Videa et al., 2009; Jia et al., 2012; Bolan et al., 2014). The Comprehensive Environmental Response, Compensation, and Liability Act permanently listed As as no. 1 and Cd as no. 7 out of 275 in its priority list of hazardous materials (ATSDR, 2007).

Arsenic and Cd toxicity cause oxidative stress by changing the composition and fluidity of membrane lipids, displacing essential metals in plant pigments or enzymes and thereby inactivating photosynthesis and respiration, and obstructing uptake and transport of water and nutrients, further reducing protein synthesis and carbohydrate metabolism along with diminished growth and yield (Zavala and Duxbury, 2008; Garg and Singla, 2011; Piršelová et al., 2011; Shao et al., 2011; Ovečka and Takáč, 2014).

Arsenic and Cd are found naturally at low concentrations in the earth's crust and may not have been recruited during evolution because of their lower abundance compared to phosphorus (P) and zinc (Zn), respectively. The similarity between these pairs make As and Cd potentially toxic for the cell because they tend to substitute for P and Zn, respectively, and consequently disturb homeostasis of essential elements in cellular metabolism and inhibit plant growth (Pigna et al., 2010). Arsenate (AsV), a nonessential element and phosphate chemical analog, can compete with phosphate during phosphorylation reaction, leading to the formation of AsV adducts that are often unstable and short-lived. The formation and rapid autohydrolysis of AsV-ADP sets in place a futile cycle that uncouples photophosphorylation and oxidative phosphorylation, decreasing the ability of cells to produce ATP (Geng et al., 2006; Srivastava and Sharma, 2013). Another important mode of action of AsV toxicity may be the substitution of inorganic phosphate (Pi) by AsV in numerous biochemical reactions, and any reaction with Pi or a Pi-ester as a substrate is a potential target for AsV disruption (Gresser, 1981; Sharma, 2013). Cd becomes toxic to plants through irreversible changes to protein conformation by forming metal thiolate bonds and through alteration of the cell

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wall and membrane permeability by binding to nucleophilic groups (Ramos et al., 2002; Verbruggen et al., 2009). Thus, plant P/As and P/Cd molar ratios can be a good index for their relative abundance and their important roles in the plant growth. Since cellular AsV is reduced to As(III) in cells, there is a degree of similarity between the toxicology and the sequestration machineries of Cd and As (Verbruggen et al., 2009).

AsV, though not a redox metalloid, can provoke cellular disruption in plants through the induction of oxidative stress caused by the generation of reactive oxygen species (ROS) during the conversion of AsV to As(III) (Gomes et al., 2012; Liu et al., 2013), while Cd, a redox metal, does not participate directly in cellular redox reactions (Clemens, 2006; Garg and Bhandari, 2014). Exposure to Cd drives oxidative injuries, such as lipid peroxidation, which leads to alteration in the membrane functionality and protein carbonylation (Schützendübel et al., 2001; Romero-Puertas et al., 2002; DalCorso et al., 2008, 2010; Garg and Aggarwal, 2012). A possible constitutive mechanism of detoxification in plants is upregulation of antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POX), and catalase (CAT) (Meharg, 2005; Gomes et al., 2012; Dave et al., 2013; Sobrino-Plata et al., 2014).

The recovery of soils degraded by metal(loid)s in excess is possible by means of several processes, of which revegetation is the most recommended strategy because of its lower cost and the resulting stabilization of the area. Moreover, successful establishment of plant coverage in these conditions requires a diverse and functional microbial community such as arbuscular mycorrhizae (AM) . The importance of AM fungi relies on their capacity to increase water and nutrient uptake, mainly uptake of P, in low-fertility soils (Schneider et al., 2013). A number of AM fungi like *Glomus*, *Gigaspora*, and *Entrophospora* inhabit metal-contaminated soils, among which *Glomus* species are the most common (Khade and Adholeya, 2009), indicating their HM tolerance (Vallino et al., 2006; Kapoor and Bhatnagar, 2007; Upadhyaya et al., 2010; Bona et al., 2011). Because of the dominance of *Funneliformis mosseae* in metal-polluted soils, its association with crop plants can be highly useful in conferring metal tolerance (Repetto et al., 2007; Andrade et al., 2008; Garg and Chandel, 2012). Many studies demonstrate the potential application of AM fungi for enhancing plant tolerance to metal(loid)s (Elahi et al., 2010; Krüger, 2013). However, the extent of mycorrhizal colonization varies with the type of plant species as well as metal concentration (Janoušková et al., 2007; Bai et al., 2008; Jankong and Visoottiviseth, 2008; Al-Ghamdi and Jais, 2012).

Most legume species are generally considered to be less tolerant to HM toxicity (Pajuelo et al., 2011). The capacity to take up metal(loid)s is species- and genotype-specific (Baroni et al., 2004; Garg and Aggarwal, 2012; Garg and Chandel, 2012). However, there is lack of information on relative potential of plant species to associate with metal(loid)-tolerant AM fungi. Therefore, the selection of plant species is the first step for an efficient process of revegetation of HM-contaminated soils. Pigeonpea (*Cajanus cajan* (L.) Millsp.) and pea (*Pisum sativum* L.) are the two most important food legumes and are nutritionally important as they contain higher levels of proteins and important amino acids like methionine, lysine, and tryptophan, as well as iron and vitamins B and C. They establish effective symbiosis with P-scavenging AM fungi and this association might improve resistance to heavy metal(loid) s in the soils. To our knowledge, little is known regarding the interspecific variation in toxicity symptoms of HM stress as well as mycorrhizal responsiveness in legume species. The aim of the present study was to compare As- and Cd-induced responses in *Cajanus cajan* (L.) Millsp. (pigeonpea) and *Pisum sativum* L. (pea) of the family Fabaceae and the phytoprotective role of AM fungi in enhancing HM tolerance.

2. Materials and methods

2.1. Biological material and plant growth conditions

The experimental material consisted of two legume species, namely pigeonpea (*Cajanus cajan* (L.) Millsp. 'Sel 85N') and pea (*Pisum sativum* (L.) 'PB 89 2008'), procured from the Pulse Laboratory, Indian Agricultural Research Institute, New Delhi and the Department of Vegetable Crops, College of Agriculture, Punjab Agricultural University, Ludhiana, India, respectively. Pigeonpea is a 'kharif' crop that grows naturally in the monsoon summer season, while pea is a 'rabi' crop that grows naturally in the winter season. Greenhouse experiments were conducted for two consecutive years (2011 and 2012) in the Department of Botany, Panjab University, Chandigarh (30.5°N, 76.5°E; 305–366 m a.s.l.), from mid-June to October for pigeonpea (minimum temperature: 22–29 °C, maximum temperature: 30–37 °C, morning relative humidity: 55%–92%, afternoon relative humidity: 42%–81%). For pea, experiments were conducted from November to February (minimum temperature: 9–18 °C, maximum temperature: 16–25 °C, morning relative humidity: 25%–31%, afternoon relative humidity: 83%–89%). Mycorrhizal inoculum of *Funneliformis mosseae* (formerly called *Glomus mosseae*) (UTMU 128 WM1/11) was obtained from The Energy and Resource Institute, New Delhi, India. The inoculum was bulked in an open-pot soil culture (Miyasaka et al., 2003; Dalpé and Monreal, 2004)

using *Zea mays* L., *Sorghum bicolor* L., and *Coriandrum sativum* L. Inocula of *Sinorhizobium fredii* AR-4 for pigeonpea and *Rhizobium leguminosarum* bv. *viciae* strain PRH-1 for pea were procured from the Department of Microbiology, Indian Agricultural Research Institute, New Delhi and the Department of Microbiology, CCS Haryana Agricultural University, Hisar, India, respectively. These rhizobial inoculations were given to all sets of treatments, including unstressed controls, in order to ensure natural fertilization for all plants.

