Chapter 5

Short-Term Response of Plants Grown under Heavy Metal Toxicity

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Additional information is available at the end of the chapter

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Abstract

Sorghum vulgare L. plants when exposed to cadmium nitrate with the concentrations of 70 and 150 ppm per kg of soil for 90 days exhibited phytotoxic responses. The observations of specific responses were dependent on treatment combinations. The significant hazardous effects and oxidative damage of cadmium nitrate (70 and 150 ppm) were evident by increased MDA content and hydrogen peroxide content. However, these responses were reversed by exogenous application of putrescine (2.5 and 5.0 mM) and mycorrhiza (*Glomus*; 150 inoculants per kg of soil), more so, in their combined treatment, at different DAS. But combined treatment of putrescine and mycorrhiza enhanced the stability of sorghum by reducing the ROS production in plant cells. On the basis of the data obtained, it is concluded that plants responded up to 70 ppm cadmium nitrate with stress-induced responses, which were ameliorated by combined application of putrescine and *Glomus* mycorrhiza.

Keywords: agriculture, biotic, cadmium, density, economic, forage

1. Introduction

Cadmium (Cd) is one of the components of the earth's crust and present in several places or in different ecosystems on earth i.e., terrestrial, aquatic and others. Benavides et al. [1] reported that cadmium is one of the most hazardous heavy metals in the atmosphere, soil and aquatic system which is finally going into our food chain and responsible for serious environmental problem leading to the health hazards in the living organisms, for instance, mutagenesis, lung cancer, convulsion and brain damage. The alleviation or inhibition of cadmium in plants has, therefore, caused extensive attention of the whole society [2, 3]. It is must to know about cadmium, its physical and chemical properties, isotopic studies In the atmosphere, Cd can enter by burning of coal, mining of metals as well as refining process which may lead to rise in Cd level in the soil by atmospheric fallout also. If we see the atmospheric fallout of Cd from atmospheric air, it follows in the order Remote area < Rural area < Urban areas. Due to long term effects of cadmium, the countries fixed its tolerance limit. The European Economic Committee proposed the concept of PTE i.e. Potential Toxic Elements. PTE for the Cadmium in soil is 1.0–3.0 mg/kg of dry soil [4]. If we think about the aging of the metal in the soil, then a distinction should be made between persistence of total metals in the soil and the persistence of bioavailable forms of metals. This aging of the metals depends on soil acidity. Evidence of the aging process is provided by studies of metal extractability and liability. There are many terms used to describe and categorize metals, including traces metals, transition metals, micronutrients, toxic metals, heavy metals. Bjerrum's [5] meaning of "heavy metals" depends on the density of the natural type of the metal, and he arranges "heavy metals" as those metals with basic densities over 7 g cm^{-3}. In 1964, the editorial manager of Van Nostrand's Worldwide Reference book of Concoction Science and in 1987, the editors of Allow and Hackh's Compound Lexicon included metals with a thickness more noteworthy than 4 g cm^{-3}. The fortune of various metals viz. Cr, Ni, Cu, Mn, Hg, Compact disc, Pb and metalloids, including As, Sb, and Se, in the encompassing outskirts is of huge misgiving [6], especially close previous mine locales, dumps, following heaps, and impoundments, yet in addition in urban and mechanical focuses. Cause of cadmium in soil is portrayed as agrarian squanders (20%), ooze (38%), manures (2%) and barometrical aftermaths (40%) [7]. Cadmium is one of the most toxic elements with reported carcinogenic effects in humans [8]. Cadmium and cadmium compounds are, compared to other heavy metals, relatively water soluble and mobile compound in most soils, generally more bio- available and tends to bio- accumulate. It induces cell injury and death by interfering with calcium (Ca) regulation in biological system. Cadmium is not essential for plant or animal life (IPCS monographs). Terrestrial plants may accumulate cadmium in the roots where it is found bound to the cell walls [9]. The pH level is one of the most important factors controlling cadmium absorption. The translocation of cadmium is significant in above ground parts with respect to copper and lead. Concentration in roots represents only 2–5 times that in the above ground parts, but cadmium is transferred only with difficulty to reproductive or storage organs of the plant [10]. The polyamine (PA) putrescine (Put) is an important modulator for the mitigation of diverse of stress in the plants [11–18], and it also plays a significant role in the apoptosis and programmed death in both animals and plants [19]. Chemically the PAs are undersized, +ve charged aliphatic amines at cellular pH values and, consequently, bind opposite charged molecules, viz. nucleic acids, acid phospholipids and proteins, consequently [14, 20]. The cellular level of free amino acids in plants depends on the synthesis and degradation of PAs, their bounding with phenolic acids and intracellular transport [14, 15, 21–23]. The inhibitory effects of Cd are manifestations of oxidative stress, which finally reduces crop productivity. Polyamines are involved in abiotic stress tolerance in plants. Increased polyamines level in stressed plants has adaptive significance because of their involvement in the regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss and stimulation of protein, nucleic acid and protective alkaloids. Interaction of polyamines with membrane phospholipids implicates membrane stability under stress conditions. Polyamines additionally shield the layer from oxidative harm as they go about as free radical foragers. Reaction to abiotic damage and mineral supplement insufficiency is related with the generation of conjugated PAs in plants. Polyamine substances are adjusted in light of the introduction to overwhelming metals. These viably balance out and secure the film frameworks against the dangerous impacts of metal particles. The mycorrhiza *Glomus* is the significant inhibitor of mobility of cadmium ion into the soil solution and defends the plants from cadmium toxicity. Colonization of AM fungal was observed in highly contaminated soil [24, 25]. *Glomus mosseae* was reported in heavy metal contaminated sites [26]. External mycelium of *Glomus mosseae* produce a type of protein called Glycoprotein (Glomalin), which has heavy metal binding sites [27]. Cadmium metals accumulate at these binding sites. The antioxidant level is also increased as a result of association of AM fungi with plants [28–30]. Some fungal strains were isolated in the past which were resistant to heavy metal contamination. Mycorrhizal fungi overcome the stress of heavy metal contamination [31, 32].

