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Arbuscular mycorrhiza mediated cadmium stress tolerance of sorghum (*Sorghum vulgare* L.)

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Arbuscular mycorrhiza (AM) is known to provide tolerance of plants to various heavy metal stresses. Accumulation of cadmium (Cd), a highly mobile and toxic heavy metal by plants is reported from various parts of the world. The present investigation carried out to observe the effect of mycorrhization on the growth and tolerance of sorghum (*Sorghum vulgare* L.) to Cd contaminated municipal solid waste (MSW) amended agricultural soil of Kolkata. Thirty days old mycorrhizal sorghum seedlings were transferred to MSW soil containing Cd at 0.0, 50.0, 100.0 mg kg^{-1} concentrations and allowed to grow for 15 days. Expectedly arbuscular mycorrhizal (M^+) sorghum showed improved roots and shoot growth significantly over non-mycorrhizal (M^-) plants. Sorghum plants both M^+ and M^- accumulated considerable amount of Cd in root tissue after 15 days exposure to Cd with significant growth reductions. Although concentrations of Cd in M^+ roots were significantly higher than in M^- roots but growth suppression effect was lower in M^+ roots. Tolerance of mycorrhizal sorghum to Cd may be attributed to greater absorption and retention of Cd in fungal body which restricted the movement and interference of Cd with the plant physiological processes as evident from lower shoot Cd concentrations of M^+ plants than M^- plants. Proline, a stress related amino acid, content found to increase significantly with the increase in Cd stress in both M^+ and M^- sorghum plants, but higher in M^+ roots than M^- roots at all Cd stress levels. Higher concentration of soluble protein in M^+ sorghum root and leaf than M^- plants was detected after 15 days of Cd stress up to 100 mg Cd kg^{-1} soil. Changes in protein banding patterns as observed by SDS-PAGE of root and leaf due to Cd exposure indicated an altered physiology in both M^+ and M^- plants due to Cd stress but with greater advantage in M^+ plants than in M^- plants.

Key words: Arbuscular mycorrhiza, Cd, MSW, sorghum

INTRODUCTION

Arbuscular mycorrhiza (AM) is an integral part of most angiospermic plants providing a direct link between the soil and plants. Mycorrhizal symbiosis results in improvements of the plants' overall physiology. Arbuscular mycorrhiza besides its role in plant phosphorus (P) and other mineral element nutrition, is also known to improve tolerance capacity of plants to various toxic heavy metals. However, high concentrations of zinc, copper, nickel, and cadmium can eliminate AM infection in contaminated soils (Gildon and Tinker, 1983; del Val *et al.,* 1999). The plants grown on municipal solid waste (MSW) amended soil contains lower mycorrhizal colonization intensity and spore density as compared to the unamended normal alluvial soil (Mandal *et al.*, 2007). AM fungal infection may decrease metal accumulation in plants growing in polluted soils and thus protect the host

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against phytotoxic metal effects (Arines *et al.,* 1989). Cadmium (Cd), a highly mobile and toxic heavy metal accumulation by plants is reported from various parts of the world due to various anthropogenic reasons. The present investigation has been carried out to observe the effect of mycorrhization on the growth and tolerance of sorghum (*Sorghum vulgare* L.) to cadmium in heavy metal contaminated municipal solid waste (MSW) amended agricultural soil of Kolkata.

MATERIALS AND METHODS

Investigations were carried out in the Laboratory for Plant and Microbial Biotechnology, Research Complex Building of BCKV, at Kalyani, Nadia, West Bengal. Investigations were carried out mainly with transported Kolkata municipal solid waste (MSW) amended agricultural soil from '*Dhapa*' disposal site in a well-ventilated nursery house under ambient light and temperature condition. Seedlings were maintained in wire net nursery under ambient temperature, light and humidity. Seedlings were fertilized with half strength Hoagland's solution without phosphorus twice at 15 days interval. All plants while in the nursery were routinely sprayed with insecticide to keep the pest incidence in check.

Cadmium chloride solution were added and mixed thoroughly in sieved (< 2.0 mm) MSW amended soil separately to dispense Cd @ 0, 50 and 100 mg kg^{-1} of soil, moistened with double distilled water and kept for one month to stabilize the Cd availability in the soil. Sorghum seedlings grown in normal soil for 30 days in small plastic pots with root-soil based mycorrhizal inoculum (MPN 28 g^{-1}) consortium (M^+) @ 2.0 g in 150 g soil comprising of (*i)Glomus epigaeum*, (ii) *G. mosseae*, (iii) *G. claroideum*, and (iv) *G. aggregatum* (1:1:1:1 v/v) (Figs. 1, 2, 3 & 4), isolated as dominant AM fungal species of experimental soil site and developed as single species culture in maize roots with 5 mg Cd kg^{-1} soil. Nonmycorrhizal (M^-) seedlings received heat killed inoculum.