2.2. Experimental design

The growing substrate (a mixture of sand and loam in a ratio of 1:1 by volume) was obtained from nearby agricultural fields [11.0 mg P kg^{-1} (Olsen and Sommers, 1982), 0.17 mEq 100 g^{-1} available potassium (K), 0.19 Na mEq 100 g^{-1}, 0.82 calcium (Ca^{2+}) mEq 100 g^{-1} (Mehlich, 1953), 0.42% total nitrogen (N) (Nelson and Sommers, 1973), pH 7.6 (soil:water; 1:1), 0.68% organic carbon (Walkley, 1947)]. It was autoclaved (121 °C, 1 h twice at 48-h intervals) to eliminate existing AM propagules. Circular earthenware experimental pots (30 × 25 × 25 cm) were disinfected with 70% ethanol before filling them with soil (Liu LZ et al., 2011). A thick wad of glass wool was placed on the central drainage hole, which was covered by a clean watch glass. The pots were lined with polythene bags to avoid leaching during irrigation and were filled with 7 kg of soil mixture. Fifty grams of soil-based inoculum of *F. mosseae* (containing segments of colonized roots, spores, and filamentous hyphal networks) was placed at a pot depth of 1.5 cm prior to sowing to facilitate fungal colonization of plant roots. Non-AM treatments received the same weight of autoclaved inoculum to obtain the same soil texture together with a 10-mL aliquot of an inoculum filtrate. Seeds were surface sterilized with 10% hydrogen peroxide (v/v) solution for a few minutes and then rinsed by soaking in sterile distilled water. Seeds were pretreated with rhizobial inoculum and were kept for drying at room temperature. Three plants per pot were maintained throughout the growth period. After 2 weeks of seedling establishment, As was applied as a water solution of sodium arsenate GR ($Na_2HAsO_4.7H_2O$), while Cd was applied as cadmium chloride AR ($CdCl_2$) at the rates of 30 and 60 mg kg^{-1} of dry soil respectively, with and without AM inoculations. Pots were arranged in a completely randomized block design with a factorial combination of 2 × 2 × 3 × 2 for two species under two metal(loid) stresses, at three concentrations, with and without AM inoculations. Deionized water was used to prepare all solutions. The control set of plants was treated with tap water only. Plants were harvested for physiological and biochemical analysis at 75 days of sowing. Samples (roots and shoots) were oven-dried at 70 °C for 72 h until they reached a constant weight. Six plants per treatment per year were analyzed and data were calculated on a per plant basis by taking the means of replicates for both years.

2.3. Mycorrhizal frequency

Mycorrhizal frequency (MF) was estimated 30 days after treatment (McGonigle et al., 1990) after staining the fungal structures with Cotton Blue (Phillips and Hayman, 1970). The samples were kept in staining solution for 24–36 h. The roots were cut into 50 small pieces of approximately 1 cm and observed under a compound light microscope. Root pieces that contained even a single vesicle or arbuscules along with hyphae were considered colonized.

$$\text{Percent root frequency} = \frac{\text{Total number of colonized roots}}{\text{Total number of roots observed}} \times 100$$

2.4. Arsenic and cadmium concentrations

Dried plant samples (roots and leaves) were digested with HNO_3-H_2O_2 (1:1 v/v) at 120 °C for 3 h to determine total As concentration (Tang and Miller, 1991). Arsenic concentrations were determined using a hydride generation atomic absorption spectrometer (PerkinElmer Analyst 300 fitted with a flow injection analysis system). Cd concentrations were determined by the method as described by Ouzounidou et al. (1992). Dried plant material was wet-digested in a nitric-perchloric (HNO_3-$HClO_4$) acid mixture (5:2 v/v) at 125–135 °C for 6 h (AOAC 1990) and metal concentration was determined by atomic absorption spectrophotometry.

2.5. Nutrient (N, P, and K) determination

The N concentration was estimated by the colorimetric method of Lindner (1944). Fifty milligrams of dried and well-ground plant material was taken in a 25-mL conical flask with 3 mL of digestion mixture. The digest was cooled and 0.5 mL of 30% H_2O_2 was added. The solutions were diluted to 100 mL with distilled water. A 0.5-mL aliquot of the diluted digest was taken and 0.3 mL of 2.5 N NaOH was added to partially neutralize the excess acid. Another 0.1 mL of 10% sodium silicate was added to the aliquot to avoid turbidity, and the final volume was attained by adding 5 mL of distilled water. After shaking thoroughly, five drops of Nessler's reagent were added. The mixture was allowed to stand for 30 min at room temperature. The optical density (OD) was recorded at 420 nm on a double-beam UV-190 spectrophotometer (Labnics Equipment) against a reagent blank. The standard curve was prepared using graded concentrations of $(NH_4)_2SO_4$. P was assayed at 420 nm with atomic spectrophotometer by the method of Chapman and Pratt (1961). Vanadate solution was added to molybdate solution and cooled to room temperature; 250 mL of concentrated HNO_3 was then added and diluted to 1 L. Next, 0.5 g of plant material (roots and leaves) was taken in 50-mL volumetric flasks

and 10 mL of vanadomolybdate reagent was added to each flask, and absorbance was taken at 420 nm after 30 min. For K estimation, 10 mL of acid mixture consisting of nitric acid, sulfuric acid, and perchloric acid at a ratio of 9:4:1 was added to 5 g of ground samples and kept overnight. The samples were then maintained at 70 °C on a hot plate for 30 min, and the temperature was increased to 120 °C for 30 min and then to 250 °C until only 3–4 mL of the sample was left. A final volume of 50 mL was maintained with distilled water and left overnight. Solution was filtered the next day using Whatman No. 1 filter paper. K concentration was estimated on a flame photometer (Chapman and Pratt, 1961) against a reagent blank.

2.6. Oxidative damage

The level of lipid peroxidation was measured by estimating malondialdehyde (MDA), a decomposition product of the peroxidized polyunsaturated fatty acid component of the membrane lipid, using thiobarbituric acid as the reactive material following the method of Heath and Packer (1968). The absorbance of the supernatant was recorded at 532 nm and the nonspecific absorbance at 600 nm was subtracted. The MDA concentration was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1}. H_2O_2 concentration was determined according to Velikova et al. (2000). The absorbance of the supernatant was measure at 390 nm with a spectrophotometer. The concentration of H_2O_2 was calculated by comparison with a standard calibration curve, plotted by using different concentrations of H_2O_2.

2.7. Antioxidant enzyme extractions and assays

Crude extracts were obtained by maceration of 0.5 g of frozen plant material (roots and leaves separately) in 50 mmol L^{-1} potassium phosphate buffer (pH 7.8) containing 1 mmol L^{-1} EDTA, 3 mmol L^{-1} 2-mercaptoethanol, and 2% (w/v) polyvinylpolypyrrolidone in a chilled mortar and pestle. The homogenate was centrifuged at 16,000 × *g* for 30 min at 4 °C and the supernatant was used for enzyme assays. SOD activity was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Dhindsa et al., 1981). One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions. Enzyme activity was expressed as µkat per mg protein (1 kat = 1 mol s^{-1} catalytic activity). CAT activity was assayed by following the decline in absorbance of H_2O_2 at 240 nm according to the method of Aebi (1984). One unit of activity was defined as the amount of enzyme that catalyzed the oxidation of 1 µmol of H_2O_2 per minute under the assay conditions and enzyme activity was expressed as µkat mg $protein^{-1}$. POX activity was assayed as the increase in optical density due to the oxidation of guaiacol to tetraguaiacol (Castillo et al., 1984). Absorbance due to the formation of tetraguaiacol was recorded at 470 nm and enzyme activity was calculated as per the extinction coefficient of its oxidation product tetraguaiacol (€ = 26.6 mM^{-1} cm^{-1}). Enzyme activity was expressed as µkat mg $protein^{-1}$.