We are interested in the study of the sorghum plant because of several reasons, which we find more relevant:

[A] The research of the poisoning effect of the heavy metal Cd on plant mainly focuses on food crops such as rice, wheat and maize, but less on sorghum.

[B] Sorghum frequently used as animal feed so quality assurance related to heavy metals is not understood very well, hence studies on contamination of heavy metal in fodder crop is required.

[C] The sensitivity level of sorghum for cadmium varies. By deciding the sensitivity one can find genes which are responsible for the same. Literature indicates that tolerance level of a heavy metal for sorghum is 1000 ppm onwards. It indicates that it has a wide range of tolerance. The genomic size of the sorghum ranges from 700 to 772 Mbp, and it has been well sequenced. So, search for genes responsible for tolerance is not that difficult now. After finding the gene, it can be transferred into the sensitive crops such as cow pea and other leafy vegetables. Finally, we can get transgenic of the heavy metal tolerance. Da-Lin et al. [33] reported some important changes in growth and physiological characteristics of the sorghum plant under cadmium stress such as height, chlorophyll content, root activities and MDA content. They concluded that Cd effect was the manifestation of oxidative stress. Low concentration of cadmium can promote growth of hard wheat, while under a relatively high concentration, the growth of wheat and tillering were both inhibited and the degree varied among the various species. Liu's [34] research also showed that corn seedling's height under cadmium treatment was reduced significantly as the concentration of cadmium increased with prolonged growth period. These studies showed that lower concentrations of Cd stimulated increase of sorghum height, which may be related to the certain resistance of the sorghum genus plant. The higher concentrations hindered their development; explanations behind the restraining impact of substantial metals to plant development were presumably due to: [A] A progression of physical and chemical responses between the overabundance substantial metal and soil parts which changes soil properties, along these lines influencing soil fruitfulness [35, 36]. For instance, substantial metal contamination can improve the obsession of soil phosphorus, which influenced the plant engrossing phosphorus, consequently influencing the development of plants [37, 38].

