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Alleviation of Cadmium Toxicity and Growth Enhancement of *Helianthus annuus* and *Triticum aestivum* Seedlings through Bacterial Inoculation

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ABSTRACT

*Two Cd-resistant bacterial growth strains MA-9 (*Aeromonas*) and MA19 (affinities uncertain), which were isolated from ICI effluents, were used to inoculate seeds of* Triticum aestivum *(wheat) and* Helianthus annuus *(sunflower). Both inoculated and non-inoculated seeds were germinated and grown under different concentrations (0, 1, 2 and 3 mM) of $CdCl_2$ for 10 days. Under Cd-stress conditions, bacteria-inoculated plants had better germination and growth than non-inoculated treatments. Bacterial growth enhancement of seedlings was associated with reduced Cd uptake.*

INTRODUCTION

Cadmium, a non-essential element and an industrial pollutant, is of serious environmental and toxicological concern. It is a by-product of zinc and lead mining industries and is used in electroplating, paints, batteries (Goyer 1986). The use of phosphate fertilizers, sewage sludge, manure and lime also increases cadmium content in the soil (Anderson 1977). Cadmium is toxic to man (Goyer 1986), animals (Agrawal and Bhattacharya 1989) and plants (Page *et al.* 1981). It remains in an active state for a long time and is readily bioavailable (Goyer 1986). Plants with high cadmium content are the major source of intake, either directly or indirectly, by man (Page *et al.* 1981). In humans, its toxicity is manifested by renal dysfunction, hypertension, carcinogenic conditions, cardiovascular and chronic pulmonary diseases (Goyer 1986). Cadmium phytotoxicity is expressed by retarded growth (Greger 1989), chromosomal (Rohr and Baughinger 1976) as well as structural abnormalities (Wong *et al.* 1989), disturbed biochemical (Poschenrieder *et al.* 1989; Satakopan and Rajendran 1990) and physiological processes (Greger 1989; Poschenrieder *et al.* 1989).

It is imperative to keep the intracellular concentration of potentially noxious heavy metal ion/s at low concentrations. Some plants combat heavy metal stress by acquiring different mechanisms (Vogeli-Lange and Wanger 1990). Bacteria have developed several metabolic dependent or independent devices to counter heavy metals (Gadd 1990; Hughes and Poole 1991). These could be utilized by man for the removal and recovery of heavy metal from industrial effluents and refuse composites (Gadd 1990). Hasnain and Yasmin (1992), Sabri *et al.* (1992), Hasnain *et al.* (1993, 1995) and Saleem *et al.* (1994) have demonstrated enhanced growth of wheat seedlings under different levels of heavy metal stress through bacterial inoculation. Here, the effect of bacterial inoculation on growth of sunflower and wheat seedlings under $CdCl_2$ stress is evaluated.

MATERIALS AND METHODS

The bacterial isolates MA-9 and MA-19 are gram negative, asporogenous and facultative anaerobic rods. MA-9 was affiliated with *Aeromonas* while affinities of MA-19 remained uncertain (Sabri *et al.* 1995). Both bacterial strains were isolated from polluted waters of outlet effluents of the ICI plant (near Sheikhupura, Pakistan). Both strains tolerate up to 500 µg ml^{-1} $CdCl_2$ in the medium. Inoculum from the overnight culture (16 h), in L B (Sambrook *et al.* 1989) at 37°C (200 rpm), was replenished with fresh L broth medium and incubated at 37°C at 200 rpm. Bacterial cells from the late logarithmic growth were collected, washed and resuspended in sterile distilled water to get a final population of 10^7 cells ml^{-1}.

