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Plant Growth Promotion by *Brevibacterium* under Chromium Stress

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Abstract: Three chromium resistant bacterial strains originally isolated from chromium-contaminated wastewater, were tested in the field experiments for the growth promotion of *Helianthus annuus* in the presence of different chromium salts. Much reduction in seed germination was observed under Cr(VI) stress but all bacterial strains promoted seed germination with respect to non-inoculated control. Inoculation increased plant length in sunflower by 14 to 26%. Sunflower plants showed medium and high increases in their fresh weight in response to inoculation with these strains. Inoculation with these strains increased dry weight up to 15% as compared to non-inoculated control. The contribution of the strains to promote auxin content in these plants varied from 88% when sunflower plants were inoculated with strain CrT-6, to 174% when strain CrM-6 was used. All the three strains showed similar effects in respect to chromium accumulation when supplied with 300 μg g^{-1} of K_2CrO_4.

Key words: PGPR, auxin, chromium, sunflower, bacteria, heavy metals, hormones

Introduction

It is now recognized that some soil bacteria called plant growth-promoting rhizobacteria (PGPR) can promote the growth of widely grown cereals (Watt *et al*., 2003). Rhizobacteria belonging to different genera, such as *Azotobacter* and *Pseudomonas* have been reported to promote the growth of diverse plants such as wheat (Egamberdiyeva and Holflich, 2003) and maize (Filya *et al*., 2003), respectively, indicating that the PGPR effect is widespread among bacteria. Soil contamination by heavy metals occurs when the concentration of these elements exceeds the background level in the substratum. Chromium is not considered an essential element for plant nutrition. Chromium occurs in oxidation states Cr^{-2} to Cr^{+6}, but only Cr^{+3} and Cr^{+6} are of biological significance. Symptoms of Cr^{+6} phytotoxicity include poor growth and leaf chlorosis (Sharma *et al*., 2003). Also, the bioavailability and the toxicity of this element in the soil depend on its speciation; the Cr(VI) form is more toxic and more mobile than the Cr(III) form (Shanker and Pathmanabhan, 2004). Recently, different heavy metal tolerance mechanisms have been discovered in various bacterial strains which involves exclusion, active removal, biosorption, precipitation, or bioaccumulation both in external and intracellular spaces (Malik, 2004). These processes can influence the solubility and the bioavailability of metal to the plant, thus modifying the efficiency of accumulation process. Present study deals with the use of Cr(VI)-reducing bacterial strains for the growth improvement of *Helianthus annuus* L. plants in pot/green house experiments.

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Materials and Methods

Present study was conducted in the year 2004-2005 at Botanical Garden, Department of Botany, University of the Punjab, Lahore, Pakistan.

Bacterial Strains

Three chromium resistant bacterial strains (CrT-6, *Brevibacterium* and CrM-6) previously isolated (Faisal and Hasnain, 2004) from effluents of tanneries were used in this study. All of them showed very high level resistance to K_2CrO_4 both on the nutrient agar (up to 40 mg mL^{-1}) as well as in acetate-minimal medium (up to 10 mg mL^{-1}).

Plant Material

Certified seeds of sunflower (*Helianthus annuus* var SF-187) were obtained from National Agriculture Research Center, Islamabad, Pakistan.

Plant Growth Experiments

Plant growth experiments were conducted in pots, each containing 3 kg of sterilized garden soil. The effects of chromium salts on the sunflower plants were studied at a concentration of 300 μg g^{-1} of trivalent ($CrCl_3$) and hexavalent chromium (K_2CrO_4). Seeds were disinfected with 5% sodium hypochlorite, rinsed with autoclaved distilled water twice and then inoculated with bacterial suspension of the strains CrT-6, *Brevibacterium* and CrM-6. Initially twelve seeds were sown in each pot and watered regularly. After germination plants were thinned to six per pot. Auxin content were measured by following Mahadevan (1984). Plants were harvested at full maturity. To check the chromium contents in the root, shoot and leaves, material was oven dried at 80°C for 24 h and was digested by following Humphries (1956). Total chromium content was measured using diphenylcarbazide.

Cr(VI) Reduction

To check whether bacterial strains as well as sunflower seedlings or both were involved in the reduction of toxic Cr(VI) into less toxic Cr(III), separate experiments were conducted. Seedlings were grown both as control and inoculated in the presence of 300 μg K_2CrO_4 mL^{-1}. After ten days, seedlings were harvested and the amount of chromate reduced was determined in the remaining nutrient solution (initially supplemented with 300 μg K_2CrO_4 mL^{-1}) present in petri dishes. All experiments were conducted in four replicates and data was analyzed statistically following Steel and Torrie (1981).