[B] Substantial metal caused a lessening in plant photosynthesis, in this way decreasing the plant water and supplement adsorption, which influenced the ordinary development and improvement of sorghum [39].

There are large reports about substantial metal contamination influencing root exercises of Graminaceous plants. For instance, through the hydroponic way, Yang et al. [40] found the impact of sewage straightforwardly flooded on root and seedlings of the wheat. The outcomes demonstrated that the worry of sewage flooded quickened the decrease of wheat seedlings and root, diminishing the root number and the root exercises essentially. Jiang et al. [41] additionally demonstrated that tainted soil made the underlying foundations of the rice seedlings yellow and red, extended the rhizome, root shading was dark colored and yellow, while Huang et al. [42] demonstrated that under framework or soil with cadmium the root exercises altogether diminished.

So, our objective in the present study was to find out the oxidative damage induced by cadmium toxicity in sorghum and to assess the role of putrescine and mycorrhiza in the mitigation of cadmium induced stress responses in sorghum.

2. Material and methods

2.1. Selection of crop sorghum (*Sorghum vulgare* L.)

Sorghum is one of the main staple foods for the world's poorest and most food-unsecured people across the semi-arid tropics. Sorghum belongs to the grass family Graminae. It is a short-day C4 plant. The optimum photoperiod which induces flower formation is between 10 and 11 h. Sorghum is one of the main staple foods for the world's poorest and most food-unsecured people across the semi-arid tropics. Globally, sorghum is cultivated on 41 million hectares to produce 64.20 million tons, with productivity having around 1.60 tonnes per hectare ([96], Directorate of Sorghum Research, Hyderabad). With exception in some regions, it is mainly produced and consumed by poor farmers. India contributes about 16% of the world's sorghum production ([96], DSR Hyderabad). It is the fifth most important cereal crop in the country. In India, this crop was one of the major staple cereals during 1950s and occupied an area of more than 18 million hectares, but has come down to 7.69 million hectares ([96], Directorate of Sorghum Research, Hyderabad). Sorghum requires warm conditions, but it can be grown under a wide range of conditions. It is grown from sea level to as high as 1500 m. It can tolerate high temperature throughout its life cycle better than any crop. It can be grown in the areas having an average annual rainfall 60–100 cm. The minimum temperature for the germination of the seed is 7–10°C. It needs about 26–30°C for optimal growth. Sorghum is characterized by a vastly diverse germplasm in terms of phenotypic and morphological traits. Sorghum can be classified into four main groups depending on their production characteristic: grain sorghum, forage sorghum (FS), high-tonnage sorghum (energy) and sweet sorghum.

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Sorghum cultivars are now being considered as candidates in the search for efficient energy crops due to an increased interest in the conversion of biomass to energy.

2.2. Experimental site

The present investigation was carried out in the polyhouse and the laboratory of the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P., India. Its geographical location lies between 25°18′ N latitude to 83°03′E longitudinal and the elevation of the experimental site from the sea level is approximately 75.7 m above the mean sea level.

2.3. Climate condition

Varanasi falls in the Northern India belt of semi-arid to sub humid climate. The normal period of onset of monsoon is the third week of June in this region, which lasts up to the end of September or sometimes third week of October. The normal annual rainfall is about 1100 mm, of which 88% are received from June to September as monsoon season rain, 5–7% in October to December as post monsoon and about 3.3% from January to February as winter season or pre –monsoon rain. The temperature fluctuated in the range of 45–19°C as maximum and 28–7°C as a minimum. The mean relative humidity of the area is about 66%, which rises up to 92% during July to September and falls down to 39% during the end of April to early June.

2.4. Plant and mycorrhizal source

Disease free and healthy, bold seeds of sorghum cv. CSV15 were obtained from the Directorate of Sorghum Research, Hyderabad. The endomycorrhiza *Glomus mosseae* was obtained from the Tata Energical Research Institute, New Delhi.