2.8. Statistical analysis

Data presented are mean values ± standard error (SE). All results were subjected to two-way analysis of variance (ANOVA) using SPSS 18.0. The significance of difference between exposed and control plants were tested by Duncan multiple comparison test ($P < 0.05$). Pearson's correlation (r_p) was used to determine the relationship between two dependent variables for different parameters.

3. Results

3.1. Growth attributes

Plants subjected to As and Cd contamination showed clear symptoms of toxicity as the dry weights of roots and leaves decreased significantly in a concentration-dependent manner (Table 1). Deleterious effects of As treatments were more pronounced as compared to those of Cd. Higher negative correlations between plant biomass and metal(loid) concentration were observed for pea as compared to pigeonpea (As: pigeonpea roots- $r_p = 0.921$, pigeonpea leaves- $r_p = 0.769$, pea roots, $r_p = 0.94$, pea leaves- $r_p = 0.797$; Cd: pigeonpea roots- $r_p = 0.898$, pigeonpea leaves- $r_p = 0.759$, pea roots- $r_p = 0.902$, pea leaves- $r_p = 0.784$). Presence of mycosymbionts alleviated the depressive effects of metal(loid)s as AM-inoculated plants had much higher root and shoot dry mass as compared to uninoculated stressed plants. However, higher positive correlation between biomass and MF was recorded in pigeonpea (roots, $r_p = 0.752$ and leaves, $r_p = 0.676$) than pea plants (roots, $r_p = 0.710$ and leaves, $r_p = 0.657$). The analysis of As × AM and Cd × AM interactions confirmed the significant role of mycorrhizae in diminishing the perilous effects of both metal(loid)s (Table 2).

3.2. Metal(loid) concentrations

Arsenic and cadmium individually and their interactions with AM inoculations showed significant impact on accumulation of As and Cd in pigeonpea and pea (Table 2). Regardless of the As and Cd substrate levels, there was large difference of As (Figure 1) and Cd (Figure 2) concentrations among species, i.e. pea accumulated more metal(loid)s than pigeonpea. The increase in As and Cd concentrations varied largely with higher buildup in the roots, suggesting that roots were the main accumulating organ. Arsenic accumulation was much higher as compared to Cd in both roots and leaves. As × AM and Cd × AM interactions showed significant contributions of mycorrhizal associations in depressing As and Cd concentration in both species.

Table 1. Effect of arbuscular mycorrhizal (AM) inoculations on shoot dry weight (SDW, g plant^{-1}), root dry weight (RDW, g plant^{-1}), and AM frequency (%) of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Treatments were designed as uninoculated controls (C) and arsenic (As) and cadmium (Cd) stress (30, 60 mg kg^{-1}) with and without arbuscular mycorrhizae (AM). Each value is the mean of six replicates ± SE. Means followed by the same letter within a column are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

Treatments	Parameters measured					
	SDW		RDW		AM frequency	
	Pigeonpea	Pea	Pigeonpea	Pea	Pigeonpea	Pea
Control (C)	$4.455^{b} \pm 0.134$	$3.341^{b} \pm 0.088$	$1.019^{b} \pm 0.021$	$0.764^{b} \pm 0.016$	-	-
C + AM	$6.093^{a} \pm 0.084$	$4.299^{a} \pm 0.123$	$1.342^{a} \pm 0.019$	$0.937^{a} \pm 0.074$	$93.27^{a} \pm 2.647$	$90.57^{a} \pm 3.126$
As	$3.402^{de} \pm 0.092$	$2.308^{d} \pm 0.106$	$0.639^{f} \pm 0.104$	$0.429^{f} \pm 0.058$	-	-
As^{30} + AM	$4.249^{c} \pm 0.093$	$2.831^{c} \pm 0.064$	$0.891^{d} \pm 0.020$	$0.579^{d} \pm 0.061$	$75.33^{c} \pm 4.756$	$69.51^{c} \pm 2.635$
As^{30}	$2.389^{g} \pm 0.092$	$1.492^{g} \pm 0.084$	$0.387^{i} \pm 0.017$	$0.230^{h} \pm 0.036$	-	-
As_{60}^{60} + AM	$2.776^{f} \pm 0.065$	$1.715^{f} \pm 0.129$	$0.524^{g} \pm 0.008$	$0.310^{g} \pm 0.040$	$64.71^{d} \pm 1.537$	$54.39^{e} \pm 2.043$
Cd	$3.548^{d} \pm 0.091$	$2.445^{d} \pm 0.096$	$0.705^{e} \pm 0.021$	$0.506^{e} \pm 0.076$	-	-
Cd^{30} + AM	$4.568^{b} \pm 0.087$	$3.062^{b} \pm 0.094$	$0.993^{c} \pm 0.019$	$0.692^{c} \pm 0.078$	$87.62^{b} \pm 4.908$	$78.5^{b} \pm 5.514$
Cd^{30}	$2.757^{fg} \pm 0.091$	$1.786^{f} \pm 0.096$	$0.479^{h} \pm 0.018$	$0.317^{g} \pm 0.031$	-	-
Cd_{60}^{60} + AM	$3.290^{e} \pm 0.095$	$2.112^{e} \pm 0.075$	$0.653^{f} \pm 0.013$	$0.429^{f} \pm 0.048$	$75.79^{c} \pm 3.055$	$64.39^{d} \pm 2.216$

Table 2. Results of two-way ANOVA test for independent variables, including arsenic (As) and cadmium (Cd) treatments, arbuscular mycorrhizal (AM) inoculations, and interactions among them. RDW: Root dry weights; SDW: shoot dry weights; L: leaves; R: roots; ns: no significant differences; *: significant differences at 95%.

Parameters measured		As		Cd		AM		As × AM		Cd × AM	
		Pea	Pigeonpea	Pea	Pigeonpea	Pea	Pigeonpea	Pea	Pigeonpea	Pea	Pigeonpea
RDW		*	*	*	*	*	*	*	*	*	*
SDW		*	*	*	*	*	*	*	*	*	*
AM frequency		*	*	*	*	-	-	-	-	-	-
Arsenic concentration	L	*	*	-	-	*	*	*	*	-	-
	R	*	*	-	-	*	*	*	*	-	-
Cadmium concentration	L	-	-	*	*	*	*	-	-	*	*
	R	-	-	*	*	*	*	-	-	*	*
Nitrogen(N)	L	*	*	*	*	*	*	*	*	*	*
	R	*	*	*	*	*	*	*	*	*	*
Potassium (K)	L	*	*	*	*	*	*	*	*	*	*
	R	*	*	*	*	*	ns	ns	ns	ns	ns
Phosphorus (P)	L	*	*	*	*	*	*	*	*	*	*
	R	*	*	*	*	*	*	*	*	*	*
P/As	L	*	*	-	-	*	*	*	*	-	-
	R	*	*	-	-	*	*	*	*	-	-
P/Cd	L	-	-	*	*	*	*	-	-	*	*
	R	-	-	*	*	*	*	-	-	*	*
H_2O_2	L	*	*	*	*	*	*	ns	ns	ns	ns
	R	*	*	*	*	*	*	*	ns	*	*
MDA	L	*	*	*	*	*	*	*	*	*	*
	R	*	*	*	*	*	*	*	*	*	*
SOD	L	*	*	*	*	*	*	*	*	*	*
	R	*	*	*	*	*	*	*	*	*	ns
CAT	L	*	*	*	*	*	*	ns	ns	*	*
	R	*	*	*	*	*	*	*	*	ns	ns
POX	L	*	*	*	*	*	*	ns	ns	ns	ns
	R	*	*	*	*	*	*	*	*	*	*