Sorghum seedlings were transferred to Cd enriched MSW soil (2.0 kg/packet) carefully without disturbing root growth and allowed to grow under normal maintenance practices in net house. Biomass of shoot, root; P, Cd concentrations in plant tissues; AM infection and other biochemical parameters were recorded after 15 days. Cd and P in soil or plant tissue were estimated following standard methods using AAS (Varian AA-575 series) and UV-VIS spectrophotometer (Systronics-119) respectively. Free proline was estimated following the method of Sadasivam and Manickam (1996). Root and leaf proteins were estimated by standard Lowry's colorimetric method (Lowry *et al.*, 1951). Sodium Dodecyl Sulfate – Poly Acrylamide Gel Electrophoresis (SDS-PAGE) of root and leaf proteins was done using equivalent protein following the methods of Laemmli (1970) and analyzed by gel documentation system (ULTRALUM).

RESULTS AND DISCUSSION

Cadmium dose dependent reduction in root and shoot biomass yield of sorghum was observed. Under soil cadmium stress higher root, shoot and total dry biomass was recorded in M^+ plants than M^- plants (Figs. 5-8). However, growth reduction in M^+ plants was lower than M^- plants in spite of greater accumulation (Figs. 6-8 & Table 1) of cadmium in root. Mycorrhization reduced cadmium accumulation in shoot compared to M^- plants. Root infection intensity reduced with increased concentration of cadmium in soil.

Although AM infection intensity reduced with increased cadmium concentration after 15 days of cadmium exposure but M^+ plants produced higher biomass and greater phosphorus accumulation in shoot than M^- plants indicated cadmium stress tolerance. Greater accumulation of cadmium in M^+ roots than M^- roots might be due to binding of the heavy metal into the fungal structures and restricted its entry into shoot tissue as indicated by lower concentration of cadmium in shoot (Table 1). Similar phenomenon was observed by earlier workers while studying on heavy metals and AM interactions (Schuepp *et al.*, 1987, Weissenhorn *et al.,* 1995).

Proline accumulation in plant tissue under various environmental stress including heavy metals is well known. However, no such report is available for mycorrhizal roots under heavy metal stress. In the present study free proline concentrations (μM g^{-1} fresh root tissue) were increased with the increasing soil concentrations of cadmium in both M^- and M^+ roots but with greater amounts in M^+ roots than in M^- roots after 15 days of exposure to cadmium (Fig. 9). It indicated that natural stress tolerance mechanisms remained operative involving proline as a biomolecule which improved further due to

Table 1 : Concentration (μg g^{-1}) of phosporus (P) and cadmium (Cd) and % root infection in AM inoculated (M+) and uninoculated (M$^-$) sorghum in MSW amended Cd enriched soil after 15 days exposure

Treatment	Shoot P* concentration (μg g^{-1})	Root Cd* concentration (μg g^{-1})	Shoot Cd* concentration (μg g^{-1})	Root Infection %
Cd_0M^-	2183 ±113.7	2.73 ±0.11	5.06 ±0.135	0
Cd_0M+	2242 ±95.6	7.15 ±0.59	2.27 ±0.125	76.3 ±3.6
$Cd_{50}M^-$	1281 ±56.7	82.15 ±3.35	15.25 ±1.046	0
$Cd_{50}M+$	1570 ±55.5	106.42 ±1.56	10.13 ±0.941	57.0 ±2.4
$Cd_{100}M^-$	1120 ±112.2	110.33 ±2.34	23.81 ±0.942	0
$Cd_{100}M+$	1447 ±89.4	140.12 ±2.91	16.35 ±1.137	51.0 ±2.2
SEm (±)	20.19	0.48	0.19	
$LSD_{0.05}$	58.94	1.41	0.55	

* Values are averages of 4 replications

Cd_0 = No Cadmium, Cd_{50}= 50 mg kg^{-1} and Cd_{100}= 100 mg kg^{-1} of soil Cadmium

Table 2 : Soluble protein concentration (mg g^{-1} dry tissue) in root and shoot of non mycorrhizal (M$^-$) and mycorrhizal (M$^+$) sorghum under Cd stress

Treatment	Root		Leaf	
	Protein concentration (mg g^{-1})	% increase over non mycorrhizal (M$^-$) root	Protein concentration (mg g^{-1})	% increase over non mycorrhizal (M$^-$) shoot
Cd_0M^-	0.748	-	3.423	-
Cd_0M^+	0.846	13.1	4.158	21.5
$Cd_{50}M^-$	0.882	-	3.042	-
$Cd_{50}M^+$	0.992	12.5	3.510	15.4
$Cd_{100}M^-$	0.931	-	2.721	-
$Cd_{100}M^+$	1.042	11.9	3.129	15.0