Certified seeds of *Triticum aestivum* var. Pak81 (wheat; Ayub Agricultural Research Institute, Faisalabad) and *Helianthus annuus* var. 256 (sunflower; Punjab Seed Corporation, Lahore) were surface sterilized by immersing in 0.1% $HgCl_2$ solution for 5-10 min. After thorough washing, seeds were soaked in bacterial suspension for 15 min, while the control seeds were drenched in sterilized distilled water for the same period. Twenty-five pretreated randomly selected seeds from each plant were spread aseptically and evenly in glass petri dishes lined with two layers of Whatman filter paper No. 1. Each plant (wheat and sunflower) was given three inoculation treatments (control, MA-9, MA-19). A total of 12 treatments were used per plant species. Fifteen ml of the respective $CdCl_2$ solutions were added to each petri dish, to ensure that the filter papers were well moistened. The petri dishes were kept in the dark at 25 ± 2°C. The dishes were regularly watered with the respective solutions. The seeds were observed daily for signs of germination. On the third day, petri dishes with germinated seeds were moved to 10 K Lux light at 25°C. Petri dishes were arranged in a completely randomized design and the position of the dishes randomized daily during the course of the experiment. An additional 15 ml of Hewitt's nutrient solution (Hewitt 1963) containing the respective $CdCl_2$ concentrations (0, 1, 2 and 3 mM) was added once to the respective treatment. The seedlings were observed daily. Growth measurements, which included length of shoot and root, fresh and dry weights of seedling, were taken 10 days after exposure to light. Presence of specific bacteria species was confirmed by isolating the bacteria from small pieces (0.5 cm) of root. The experiments were repeated four times. Data obtained were subjected to statistical analysis (means, standard error of the means, standard deviation, least significant difference, analysis of variance) adopting the method of Steel and Torrie (1981). Cadmium content in the seedlings was determined using the atomic absorption method of Rand *et al.* (1979).

RESULTS AND DISCUSSION

Seedling Germination Experiments

Hasnain and Yasmin (1992) had earlier demonstrated that Cd-resistant bacteria from the histoplane of *Suaeda fruticosa*, *Cynodon dactylon* and *Typha* could stimulate *Triticum aestivum* seedlings grown under Cd-stress conditions. Results from the present study showed that $CdCl_2$ treatments adversely affect germination of both *Triticum aestivum* and *Helianthus annuus* seeds, with a linear decrease in percentage germination as the concentration of $CdCl_2$ increased (*Fig. 1*). $CdCl_2$ at 3 mM concentration resulted in 25 and 33% decrease in percentage germination of *Triticum* and *Helianthus*, respectively, compared to control. The inhibitory effect of cadmium on germination has been reported in many plant species (Renjini and Janardhanan

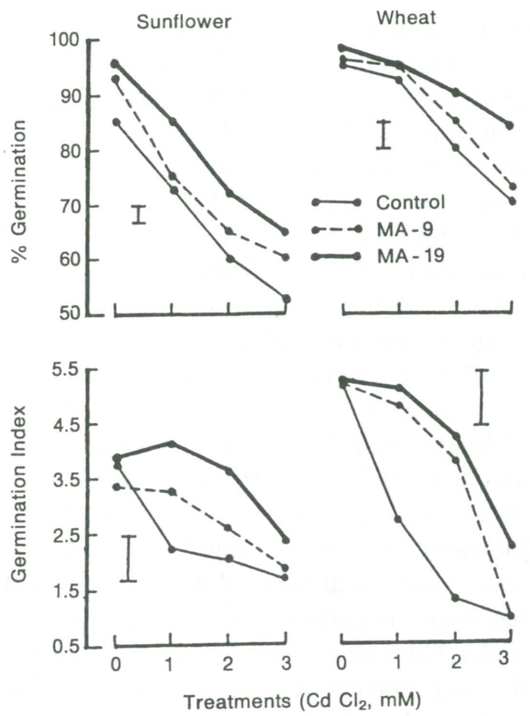

Fig. 1: Effect of bacterial inoculation on the percentage germination and germination index of sunflower and wheat seeds under different concentrations of $CdCl_2$. (Figures based on means of four replicates. Variability within means was less than 10%. Vertical bar represents least significant difference at P=0.05)