Results

Plant Growth Experiments

All the three bacterial strain improved germination when compared to non-inoculated respective control. Application of both chromate salts ($CrCl_3$ and K_2CrO_4) leads to a reduction in this parameter but the effects of K_2CrO_4 were more severe. Almost 10 and 44% reduction in seed germination was observed under 300 μg mL^{-1} of $CrCl_3$ and K_2CrO_4, respectively. In case of 300 μg mL^{-1} of trivalent chromium treatment, all bacterial inoculations stimulate germination. Strains *Brevibacterium* and CrM-6 cause relatively more stimulation where almost 58 and 10% increases in seed germination were recorded, respectively (Table 1). Seedling length was severely affected by the application of chromate salt especially with the Cr(VI). About 20 and 61% reduction in seedling length, respectively

Table 1: Effect of inoculation of chromium resistant bacteria on germination and plant length of *Helianthus annuus* var SF-187 plants at 0 and 300 μg g^{-1} of $CrCl_3$ and K_2CrO_4 concentrations (n = 4)

	% Germination			Plant length (cm)		
Strains	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	98±3.4	88±2	55±4.1	25.4±1.4	20.6±1.34	9.8±1.08
CrT-6	100±4.2	92±5	56±5.5	28.9±1.3	21.3±1.24	11.1±0.98
Brevibacterium	100±0.0	100±0.0	87±4.1	40.8±3.4	38.4±2.5	23.3±1.02
CrM-6	100±5.3	97±3	70±5.1	30.5±1.8	25.4±2.1	13.2±0.58
LSD at 0.05						
For strains		3.10			2.51	
For treatment		1.20			2.24	

Table 2: Effect of inoculation of chromium resistant bacteria on number of roots and leaf area of *Helianthus annuus* var SF-187 plants at 0 and 300 μg g^{-1} of $CrCl_3$ and K_2CrO_4 concentrations (n = 4)

	Number of roots			Leaf area (cm)		
Strains	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	5±0.34	5±0.48	9±0.21	4.8±0.14	4.3±0.10	2.2±0.10
CrT-6	5±0.26	5±0.36	9±0.35	4.9±0.25	4.3±0.13	2.2±0.09
Brevibacterium	6±0.42	6±0.25	9±0.74	5.13±0.9	5.09±0.7	1.90±0.1
CrM-6	5±0.30	6±0.67	9±0.50	5.2±0.16	4.6±0.14	2.8±0.10
LSD at 0.05						
For strains		0.98			0.75	
For treatment		0.34			0.28	

Table 3: Effect of inoculation of chromium resistant bacteria on fresh weight and dry weight of *Helianthus annuus* var SF-187 plants at 0 and 300 μg g^{-1} of $CrCl_3$ and K_2CrO_4 concentrations (n = 4)

	Fresh weight (g)			Dry weight g^{-1} fresh weight (g)		
Strains	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	8.9±0.84	7.4±0.24	3.8±0.21	0.139±0.001	0.165±0.003	0.291±0.006
CrT-6	9.6±0.65	8.6±0.36	4.8±0.14	0.160±0.001	0.152±0.002	0.232±0.003
Brevibacterium	12.7±0.25	9.2±0.25	5.5±0.13	0.120±0.01	0.153±0.06	0.194±0.07
CrM-6	10.9±0.35	8.9±0.23	5.3±0.21	0.119±0.002	0.152±0.002	0.212±0.002
LSD at 0.05						
For strains		0.52			0.00	
For treatment		0.13			0.00	

under 300 μg mL^{-1} of $CrCl_3$ and K_2CrO_4 was observed. All bacterial inoculations stimulated plants length as compared to non-inoculated control. *Brevibacterium* caused maximum stimulation where 61% increase was recorded when compared to its respective non-inoculated control (Table 1). Hexavalent chromium caused significant increase in root numbers while trivalent chromium had no effect on this parameter. Except strain *Brevibacterium*, other inoculation had no effect on number of roots and they remain same as that of control (Table 2). Much reduction in leaf area was observed especially in case of Cr(VI) treatment (Table 2). 300 μg mL^{-1} of K_2CrO_4, significantly reduce the fresh biomass of plants as compared to control and 300 μg mL^{-1} $CrCl_3$. Almost 17% and 57% reductions, over control, in fresh weight of plants were observed at 300 μg mL^{-1} of $CrCl_3$ and K_2CrO_4, respectively (Table 3). All bacterial inoculations caused an increment in fresh weight of wheat seedling. Strains CrT-6, *Brevibacterium* and CrM-6 promote the fresh biomass of plants up to 9, 43 and 22%, respectively, when compared with non-inoculated respective control.