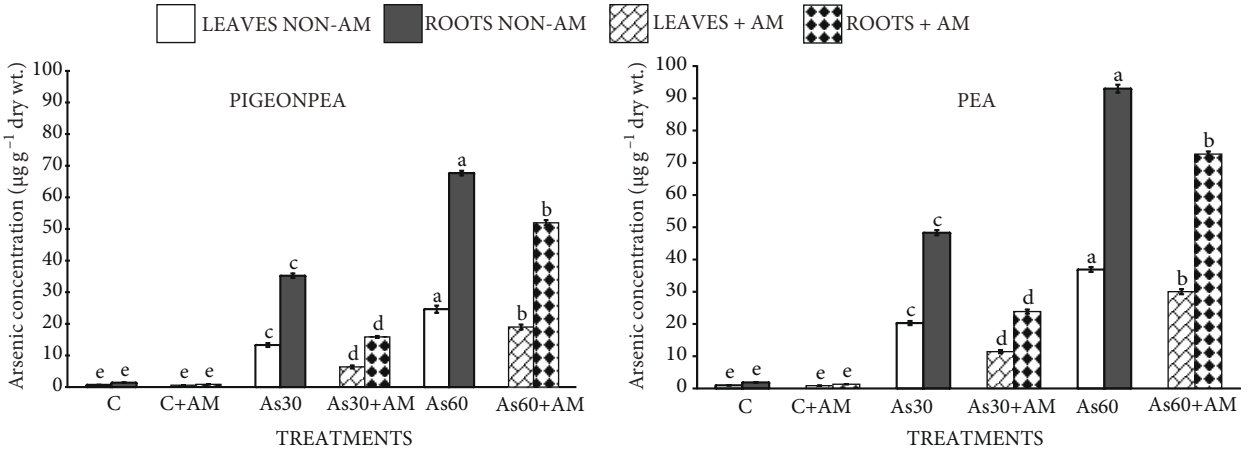

Figure 1. Effect of arbuscular mycorrhizal (AM) inoculations on arsenic (As) content (µg g^{-1} DW) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

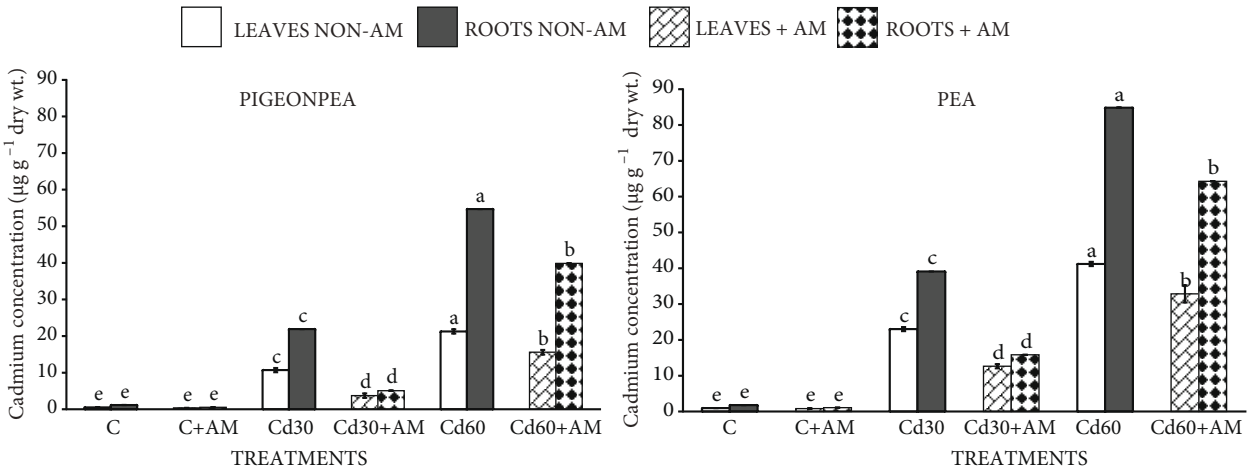

Figure 2. Effect of arbuscular mycorrhizal (AM) inoculations on cadmium (Cd) content (µg g^{-1} DW) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

3.3. Mycorrhizal frequency

No root colonization was observed in microscopic assessments of uninoculated plants. Percentage of mycorrhizal colonization declined with increase in metal(loid) concentration; however, the decrease was greater in pea than pigeonpea. Moreover, the mycorrhizal association with the root systems of plants was significantly higher in Cd-contaminated soil when compared with As (Table 1). There was lower negative correlation between MF and metal(loid) concentration in pigeonpea [As- roots (r_p): –0.833, leaves (r_p): –0.813; Cd- roots (r_p): –0.755, leaves (r_p): –0.729] than pea [As- roots (r_p): –0.918, leaves (r_p): –0.886; Cd- roots (r_p): –0.844, leaves (r_p): –0.807].

3.4. Nutrient status

Presence of As or Cd significantly deteriorated nutrient (N, P, K^+) status and the deterioration considerably varied depending on metal(loid) concentration, species, and tissue. Compared with the control treatment, percent nutrient loss in the roots and leaves increased with increasing concentrations of As and Cd in the soil and the rate of increase was higher in plants exposed to As (Figures 3–5). P/As and P/Cd ratios declined with increasing metal(loid) concentrations, with higher decline in pea as compared to pigeonpea (Table 3). Further, P/As ratio was more negatively affected than P/Cd ratio and was better maintained in leaves than roots. However,

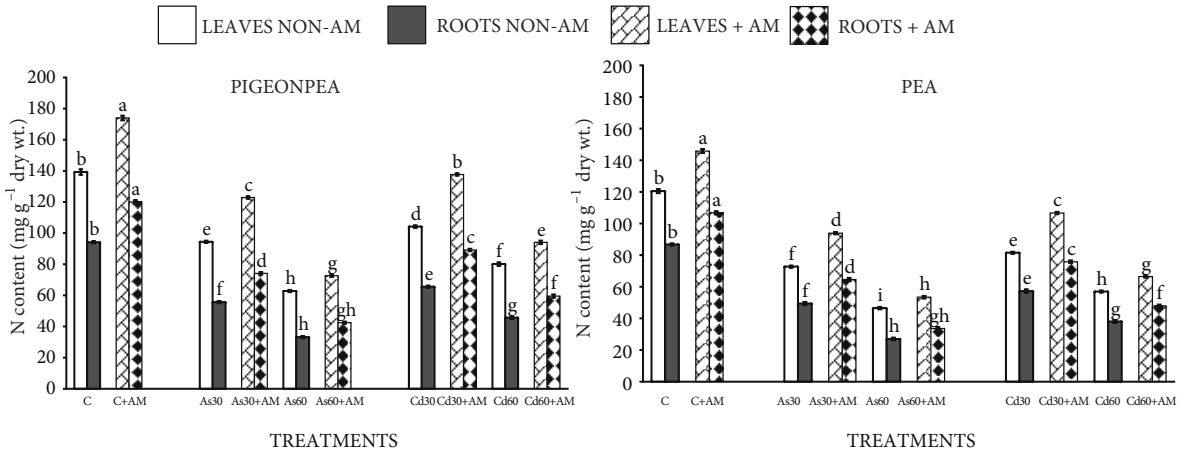

Figure 3. Effect of arbuscular mycorrhizal (AM) inoculations on nitrogen (N) content (mg g^{-1} DW) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

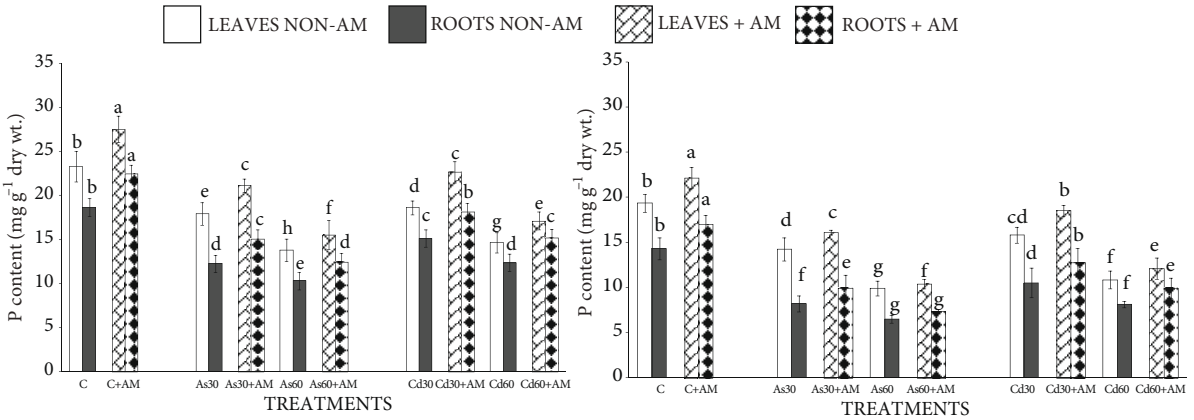

Figure 4. Effect of arbuscular mycorrhizal (AM) inoculations on phosphorus (P) content (mg g^{-1} DW) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