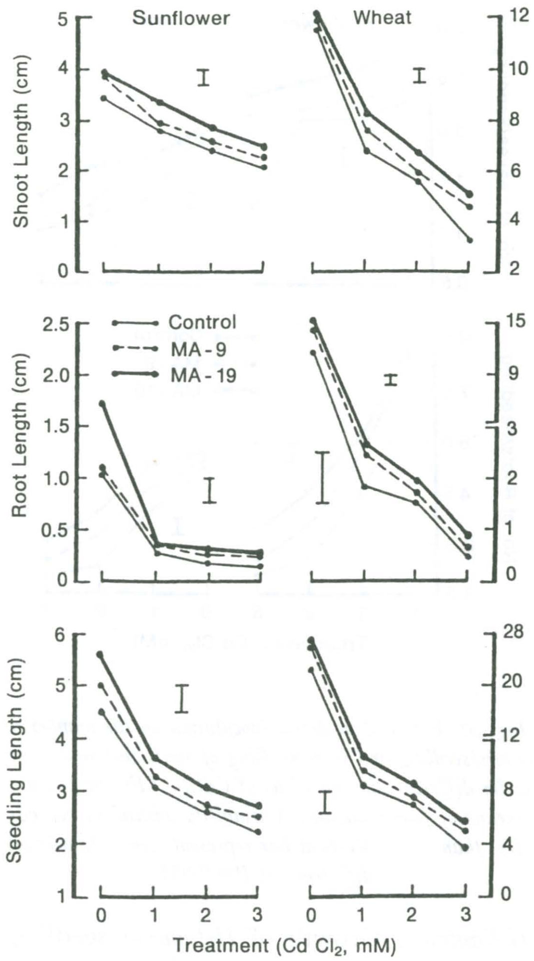

Fig. 2: Effect of bacterial inoculation on length parameters (shoot, root, seedling) of sunflower and wheat under different concentrations of $CdCl_2$. (Figure based on means of four replicates. Variability within means was less than 10%. Vertical bar represents least significant difference at P=0.05)

1989; Satakopan and Rajendran 1990; Hasnain and Yasmin 1992). This adverse effect may be attributed to increased uptake of cadmium which may disturb nuclear division and hinder cytokinesis (Vauline *et al.* 1978). *Fig. 1* shows inoculation of seeds with MA-9 and MA-19 enhanced and increased germination of both wheat (2-5% with MA-9; 2-14% with MA-19) and sunflower (2-8% with MA-9; 10-13% with MA-19). The stimulatory effect of the bacterial inoculum was more pronounced in the presence of cadmium.

Seedling Growth Experiments

The adverse effects of cadmium were also manifested in other growth parameters (shoot and root lengths, number of leaves and number of roots) of both wheat and sunflower seedlings (*Fig. 2, 3*). Presence of $CdCl_2$ also caused significant reduction in seedling lengths. At 3 mM, $CdCl_2$ shoot growth was reduced by 71% in *Triticum* and 38% in *Helianthus*. *Fig. 2* shows shoot length was relatively less curtailed compared with root growth (with 95% reduction in *Triticum* and 80% reduction in

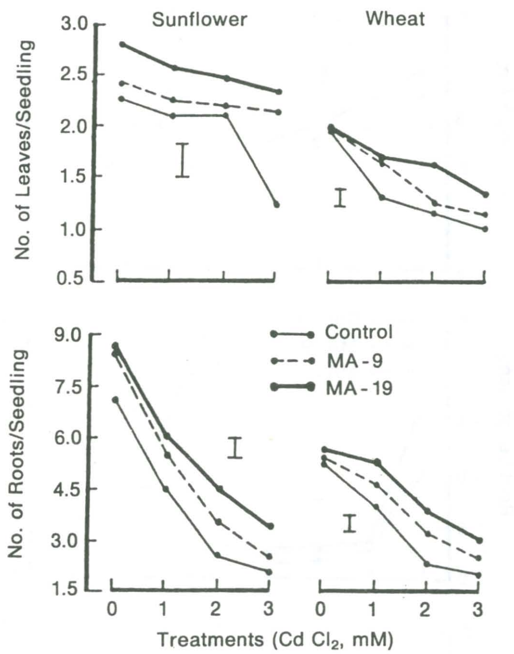

Fig. 3: Effect of bacterial inoculation on the number of leaves/seedling and roots/seedling of sunflower and wheat under different concentrations of $CdCl_2$. (Figure based on means of four replicates. Variability within means was less than 10%. Vertical bar represents least significant difference at P=0.05)

Helianthus). Growth of *Helianthus* seedlings (root and shoot) gradually decreased with increase in $CdCl_2$ concentrations (*Fig. 2*), i.e. at 1, 2 and 3mM there was a decrease of 31, 42 and 49% respectively compared with the control (0 mM). In *Triticum*, reduction in seedling length was 63, 69 and 84%, at the respective $CdCl_2$ concentrations of 1, 2, 3 mM. Affected seedlings had smaller leaves and roots, and looked unhealthy.