Cd_0 = No Cadmium, Cd_{50}= 50 mg kg^{-1} and Cd_{100}= 100 mg kg^{-1} of soil Cadmium

mycorrhization. In *Sileue vulgaris* under cadmium stress higher proline accumulation was recorded by Schat *et al.* (1997). Proline has several roles on heavy metal tolerance of plants. It was found to play a role in scavenging hydroxyl radical (ROS) produced under heavy metal stress (Smirnoff and Cumbes, 1989), protecting enzymes (Shah and Dubey, 1997), and protecting macromolecules preventing denaturation (Schobert and Tschesche, 1978) and also increases the rigidity of cell wall (Munoz *et al.,* 1998). The exact relation between heavy metal stress and higher proline accumulation in mycorrhizal plants could not be explained.

Concentrations of soluble root protein increased and leaf protein decreased with increase in soil cadmium in both M$^-$ and M$^+$ plants (Table 2). But M$^+$ plants had higher soluble protein than M$^-$ plants at all the levels of soil cadmium stress. The increasing trend of root protein concentrations may be an indication of synthesis of extra amount of proteins (enzymes) due to exposure to cadmium as defen-

Fig. 1 : *Glomus epigaeum*

Fig. 2 : *Glomus mosseae*

sive weapons which were further improved by mycorrhization. Improvement or alteration in physiology of plants and root architecture towards better performance was correlated with such quantitative changes in root proteins in mycorrhizal plants (Bonfante and Perotto, 1995; Harrison, 1999).

Fig. 3 : *Glomus claroideum*

The results of improved cadmium stress tolerance of mycorrhizal sorghum, indicated that some changes in regulation of protein synthesis might be induced by mycorrhiza. Such change in regulation was further verified by qualitative analysis of soluble proteins by SDS-PAGE (Fig.10). A distinct down-regulation of high molecular protein (> 50 kDa) synthesis in root and leaf of both M^- and M^+ plants with corresponding up regulation of low molecular weight proteins (14 – 50 kDa). The presence of four low molecular weight protein bands exclusive to M^+ roots at 100 mg kg^{-1} cadmium stress indicated the synthesis of new proteins in root tissue were regulated by mycorrhiza under high cadmium stress. Similar low molecular weight proteins were found to be produced in plants under heavy metal stress as observed earlier by Choi *et al.* (1998) under heavy metal stress. They found a 14 kDa protein in root and a 11 kDa protein in green tissue of plants under heavy metal stress.

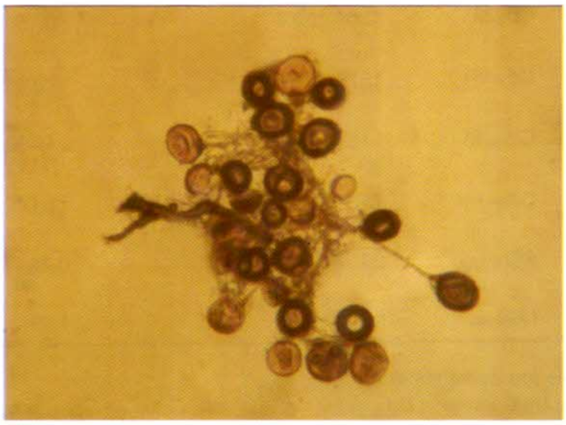


Fig. 4 : *Glomus aggregatum*

Fig. 5 : Sorghum plants (M^- and M^+) in 100 mg Kg^{-1} of soil Cd stress

Some protein bands appeared in both M^+ and M^- sorghum roots only at high cadmium stress indicated that there are some proteins in plants that are synthesized only under high cadmium stress *i.e.* stress dependent regulation. These results of protein analyses of M^+ and — sorghum root and leaf under cadmium stress suggested changes in host and/or AM fungal genes expression in plants under heavy metal stress.

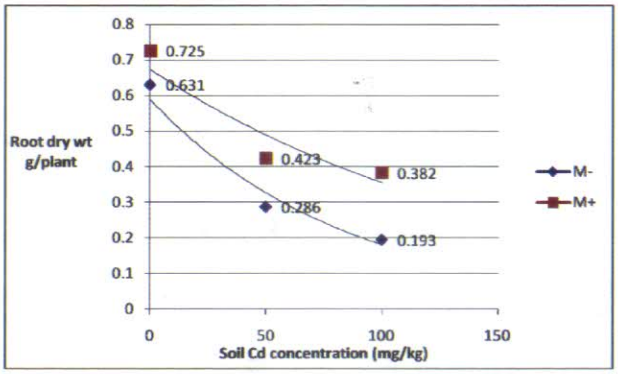


Fig. 6 : Root dry matter production of sorghum seedlings with AM (M^+) and without AM (M^-) under soil Cd stress