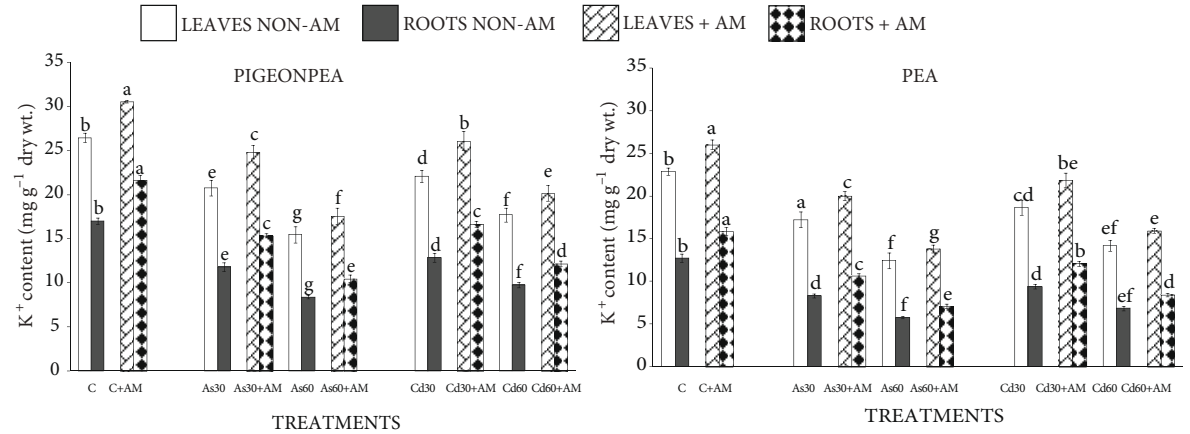

Figure 5. Effect of arbuscular mycorrhizal (AM) inoculations on potassium (K) content (mg g^{-1} DW) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

mycorrhizal pigeonpea plants maintained better nutrient status as compared to mycorrhizal pea plants, which was also depicted by better positive correlation between root P and MC in pigeonpea (r_p = 0.967) than pea (r_p = 0.955). Superior nutrient status was also interpreted by higher P/As and P/Cd ratios in mycorrhizal plants as compared to the respective nonmycorrhizal plants (Table 3). Statistically significant As × AM and Cd × AM interactions confirmed mycorrhiza-mediated amelioration of As- and Cd-induced nutrient imbalance (Table 2).

3.5. Oxidative burden

The inhibition of growth was accompanied by an increase in oxidative stress indicators (MDA, H_2O_2), which were positively correlated with As and Cd. MDA concentration considerably increased in stressed plants in concurrence with metal(loid) doses. Increased oxidative stress led to higher membrane disintegration in pea than in pigeonpea. Moreover, in both species, As was more injurious (pigeonpea roots, r_p = 0.903; leaves, r_p = 0.830; pea roots, r_p = 0.928; leaves, r_p = 0.889) than Cd (pigeonpea roots, r_p = 0.859; leaves, r_p = 0.775; pea roots, r_p = 0.892; leaves, r_p = 0.885) in decreasing the membrane permanence with respect to tissue susceptibility, i.e. roots induced more lipid peroxidation than leaves (Table 4). Higher concentrations of both metal(loid)s (60 mg kg^{-1}) caused more detrimental effects than lower concentrations (30 mg kg^{-1}). Association with *F. mosseae* decreased membrane peroxidation, which was reflected by lower MDA concentrations in AM plants. Significant As × AM and Cd × AM interactions highlighted the positive influence of AM in maintaining the membrane viability in response to As and Cd treatments (Table 2). Increasing concentrations of As caused more rigorous oxidative stress than Cd (pigeonpea roots, r_p = 0.699; leaves, r_p = 0.781; pea roots, r_p = 0.839; leaves, r_p = 0.856), which was reflected in the form of higher induction of H_2O_2 in As-stressed plants (pigeonpea roots, r_p = 0.914; leaves, r_p = 0.864; pea roots, r_p = 0.967; leaves, r_p = 0.912). Higher P uptake by mycorrhizal plants assisted in protecting their roots and leaves from oxidative bursts of ROS by decreasing MDA and H_2O_2, as indicated by a negative correlation between them (MDA: pigeonpea roots, r_p = 0.793 and leaves, r_p = 0.748; pea roots, r_p = 0.866 and leaves, r_p = 0.797; H_2O_2: pigeonpea roots, r_p = 0.895 and leaves, r_p = 0.825; pea roots, r_p = 0.912 and leaves, r_p = 0.904).

3.6. Antioxidants

In order to understand the antioxidant defense mechanisms under As and Cd stress, SOD (Figure 6), CAT (Figure 7), and POX (Figure 8) activities were analyzed in pigeonpea and pea. Results indicated that pigeonpea was more capable in combating metal(loid)-induced oxidative damage, with more efficient antioxidant machinery than pea. Moreover, Cd proved to be more potent inducer of these antioxidant metabolites as compared to As. The presence of As and Cd and their interactions with AM fungi displayed their significant effects on the production and accumulation

Table 3. Effect of arbuscular mycorrhizal (AM) inoculations on P/AS and P/Cd of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Treatments were designed as uninoculated controls (C) and arsenic (As) and cadmium (Cd) stress (30, 60 mg kg^{-1}) with and without arbuscular mycorrhizae (AM). Each value is the mean of six replicates ± SE. Means followed by the same letter within a column are not significantly different at P < 0.05 as determined by Duncan's multiple range test.

Treatments	Parameters measured							
	P/As				P/Cd			
	Pigeonpea		Pea		Pigeonpea		Pea	
	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves
Control (C)	13.23^{b} ± 0.544	31.95^{b} ± 1.039	7.66^{b} ± 0.799	19.75^{b} ± 0.786	15.94^{b} ± 0.977	45.92^{b} ± 1.566	8.05^{b} ± 0.765	20.55^{b} ± 0.958
C + AM	25.73^{a} ± 0.954	46.71^{a} ± 1.567	12.52^{a} ± 0.745	25.50^{a} ± 0.996	42.41^{a} ± 1.897	74.31^{a} ± 3.495	16.00^{a} ± 1.033	26.96^{a} ± 1.044
As_{30}	0.34^{d} ± 0.045	1.35^{d} ± 0.056	0.17^{d} ± 0.056	0.70^{d} ± 0.022	-	-	-	-
As_{30} + AM	0.95^{c} ± 0.086	3.32c ± 0.236	0.41^{c} ± 0.014	1.41^{c} ± 0.192	-	-	-	-
As_{60}	0.15^{ef} ± 0.013	0.55^{f} ± 0.047	0.07^{f} ± 0.009	0.26^{f} ± 0.034	-	-	-	-
As_{60} + AM	0.23^{e} ± 0.019	0.81^{e} ± 0.036	0.10^{e} ± 0.032	0.34^{e} ± 0.045	-	-	-	-
Cd_{30}	-	-	-	-	0.69^{d} ± 0.078	1.74^{d} ± 0.234	0.26^{d} ± 0.019	0.68^{d} ± 0.039
Cd_{30} + AM	-	-	-	-	3.56^{c} ± 0.345	6.07^{c} ± 0.457	0.80^{c} ± 0.095	1.46^{c} ± 0.096
Cd_{60}	-	-	-	-	0.22^{ef} ± 0.0342	0.68^{ef} ± 0.035	0.09^{ef} ± 0.004	0.26^{ef} ± 0.005
Cd_{60} + AM	-	-	-	-	0.38^{e} ± 0.0696	1.10^{e} ± 0.166	0.15^{e} ± 0.009	0.36^{e} ± 0.008

Table 4. Effect of arbuscular mycorrhizal (AM) inoculations on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentration (nmol gm^{-1} FW) in leaves (L) and roots (R) of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Treatments were designed as uninoculated controls (C) and arsenic (As) and cadmium (Cd) stress (30, 60 mg kg^{-1}) with and without arbuscular mycorrhizae (AM). Each value is the mean of six replicates ± SE. Means followed by the same letter within a column are not significantly different at P < 0.05 as determined by Duncan's multiple range test.