Growth inhibitory effects of cadmium have variously been ascribed to (i) its ability to decrease availability and transportation of calcium for various growth processes (Greger 1989) (ii) decrease in root absorption area, (iii) inhibition in cell division since Cd interferes with elongation and enlargement of cells, (iv) Cd interference with ABA metabolism and with other physiological processes, and (v) decrease in cell wall elasticity and expansion (Poschenrieder *et al.* 1989). Roots are more severely curtailed than shoots, probably due to higher levels of Cd absorbed, which reduce transportation of cytokinins from roots to shoot (Marchner 1986). Reduction in shoot and root lengths was reported for *Triticum* (Hasnain and Yasmin 1992) and for groundnut, sunflower and gingelly (Renjini and Janardhanan 1989).

Both bacteria strains, MA-9 and MA-19, promoted shoot length by 7-12% in *Helianthus* and 3-56% in *Triticum* and root length by 9-75% in *Helianthus* and by 16-80% in *Triticum* (*Fig. 2*). Significant increases in seedling length of wheat (7-59%) and sunflower (8-25%) with both bacterial inoculations were also observed under Cd stressed conditions compared to the non-inoculated treatment (*Fig. 2*). The seedling length in wheat was enhanced more than in sunflower.

Inoculation of plants with other bacterial strains has been shown to promote plant growth (Hasnain *et al.* 1993, 1995; Galiana *et al.* 1994; Saleem *et al.* 1994). Inoculating plants with specific bacterial strains increases root length, and density number, as well as the number of deformed root hairs (Bashan and Levanony 1990). Seedling growth promotion is probably the result of the bacterial cells anchoring on root surfaces (Bashan and Holguin 1993) or by changing root membrane potential for better absorption of nutrients (Bashan and Levanony 1990; Bashan 1991). The presence of various levels of $CdCl_2$ resulted in decrease in leaf number of sunflower (47%) and wheat (51%). This subsequently resulted in decrease in root number in sunflower (by 70%) and wheat (by 46%) (*Fig. 3*). This decrease in leaf number was accompanied by the appearance of brownish spots on the leaves while root tips became brown. In contrast, bacterial inoculation resulted in significant increase in the number of leaves (5-91% in wheat) compared to the non-inoculated plants. The presence of high Cd in soils has also been associated with decrease in chlorophyll content and reduced lateral roots (Padmaja *et al.* 1990). Other symptoms observed in addition to chlorosis included wilting of leaves, and severe constriction of the stems. Wong *et al.* (1989) reported that Cd damaged the plant growth through narrowing of the vessels and pits and deposition of debris, which blocked water translocation.

The presence of $CdCl_2$ also caused significant linear reduction in fresh and dry weight of seedlings (*Fig. 4*). With 1, 2, and 3 mM $CdCl_2$ concentrations, decrease in fresh weight of sunflower was in the order of 22, 50 and 75%, while decrease in wheat was in the order 43, 59 and 90% (*Fig. 4*). A subsequent decrease in dry weights of these two species was also observed. Accumulation of dry weight, indicated by dry weight per gram fresh weight, was more pronounced with increasing concentrations of $CdCl_2$, with maximum dry weight accumulation recorded at 3 mM (*Fig. 4*). Inoculation of plants with bacteria resulted in an increase of both fresh (3-200% in wheat and 28-114% in sunflower) and dry weights (4-233% in wheat and 40-2700% in sunflower) which supersedes the weights from the respective non-inoculated treatments. Accumulation of seedling dry weight is correlated with accumulation of Cd in the seedlings (*Fig. 5*). At 3 mM concentration, Cd content in sunflower seedlings was six-fold relative to that of wheat. With both bacterial inoculations dry weight per gram fresh weight increased slightly over respective non-inoculated treatments in both plants, but significantly decreased in Cd-content of seedlings compared with respective treatments. It seems that bacterial inoculation causes a decrease in the uptake of Cd by seedlings, which ultimately induces stimulated growth, over respective non inoculated treatment, under Cd stress conditions. Bacteria exhibit detoxification mechanisms, such as metal chelating complex (Gadd 1990; Hughes and Poole 1991), Cd-peptide complexes (Konya *et al.* 1990), extracellular sequester-