2.5. Treatments detail

The pot experiment was conducted in the poly house of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, with one genotype of sorghum CSV 15. The pot size for the experiment was in the diameter of 30 and 25 cm in height and each of capacity with 10 kg of soil, with a small hole at the bottom. Pots containing soil mix (Soil + FYM in 3:1) are inoculated with seeds of sorghum. Targeted pots were inoculated with Endomycorrhiza *Glomus sp*. (150 inoculants per kg of soil), after that heavy metal stress was created in the plant by the exogenous application of cadmium nitrate in soil. Two best concentrations of heavy metals on the basis of initial screening were selected i.e., 70 and 150 ppm per kg of soil. Putrescine was applied at the rate of 2.5 and 5.0 mM through foliar spray in 7 day interval, starting from seven DAS up to a weak before 90 DAS. The various observations were taken in three stages such as 30, 60 and 90 days after sowing in the concerned pots. The detailed plan of treatments were: Control (T0),Control + Mycorrhiza (T1), Control + 2.5 mM Putrescine (T2), Control +5 mM Putrescine (T3), Control +2.5 mM Putrescine + Mycorrhiza (T4), Control +5 mM Putrescine + Mycorrhiza (T5), 70 ppm $Cd(NO_3)_2$ (T6), 70 ppm $Cd(NO_3)_2$ + Mycorrhiza (T7), 70 ppm $Cd(NO_3)_2$ + 2.5 mM Putrescine (T8), 70 ppm $Cd(NO_3)_2$ + 5 mM Putrescine (T9), 70 ppm $Cd(NO_3)_2$ + 2.5 mM Putrescine + Mycorrhiza (T10), 70 ppm $Cd(NO_3)_2$ + 5 mM Putrescine + Mycorrhiza (T11), 150 ppm $Cd(NO_3)_2$ (T12), 150 ppm $Cd(NO_3)_2$ + Mycorrhiza (T13), 150 ppm $Cd(NO_3)_2$ + 2.5 mM Putrescine (T14), 150 ppm $Cd(NO_3)_2$ + 5 mM Putrescine (T15), 150 ppm $Cd(NO_3)_2$ + 2.5 mM Putrescine + Mycorrhiza (T16), 150 ppm $Cd(NO_3)_2$ + 5 mM Putrescine + Mycorrhiza (T17).

2.6. Design and layout of experiment

The experiment was laid out in completely randomized design (CRD). There were 18 treatments including control. Each treatment was replicated five times.

2.7. Measurement of oxidative damage

Estimation of Lipid Peroxidation (malondialdehyde (MDA) content) in terms of Thiobarbituric Acid Reducing Substances (TBARS) content was estimated by the method given by Heath and Packer [43]. About 0.5 g of leaf tissues from control and treated groups were cut into small pieces and homogenized by the addition of 5 ml of 5% trichloroacetic acid (TCA) solution. The homogenates were then transferred into fresh tubes and centrifuged at 12,000 rpm for 15 min at room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA solution were added into a new tube and boiled at 96°C for 25 min. The tubes were transferred into an ice bath and then centrifuged at 10,000 rpm for 5 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm, 0.5% TBA in 20% TCA solution was used as the blank. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient as 155 M^{-1} cm^{-1}. Values of MDA contents were taken from measurements. Results were presented as μmoles MDA g^{-1} FW.

The H_2O_2 content was measured by the method given by Jana and Choudhuri [44]. One hundred mg root samples were extracted using 3 ml of 50 mM sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 6000 g for 15 min. To determine hydrogen peroxide levels, 3 ml of the extracted solution was mixed with 1 ml of 0.1% titanium sulfate in 20% (w/v) sulfuric acid and then centrifuged at 6000 g for 15 min. The intensity of the yellow color in the supernatant was measured at 410 nm. The hydrogen peroxide level was calculated using extinction coefficient 0.28 μM^{-1} cm^{-1} and was expressed as nmol H_2O_2 g^{-1} tissue FW.