Treatments	Parameters measured							
	H_2O_2				Malondialdehyde (MDA)			
	Pigeonpea		Pea		Pigeonpea		Pea	
	L	R	L	R	L	R	L	R
Control (C)	$3.040^{d} \pm 0.108$	$4.060^{de} \pm 0.151$	$3.780^{e} \pm 0.116$	$4.890^{f} \pm 0.091$	$10.37^{ef} \pm 0.577$	$15.78^{f} \pm 1.16$	$13.77^{f} \pm 0.881$	$18.76^{e} \pm 1.460$
C + AM	$2.196^{f} \pm 0.103$	$3.187^{f} \pm 0.093$	$3.084^{f} \pm 0.136$	$4.290^{g} \pm 0.153$	$9.25^{f} \pm 0.608$	$10.64^{g} \pm 1.632$	$12.83^{f} \pm 0.577$	$14.94^{f} \pm 1.59$
As_{30}	$3.816^{b} \pm 0.084$	$5.279^{c} \pm 0.140$	$4.986^{c} \pm 0.287$	$6.749^{c} \pm 0.250$	$15.44^{c} \pm 0.577$	$23.85^{c} \pm 1.54$	$21.29^{c} \pm 0.697$	$33.11^{b} \pm 1.678$
As_{30} + AM	$3.128^{c} \pm 0.160$	$3.960^{e} \pm 0.183$	$4.163^{de} \pm 0.229$	$5.450^{e} \pm 0.187$	$13.15^{d} \pm 1.58$	$19.11^{e} \pm 1.008$	$18.74^{de} \pm 2.075$	$28.36^{c} \pm 0.286$
As_{60}	$4.265^{a} \pm 0.131$	$6.324^{a} \pm 0.166$	$5.564^{a} \pm 0.070$	$8.286^{a} \pm 0.174$	$18.14^{a} \pm 0.635$	$27.76^{a} \pm 1.756$	$25.21^{a} \pm 1.050$	$37.63^{a} \pm 0.548$
As_{60} + AM	$3.77^{bc} \pm 0.096$	$5.45^{b} \pm 0.154$	$5.08^{b} \pm 0.156$	$7.48^{b} \pm 0.097$	$16.55^{bc} \pm 0.753$	$24.84^{b} \pm 1.873$	$23.97^{b} \pm 0.764$	$35.82^{b} \pm 1.266$
Cd_{30}	$3.427^{c} \pm 0.230$	$4.917^{cd} \pm 0.126$	$4.440^{d} \pm 0.145$	$6.397^{d} \pm 0.270$	$14.04^{d} \pm 1.154$	$20.56^{d} \pm 2.558$	$19.12^{d} \pm 0.577$	$28.04^{c} \pm 0.898$
Cd_{30} + AM	$2.547^{e} \pm 0.194$	$3.271^{f} \pm 0.194$	$3.423^{ef} \pm 0.145$	$4.738^{f} \pm 0.282$	$11.15^{e} \pm 0.909$	$13.28^{f} \pm 1.609$	$16.63^{e} \pm 2.120$	$21.89^{d} \pm 0.804$
Cd_{60}	$3.834^{b} \pm 0.156$	$5.688^{b} \pm 0.103$	$4.996^{b} \pm 0.080$	$7.304^{b} \pm 0.206$	$17.28^{b} \pm 1.155$	$23.46^{c} \pm 0.752$	$23.82^{b} \pm 0.370$	$32.32^{bc} \pm 1.06$
Cd_{60} + AM	$3.27^{c} \pm 0.116$	$4.33^{d} \pm 0.254$	$4.36^{d} \pm 0.147$	$6.33^{d} \pm 0.233$	$15.12^{c} \pm 0.954$	$18.89^{e} \pm 0.675$	$21.78^{c} \pm 1.990$	$28.49^{c} \pm 0.618$

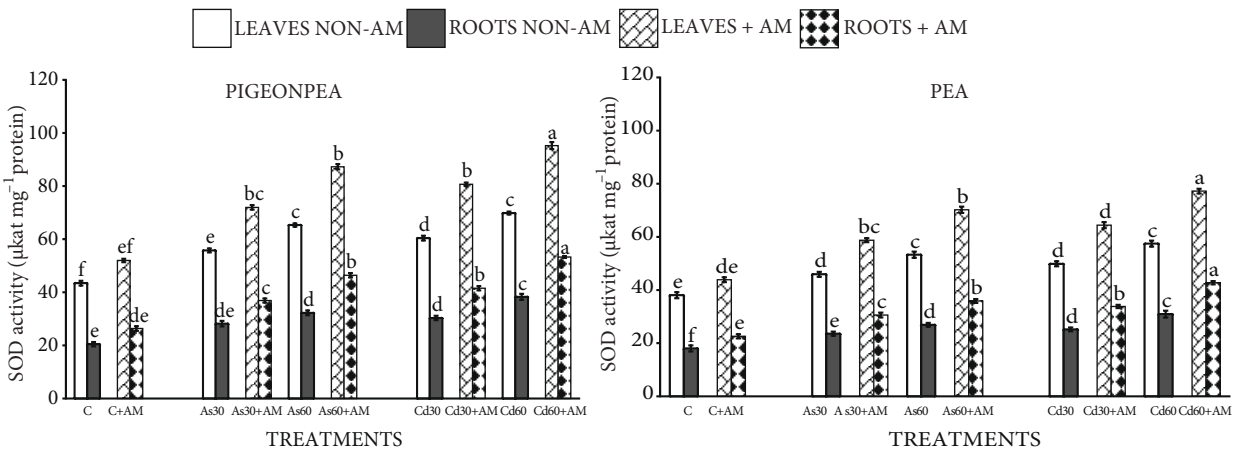

Figure 6. Effect of arbuscular mycorrhizal (AM) inoculations on superoxide dismutase (SOD) activity (µkat mg^{-1} protein) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at P < 0.05 as determined by Duncan's multiple range test.

of antioxidants. Pearson's correlation coefficient revealed that H_2O_2 was positively correlated (P < 0.05) with SOD (pigeonpea roots, $r_p = 0.623$ and leaves, $r_p = 0.586$; pea roots, $r_p = 0.621$ and leaves, $r_p = 0.513$), CAT (pigeonpea roots, $r_p = 0.830$ and leaves, $r_p = 0.805$; pea roots, $r_p = 0.802$ and leaves, $r_p = 0.663$), and POX (pigeonpea roots, $r_p = 0.810$ and leaves, $r_p = 0.731$; pea roots, $r_p = 0.720$ and leaves, $r_p = 0.641$). For MDA also, correlations were positive with all three antioxidative enzymes, SOD (pigeonpea roots, $r_p = 0.799$ and leaves, $r_p = 0.740$; pea roots, $r_p = 0.644$ and leaves, $r_p = 0.545$), CAT (pigeonpea roots, $r_p = 0.971$ and leaves, $r_p = 0.746$; pea roots, $r_p = 0.723$ and leaves, $r_p = 0.555$), and POD (pigeonpea roots, $r_p = 0.732$ and leaves, $r_p = 0.571$; pea roots, $r_p = 0.719$ and leaves, $r_p = 0.516$). Association with *F. mosseae* further boosted the activity of these ROS scavengers, especially SOD, with higher activity