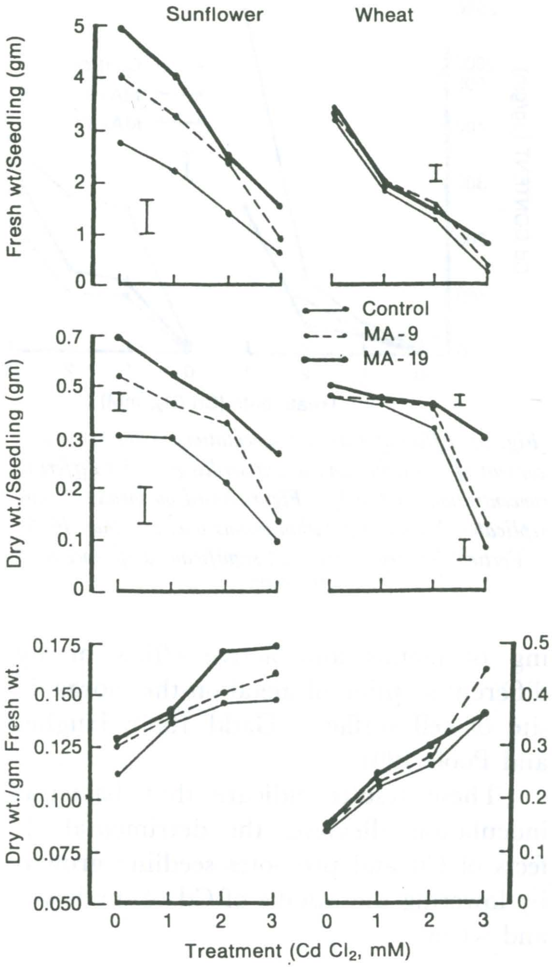

Fig. 4: Effect of bacterial inoculation on the weight parameters (fresh weight, dry weight, dry weight/gm fresh weight) of sunflower and wheat seedlings under different concentrations of $CdCl_2$. (Figure based on means of four replicates. Variability within means was less than 10%. Vertical bar represents least significant difference at P=0.05

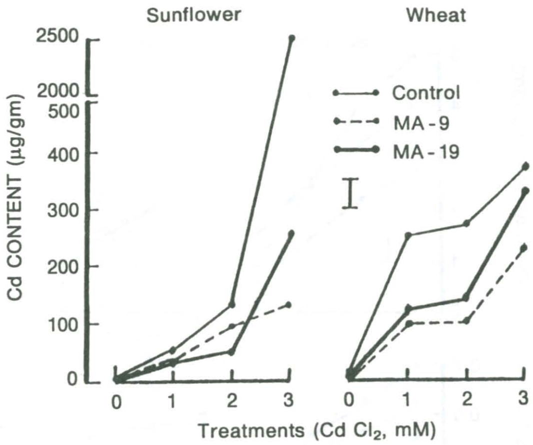

Fig. 5: Effect of bacterial inoculation on the cadmium content of sunflower and wheat seedlings under different concentrations of $CdCl_2$. (Figure based on means of four replicates. Variability within means was less than 10%. Vertical bar represents least significant difference at $P=0.05$)

ing of metals and active efflux or by different sorption of metal on the inorganic site of cell surfaces (Gadd 1990; Hughes and Poole 1991).

These results indicate that bacterial inoculation alleviates the detrimental effects of Cd and promotes seedling growth by lowering the uptake of Cd of sunflower and wheat.

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