2.8. Statistical analysis

All the numerical data obtained were analyzed through Statistical package of Origin 6.1-advance scientific graphing and data analysis [OriginLab Corporation, One RoundHouse Plaza, Northhampton, MA 01060]. Two-way ANOVA was performed for interaction between mycorrhiza and cadmium treatments. One Way ANOVA was performed for comparing the significance difference among individual means.

3. Results

3.1. Malondialdehyde (MDA) [nmole/ml FW] content

Effect of polyamine (putrescine), mycorrhiza and their combination on MDA [nmole/ml FW] was studied in sorghum variety CSV15 under the cadmium stress. Data were recorded at 30, 60 and 90 days after sowing (DAS) (**Figure 1**). It is evident that the average MDA was significantly increased by 35.64, 41.39 and 64.02% when exposed to heavy metal stress (T6) as compared to control (T0) at 30, 60 and 90 DAS of the interval. Similarly, when plants were exposed to higher doses of heavy metal (T12) then MDA was significantly increased by 62.28, 59.44 and 73.67% as compared to control (T0) on the dates of proposed interval. Exogenous application of endomycorrhiza in the soil (T7) showed the mitigation effect by reducing the MDA by 6.05, 2.87 and 7.87% as compared to T6 at 30, 60 and 90 DAS. During treatment, T13 was compared to T12, the MDA reduced significantly by 12.92, 9.85 and 9.63% at proposed DAS. In comparison to T6, the exogenous application of putrescine (T8) showed the mitigating effect by decreasing MDA by 1.84, 6.64 and 14.90% on proposed DAS. The average MDA was significantly reduced as compared to T6 by 11.15, 7.24 and 29.68% when treated with high dose of putrescine (T9) with respect to T8. Similarly, when treatment, T14 was compared with T12, the MDA reduced significantly by 21.47, 17.32 and 18.45% at proposed DAS. The average MDA was significantly reduced as compared to T12 by 33.17, 23.72 and 20.39% when treated

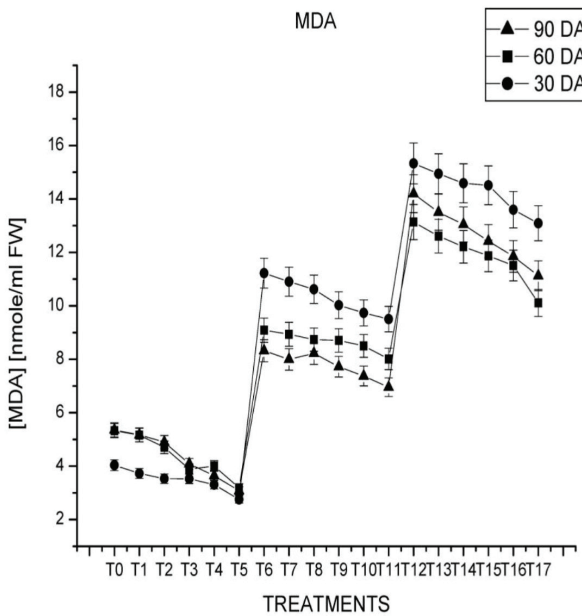

Figure 1. Effects of putrescine and *Glomus* on MDA content in sorghum grown in cadmium contaminated soil.

with high dose of putrescine (T15) with respect to T14. The combination of putrescine and mycorrhiza showed the best mitigation effect in treatment T10 by reducing MDA by 17.65, 11.03 and 36.89% with respect to treatment T6 at proposed DAS. When treatment T11 was compared with treatment T6 then MDA was reduced by 25.51, 20.21 and 42.59%, respectively. A similar effect was seen in the treatment (T16) with respect to treatment T12. In this treatment (T16), the MDA was found to decrease significantly by 43.79, 30.52 and 42.96%, respectively at proposed DAS. The treatment T17 was found to show better results; significant decrease in MDA by 57.32, 56.76 and 55.50% with respect to T12 was observed. So, the combination of putrescine and mycorrhiza showed the best combination for the mitigation of cadmium toxicity for the malondialdehyde.