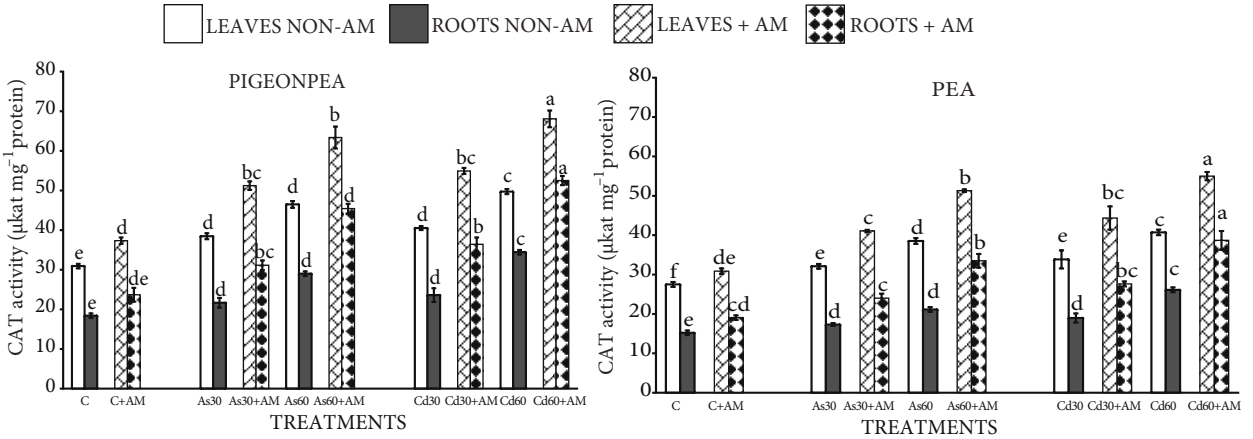

Figure 7. Effect of arbuscular mycorrhizal (AM) inoculations on catalase (CAT) activity (µkat mg^{-1} protein) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

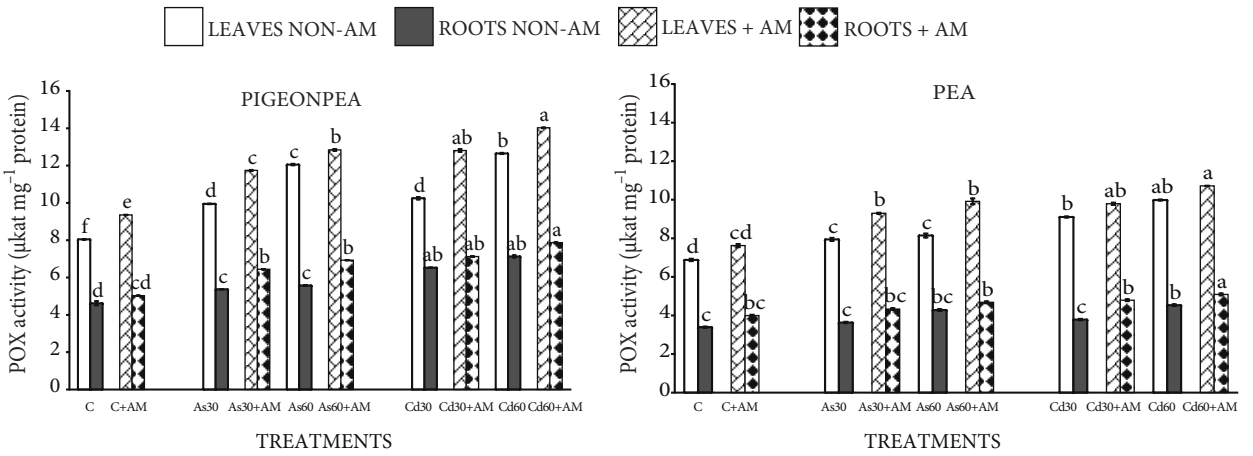

Figure 8. Effect of arbuscular mycorrhizal (AM) inoculations peroxidase (POX) activity (µkat mg^{-1} protein) in roots and leaves of pigeonpea (Sel 85 N) and pea (PB 89 2008) plants under arsenic (As) and cadmium (Cd) stress. Each value is the mean of six replicates ± SE. Means followed by the same letter within an organ in each species are not significantly different at $P < 0.05$ as determined by Duncan's multiple range test.

in Sel 85N plants thereby helping them to cope better than PB 89 2008 plants. Plants were able to withstand oxidative stress more competently than stressed non-AM plants as mycorrhizal symbiosis helped in more evident buildup of enzymatic antioxidants in colonized plants. Statistical analysis indicated that levels of antioxidant enzymes were significantly augmented by As, Cd, AM fungi, and their interactions, augmentation being concentration- and tissue-dependent (Table 2).

4. Discussion

A significant decline in growth was observed under As and Cd stress in both pigeonpea and pea. Decline in growth was proportionate to metal(loid) uptake and accumulation. Although the metal(loid) concentrations applied were the same, As was found to be significantly more phytotoxic as compared to Cd. The distinct action of each metal(loid) could be due to the different chemical properties and/or different rate of accumulation in plant tissues (Rellán-Álvarez et al., 2006). The reason behind higher toxicity of As may be the fact that As is more readily taken up by the roots than Cd and is easily translocated through the apoplast to the photosynthetic organs, indicating its higher mobility in the soil–root interface. Since As and Cd exist in solution as ions of different charges, they may interact on the root surface and then affect uptake of other element by plants (Cao et al., 2007). Most As and Cd appeared to be accumulated in roots and only a limited

amount was translocated to the aboveground parts, suggesting their low mobility in both species. Greater capacity of a species to accumulate appreciable amounts of metal(loid)s in roots and their restricted transfer to the aerial tissue signifies its better ability to tolerate metal(loid) stress (Ishtiaq and Mahmood, 2011; Garg and Singla, 2012; Talukdar, 2013). Tiwari et al. (2014) found enhanced expression of transporters (*OsNRAMP1*) in transgenic lines of rice, which might have contributed to considerable accumulation of Cd and As in roots. Thus, roots function as trap organs for metal(loid)s, acting as an efficient barrier against metal(loid) translocation to shoots where several physiological and metabolic processes are active (Metwally et al., 2005; Singh N et al., 2006; Singh S et al., 2006; Aibibu et al., 2010; Lee and Yu, 2012; Garg and Kaur, 2013b; Campos et al., 2014). Disorders in mineral metabolism and ion homeostasis under metal(loid) stress were of higher order in pea than pigeonpea, with greater variations observed in As-stressed plants than Cd. Lower P/As ratio under stress reinforces the suggestion that there is competition for uptake between arsenate and phosphate. Higher tolerance of pigeonpea may be related to less metal(loid) absorption and higher phosphate uptake and translocation than in pea. Higher accumulation of P in tolerant species as compared to As toxicity could be due to the presence of P transporters with lower As affinity (Lee et al., 2003; Zhao et al., 2010; Campos et al., 2014). However, less decline of P/Cd than P/As could be due to no direct competition for uptake between cadmium and phosphate. Reduction of ion uptake could be due to inhibition of root function since root growth was severely affected in the presence of metal(loid)s. It has been suggested that macronutrient uptake and distribution are affected by the presence of metal(loid)s through antagonistic processes mediated either by competition for binding sites or transporters that involve sulfhydryl, carboxylic groups, and Pi transporter proteins (Esteban et al., 2003; Tu and Ma, 2003; Luan et al., 2008; Gomes et al., 2012).