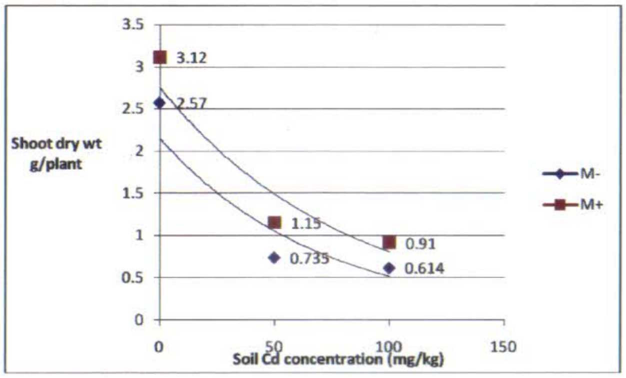


Fig. 7 : Shoot dry matter production of sorghum seedlings with AM (M^+) and without AM (M^-) under soil Cd stress

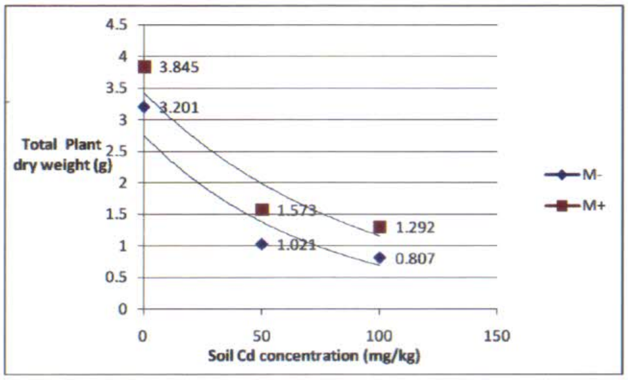


Fig. 8 : Total dry matter production of sorghum seedlings with AM (M^+) and without AM (M^-) under soil Cd stress

The cadmium stress tolerance benefit derived by sorghum plants due to mycorrhization as presented here have some practical utility which can be considered in formulating cost effective, environmentally safe phytoremediation technology for heavy metal polluted soils integrating arbuscular mycorrhiza.

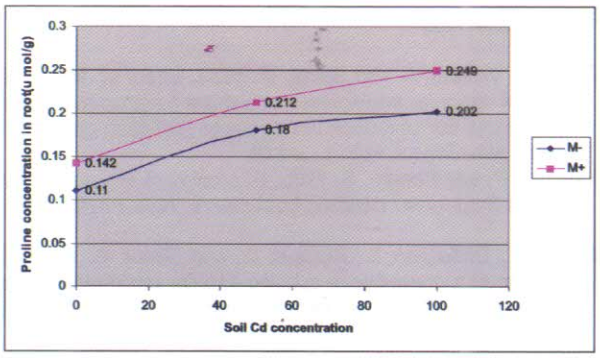
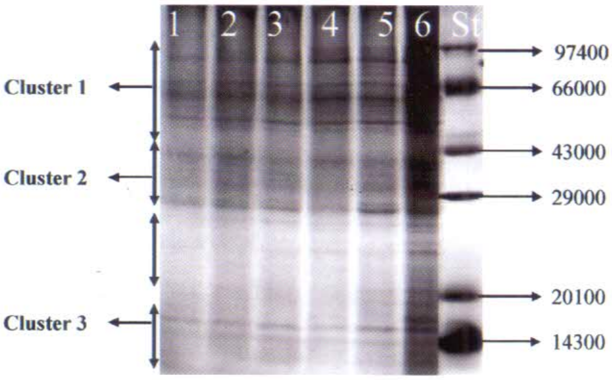


Fig. 9 : Proline accumulation in AM uninoculated (M^-) and AM inoculated (M^+) sorghum roots under soil Cd stress



Lane : 1.M^-Cd_0, 2.M^+Cd_0, 3. M^-Cd_{50}, 4. M^+Cd_{50}, 5. M^-Cd_{100}, 6.M^+Cd_{100}, 7.Standard

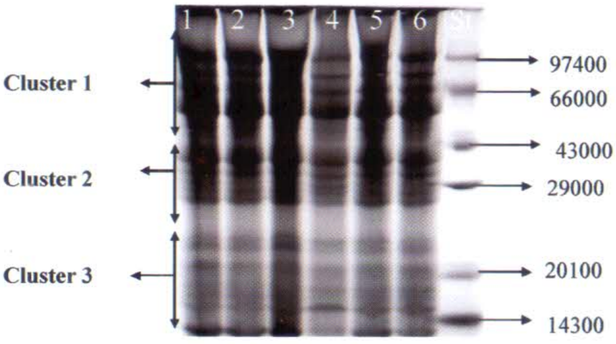


Fig. 10 : Protein banding pattern of non mycorrhizal (M^-) and mycorrhizal (M^+) sorghum root (a) and leaf (b) under Cd exposure (SDS-PAGE Gel)

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