3.2. H_2O_2 [μ mole/g FW] content

H_2O_2 [μ mole/g FW] content was studied in sorghum variety CSV15 under the cadmium stress. Data were recorded at 30, 60 and 90 days after sowing (DAS) (**Figure 2**). It is evident that the average H_2O_2 was significantly increased by 34.09, 32.02 and 19.37% when exposed to heavy metal stress (T6) as compared to control (T0) at 30, 60 and 90 DAS of the interval. Similarly, when plants were exposed to high doses of heavy metal (T12) then its H_2O_2 was significantly increased by 30.9, 37.55 and 34.66% as compared to control (T0) on the dates of proposed interval. Exogenous application of endomycorrhiza in the soil (T7) showed the mitigation effect by reducing the H_2O_2 by 32.47, 14.21 and 0.54% as compared to T6 at 30, 60 and 90 DAS. During treatment, T13 was compared to T12, the H_2O_2 reduced significantly by 1.13, 2.00 and 1.25% at proposed DAS. In comparison to T6, the exogenous application of

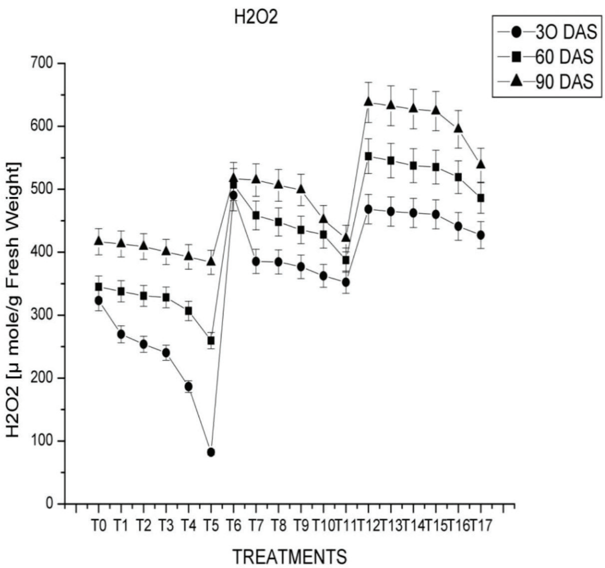

Figure 2. Effects of putrescine and *Glomus* on H_2O_2 content in sorghum grown in cadmium contaminated soil.

putrescine (T8) showed the mitigating effect by decreasing H_2O_2 by 32.74, 17.24 and 2.52% on proposed DAS. The average H_2O_2 was significantly reduced as compared to T6 by 35.10, 20.87 and 4.30% when treated with high dose of putrescine (T9) with respect to T8. Similarly, when treatment, T14 was compared with T12, the H_2O_2 reduced significantly by 1.82, 4.33 and 2.50% at proposed DAS. The average H_2O_2 was significantly reduced as compared to T12 by 2.5, 4.98 and 3.28% when treated with high dose of putrescine (T15) with respect to T14. The combination of putrescine and mycorrhiza showed the best mitigation effect in treatment T10 by reducing H_2O_2 by 17.65, 11.03 and 36.89% with respect to treatment T6 at proposed DAS. When treatment T11 was compared with treatment T6 then significant H_2O_2 was reduced by 42.70, 34.80 and 22.80%, respectively. A similar effect was seen in the treatment (T16) with respect to treatment T12 where H_2O_2 was found to decrease significantly by 8.41, 9.66 and 10.21%, respectively at proposed DAS. The treatment T17 was found to show better results; significant decrease in H_2O_2 by 12.70, 19.24 and 23.89% with respect to T12 was observed. So, the combination of putrescine and mycorrhiza showed the best combination for the mitigation of cadmium toxicity for the hydrogen peroxide.