The present investigations indicated that metal(loid)s provoked cellular disruption in plants through the induction of oxidative stress caused by increased generation of H_2O_2 and MDA. ROS overproduction under metal(loid) stress is responsible for oxidative damage, which can trigger signal transduction events (Stoeva et al., 2005; Smeets et al., 2008; Gunes et al., 2009; Deng et al., 2010; Liu YT et al., 2011; Cho et al., 2012). ROS stimulate a chain-like peroxidation of polyunsaturated fatty acids of the lipid bilayer in cellular membranes that increases the amount of MDA in plants (Hatata and Abdel-Aal, 2008; Shri et al., 2009). Oxidative DNA damage could lead to molecular and genetic instability (Peralta-Videa et al., 2009; Duquesnoy et al., 2010), which could be the reason for more damage in pea than pigeonpea.

With application of metal(loid)s in the present study, an activation of the antioxidant apparatus in pigeonpea as well as pea indicated that increased oxidative burst is not attributed to the regulation of only one enzyme, but rather to the complex upregulation of several ROS-scavenging enzymes (SOD, CAT, POX). An increase in the activity of antioxidant enzymes can be attributed to the induced transcription of their genes, probably mediated by free radicals (Duquesnoy et al., 2010; Nguyen et al., 2014; Ovečka and Takáč, 2014). In the present study, antioxidative activities varied among different organs, which could be related to the amount of metal(loid)s that accumulated differentially in the analyzed organs, to a much higher extent in roots than in leaves. Moreover, higher enzyme activities in pigeonpea than pea indicated that the former had superior capacity to adapt under metal(loid) toxicity by developing an efficient antioxidant defense system.

The present investigations revealed that colonization of pigeonpea and pea plants by *F. mosseae* was efficient, suggesting that roots provided an adequate environment protecting fungal growth from the toxic effects of metal(loid)s. Thus, mitigation of depressive effects of metal(loid)s was possibly due to an effective symbiosis of plant roots with mycorrhizal endosymbionts. Results depicted that MF was reduced in As-treated plants more severely than in Cd-treated plants. Decrease in colonizing frequency was more pronounced in pea as compared to pigeonpea. A gradual reduction in mycorrhizal colonization by metal(loid)s was reported by Engqvist et al. (2006), Andrade et al. (2008), Garg and Aggarwal (2011), Garg and Bhandari (2012), and Garg and Singla (2012). In the present investigation, mycorrhizal colonization significantly increased the biomass of pigeonpea and pea plants under both toxicities, suggesting that inoculation with mycorrhizae may be a viable technology to attenuate the negative effects of both the metal(loid)s and improve the overall health of mycorrhizal plants. Maximum metal(loid) tolerance was achieved through mycorrhizal inoculation at 30 mg kg^{-1} metal(loid)s in the rooting medium, where almost complete amelioration of negative effects of the metal(loid)s was observed. The significant interaction of species and AM fungi on the concentrations of N, P, and K^+ indicated that symbiosis between the two symbionts resulted in differential nutrient uptake efficiencies for different AM fungi and host plant combinations. Enhanced growth of mycorrhizal plants in metal(loid) environments has been related to mycorrhizae-mediated enhancement of mineral nutrition as they can translocate inorganic phosphate by increasing the interface between plants and the soil environment through extensive extraradical hyphal networks (Ezawa et al., 2002; Pichardo et al., 2012; Chan et al., 2013; Zaefarian

et al., 2013), dilution effect due to significant increase in root dry weight (Dong et al., 2008; Bona et al., 2011; Chen et al., 2013; de Melo Rangel et al., 2014), and superior branching pattern of roots (Vogel-Mikuš and Regvar, 2006; Smith and Read, 2008). Constitutive expression or induction of nutrient transporters during symbiosis could improve translocation of mineral elements to the plant (Harrison et al., 2002; Giasson et al., 2008; Wang et al., 2008; Christophersen et al., 2012). This alleviative influence can be attributed not only to AM-mediated nutritional effects, but also to the impact of AM fungi on metal(loid) distribution at the soil–fungus–plant interface. From our study, it was deduced that mycorrhizal symbionts caused reduction in metal(loid) concentrations and alleviated metal(loid)-induced nutritional disturbances and oxidative stress. Both As and Cd were found to be immobilized by efficient mycorrhizal symbiosis and mainly retained in the roots (as shown by higher negative correlation between MF and metal(loid) concentrations in roots than leaves), with pigeonpea showing higher metal(loid) retention than pea. Lower negative correlation between MF and metal(loid) concentrations in pigeonpea indicated that relatively less decline of MF under stress led to lower uptake of metal(loid)s. Lower negative correlation of MF with Cd as compared to As indicated that mycorrhizal colonization restricted Cd uptake more efficiently than that of As. Fungi may immobilize metals in several ways, including secretion of special compounds such as glomalin (González-Chávez et al., 2009; Redon et al., 2009; Miransari, 2010), as organic substances as methyl donors for biomethylation of metal(loid)s into less toxic methylated forms (Mukhopadhyay et al., 2002; Schmidt et al., 2004); precipitation of HMs in polyphosphate granules in the soil (Gaur and Adholeya, 2004; Saraswat and Rai, 2011; Muleta and Woyessa, 2012); binding of metals to chitin in the fungal walls (Joner et al., 2000; Rivera-Becerril et al., 2002; Lingua et al., 2008; Miransari, 2011, Danesh et al., 2013); and reduction of metal by extraradical hyphae followed by complexation with reduced iron(II) carbonate or hydroxides (González-Chávez et al., 2014),which may physically minimize or exclude the entry of metals into host plants. Increased tolerance to As due to high P/As ratio in mycorrhizal plants was reported by Xu et al. (2008), Wu et al. (2013), and de Melo Rangel et al. (2014). AM inoculation minimizes the translocation of As to the shoots while promoting P uptake (Ultra et al., 2007; Chen et al., 2007). Mycorrhizal roots could solubilize P, thereby increasing its availability for roots. Thus, release of phytoavailable As due to the displacement of adsorbed AsV by phosphate could be hard to detect in mycorrhizae-treated rhizosphere, thereby increasing the plant P/As ratio (Sakurai et al., 2001; Christophersen et al., 2009). The AM Pi-uptake pathway can compensate for any loss of activity of the direct uptake pathway in P absorption under As toxicity (Christophersen et al., 2009; Chen et al., 2013; Grønlund et al., 2013). On the other hand, improved P/Cd ratio in mycorrhizal plants could be due to increased uptake of P and reduced Cd adsorption. Our investigations revealed that AM fungi alleviated oxidative stress generated by both As and Cd, which was evident in the form of decreased H_2O_2 and MDA levels and enhanced antioxidant levels, thus restoring membrane integrity and functionality. This could be due to the high P-status of mycorrhizal plant that protects plant membranes from metal(loid)-induced oxidative stress (Gunes et al., 2009; Yu et al., 2009) and/or modulation of mycorrhizal colonization on the performance of plants grown under metal(loid) stress (Ouziad et al., 2005; Hildebrandt et al., 2007; Azcón et al., 2009; Lui LZ et al., 2011; Bhaduri and Fulekar, 2012; Garg and Aggarwal, 2012; Garg and Kaur, 2013a).

The mutualistic nature of AM associations significantly modulated growth of pigeonpea and pea plants by stimulating activity of the antioxidative enzymatic pool, thereby lowering oxidative stress markers and augmenting nutritional status under stressed environments. The ability of pigeonpea to survive better in a metal(loid)-contaminated environment than pea indicated its better genetic and metabolic strength. Differential symbiotic response of the two species and their correlations with metal(loid) toxicity suggest exploration of tolerant species with effective mycorrhizal colonization for further bioremediation studies. The differential metal tolerance as well as mycorrhizal benefits observed in these two species of the same family suggests that host plant inherent tolerance as well as mycorrhizal symbiotic efficiency are fundamental for survival in polluted soils.

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