4. Discussion

The ROS is conceivably unsafe to cell layers, bringing about oxidative corruption of film lipids (lipid peroxidation). Malondialdehyde (MDA) is one of the end breakdown results of lipid peroxidation and can be utilized as a marker of in vivo lipid peroxidation. Cadmium uptake triggered oxidative stress resulting in increased generation of Hydrogen peroxide and lipid peroxidation as reported in earlier studies [45, 46]. To cope with enhanced levels of oxidative stress, plants are equipped with antioxidant system that gets activated under cadmium stress [46, 47]. Ascorbate peroxidase, Peroxidase and Catalase play a crucial role during the degradation of the plant sample grown under cadmium stress. The statistical analysis in the treatment T17 showed significant reduction in MDA content because the most important property of PA conjugates with phenolic acids were their antioxidant characteristic, required by the plants to adapt under stress condition. Bors et al. [48] showed that conjugates with coffeic, cinnamic and ferulic acids displayed higher constraint of binding to reactive oxygen species (ROS) i.e., free polyamines are less efficient radical scavengers than their conjugates. Polyamines conjugation by transglutaminases, especially to Rubisco seems to have a significant role in protecting this protein from protease action, thus protecting its photosynthetic efficiency [49]. Therefore, PAs are likely to play a role in photosynthesis since they are capable of reversing stress-induced damage in the photosynthetic apparatus. Cross-linking of PAs mediated by transglutaminases might play a significant role in polyamine bioactivity for flower development and compatibility in reproduction [50]. Thus, conjugates involved in defense mechanisms against cadmium toxicity and also mediate the regulation of certain growth and developmental events [51, 52]. Similarly, the mycorrhiza *Glomus* acted as the potential inhibitor of mobility of cadmium ion into the soil solution and protected the plants from cadmium toxicity. AM fungal colonization was observed in highly contaminated soil [24, 25]. The presence of *Glomus mosseae* was also reported in heavy metal contaminated sites by some researchers [26]. The external mycelium of certain AM fungi produce a type of protein called Glycoprotein (Glomalin), which has heavy metal binding sites [53]. Heavy metals accumulate at these binding sites. The antioxidant level is also increased as a result of association of AM fungi with plants [28–30]. Some fungal strains were isolated in the past which were resistant to heavy metal contamination. Many mycorrhizal fungi overcome the stress of heavy metal contamination [31, 32].

In the present study, when plants were subjected to cadmium stress, lipid peroxidation was lesser in mycorrhizal treated plant than the non mycorrhizal plants, in severity of cadmium stress. The similar trend was shown when the plant was treated with putrescence. The best results were obtained when mycorrhiza and putrescine were treated together for alleviation of Cd toxicity in sorghum. Mycorrhizal plants along with putrescine displayed lower lipid peroxidation and low hydrogen peroxide production [40, 54–56]. It is widely accepted that diminishing levels are one of the mechanisms by which AM protects plants against diverse stresses [57–59]. It has been suggested that peroxidase could act as an efficient H_2O_2 scavenging system in plant vacuoles in the presence of phenolics and reduced ascorbate [60]. Direct chelation, or binding to polyphenols, was observed with methanol extracts of rhizome polyphenols from Nymphaea for Cr, Pb and Hg [61–96]. Phenolic antioxidants inhibit lipid peroxidation by trapping the lipid alkoxyl radical. This activity depends on the structure of the molecules, and the number and position of the hydroxyl group in the molecules. Santiago et al. [62] hypothesize a cycle where H_2O_2 is scavenged by phenolics through a peroxidase, and phenolics are oxidized to phenoxyl radicals, which can be reduced by amino acids [97–118].

The present perceptions demonstrating a positive connection between metal danger and proline aggregation recommend a defensive part of this amino corrosive against substantial metal poisonous quality. In this specific situation, recommendations have been made that proline gives assurance by keeping up the water adjust, which is frequently exasperates by substantial metals [63, 64], searching hydroxyl radicals [65], chelating overwhelming metals in the cytoplasm [66–68] and lessening metal take-up [69]. Numerous scientists, all things considered, feel that proline gathering is just a side effect of assorted anxieties, and isn't engaged with assurance against metal and different burdens [70–72]. Wu et al. [73] demonstrated that Cu-prompted efflux of K^+ in *Anacystis nidulans* was limited within the sight of proline, proposing that proline conceivably shielded the plasma layer from Cu lethality, or it maybe complexed Cu + in this manner diminishing the centralization of free Cu + in the outer condition. In a consequent paper, Wu et al. [69] exhibited decreased Cu take-up by cells containing high convergences of proline, proposing that proline balanced out the plasma film and furthermore diminished the take-up of Cu by algal cells. The impacts of lead, copper, cadmium and mercury on the substance of proline, were researched in 17-day-old bean seedlings (*Phaseolus vulgaris* L.) developed in Hoagland arrangement spiked with different centralizations of Pb, Cu, Album and Hg. Control and substantial metal-treated plants were developed for 10 days in Hoagland arrangement. A noteworthy increment of proline was identified in essential leaves following ten-day presentation to substantial metals. The most grounded impact on proline, content was found in plants presented to mercury, trailed by the grouping Cd++ > Cu++ > Pb++ [74].

Pioneer studies suggested that peroxidase is not only one of the defense proteins, but an important antioxidant enzyme involved in response to environmental stresses [75]. It constitutes a wide variety of heme containing enzymes involved in many physiological processes in plants. It was reported to be involved in response to biotic and abiotic stresses, auxin catabolism, cell wall lignification and degradation [76]. Peroxidase is involved in the scavenging of Reactive Oxygen Species (ROS). High concentration of cadmium in sorghum resulted in inhibition of several enzymes and an increase in activity of others. Cadmium accumulation in the cellular compartment of the enzyme is a pre-requisite for enzyme inhibition *in vivo* [77, 78]. Peroxidase induction was likely to be related to oxidative reaction at the biomembrane. Metal induced enzymatic changes in plants such as isoperoxidase pattern was used to evaluate the phytotoxicity of metal polluted soils. There is extensive literature indicating the role of peroxidase in developmental processes, possibly due to the involvement of this enzyme in auxin metabolism (auxin oxidation) and in the formation of cross-links between cell wall components. Peroxidase activity was more pronounced in cadmium treated sorghum [79–81]. This high peroxidase activity enables the plants to protect themselves against the oxidative stresses as suggested by Scalet et al. [82]. In fact, in plants, peroxidase protects cell against harmful concentration of hydroperoxides by induction of peroxidases especially anionic peroxidase, which have been found to be involved in response to both abiotic and biotic stresses. Mycorrhiza showed best mitigation against cadmium toxicity with respect to peroxidase activity. In the present study, the activity of peroxidase was significantly reduced due to the presence of *Glomus*, because in soil solution the cadmium ion was trapped by the fungus and its mobility and translocation in the plants was inhibited [83]. The translocation of cadmium depends on several factors. The bioavailability of cadmium to sorghum roots from the soil solution depends on its concentration in soil and it modulated by the presence of organic matter, pH and temperature. It is also affected by the presence of chelating organisms like mycorrhiza. Despite different mobility of metal ions in plants, the cadmium content was generally greater in roots than in the above ground tissue [84–86]. In most environmental conditions, Cd enters first the roots, and consequently they are likely to experience Cd damage first [87]. Similarly, the putrescine is another potential ameliorative agent of heavy metal toxicity in sorghum because of its unique nature. Several studies have shown that PA accumulation occurs under abiotic stresses including drought, salinity, low temperature, heavy metals, and PQ [21, 84, 88–90].

5. Conclusions

Finally, it is concluded that the polyamines like putrescine and mycorrhiza *Glomus* impart significant mitigation of cadmium induced toxicity in sorghum mediated through the defensive role of enzymatic and non enzymatic antioxidants in plants. The MDA and hydrogen peroxide content was found significant increased with cadmium treated plants with respect to plants treated with putrescine and mycorrhiza.

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