Chromium treatments, stimulated more synthesis of auxin content in *Helianthus annuus* plants. Almost 104 and 246% increases in auxin content were observed, respectively, with 300 μg mL^{-1} of $CrCl_3$ and K_2CrO_4 (Table 4). Marked increase in auxin content was manifested with CrT-6

Table 4: Effect of inoculation of chromium resistant bacteria on auxin content (μg g^{-1} fresh weight) and chromium content (mg g^{-1} dry weight) of *Helianthus annuus* var SF-187 plants at 300 μg g^{-1} of $CrCl_3$ and K_2CrO_4 (n = 4)

	Auxin content (μg g^{-1} fresh weight)			Cr Uptake (mg g^{-1})	
	0	$CrCl_3$	K_2CrO_4	$CrCl_3$	K_2CrO_4
Control	0.62±0.02	1.27±0.04	2.15±0.02	0.404±0.02	3.241±0.1
CrT-6	1.13±0.04	1.96±0.05	3.21±0.02	0.368±0.03	2.128±0.4
Brevibacterium	1.70±0.02	2.32±0.06	4.93±0.04	0.367±0.03	1.515±0.6
CrM-6	1.21±0.04	2.19±0.08	3.90±0.01	0.407±0.06	1.298±0.5
LSD at 0.05					
For strains	0.72		0.45		
For treatment	0.28		0.18		

Table 5: Cr(VI) reduction by the bacterial strains and sunflower seedlings present in the nutrient solution within 10 days. In controls only sunflower seedlings present but in other four plates, plants were inoculated with bacterial strains (n = 4). P stands for sunflower plant

Strains	Cr(VI) reduction (%)
Control (P)	3.1
CrT-6+P	46.2
Brevibacterium	64.4
CrM-6+P	71.2

(88%), *Brevibacterium* (95%) and CrM-6 (174%) over non-inoculated respective control. Generally at 300 μg mL^{-1} $CrCl_3$ and K_2CrO_4, all bacterial inoculations caused an increment in auxin content when compared to their respective control (Table 4). In general there was a reduction in chromium uptake with the bacterial inoculation (Table 4). Plants which were grown under 300 μg mL^{-1} K_2CrO_4, accumulate more chromium content as compared to $CrCl_3$ treated plants. In case of 300 μg mL^{-1} K_2CrO_4 treatment, all bacterial strains CrT-6, *Brevibacterium* and CrM-6 manifested a reduction in the chromium content of plants when compared to respective non-inoculated control treatment (Table 4).

Cr(VI) Reduction

Table 5 showed that under control conditions where no bacterial inoculation was applied, sunflower seedlings were unable to reduce Cr(VI) but only a fewer amount of Cr(VI) was reduced to Cr(III). All bacterial strains significantly reduced toxic Cr(VI) in to less toxic Cr(III) in the presence of sunflower seedlings (Table 5). CrM-6 was able to reduced maximum Cr(VI) in to Cr(III) 71.2% while this effect was only 3.1% in non-inoculated control seedlings (Table 5).

Discussion

In the present study, chromium stress caused significant reduction in the seed germination. Germination was more drastically hampered by the Cr(VI) salt as compared to Cr(III). However inoculation of bacterial strains resulted enhancement in germination. One possible explanation for stimulation in germination and growth parameters might be that bacteria take up chromium in their cells and may be capable of reducing the availability to seeds. Thus bacteria by lessening the harmful effects of chromium provoked germination as well as seedling growth. Being the natural inhabitants, bacteria are found to colonize the rhizosphere and/or plant roots (Dong *et al*., 2003), have capability to increase the availability of nutrients to plants and regenerate the quality of soil (Alami *et al*., 2000). Significant reduction in plants height was observed under chromium stress. Chromium stress caused much reduction in the number of leaves. Under 300 μg g^{-1} of K_2CrO_4 much reduction in the number of leaves

was observed as compared to chromium free control. Bacterial inoculation resulted increment in the number of leaves not only in chromium free but also in the presence of trivalent and hexavalent chromium. A major effect of chromium stress on roots was shortening and thickening due to a decrease in the rate of cell elongation and growth. This morphological adaptation of roots is a typical change under stress environment. Plant growth promoting rhizobacteria in addition to stimulating plant growth (length and weight parameters) and nutrient uptake; also enhance number and length of root hairs (Bertrand *et al.*, 2000). A similar trend with respect to reduced germination, growth and yield of other plants under chromium stress has been reported (Khan and Abdullah, 2003). In the presence of chromate, weight parameters of *H. annuus* were severely affected while bacterial strains caused increase in fresh weight both under normal and chromium stress conditions when compared with non-inoculated respective control. Plant fresh weight of *Salvinia minima* was significantly reduced as the Cr(VI) concentration increased in the growth media (Nichols *et al.*, 2000). Raza *et al.* (2001) reported that rhizobial strains positively influenced fresh weight, shoot dry weight, total N content of Lupin cultivars.

Several bacterial strains are capable of producing auxin, gibberellins, ethylene or abscisic acid (Gutierrez-Manero *et al.*, 2001). Auxin appears to be a master hormone, exercising regulatory action over many of other plant hormones. In the present study amount of auxin content increased in sunflower plants under chromium stress. According to Campbell (1985), bacterial strains stimulate plant growth by synthesizing and liberating growth hormones. Enhancement in auxin content with bacterial inoculation was also reported previously (Munir *et al.*, 2003). Mutaftchier *et al.* (1993) described the action of growth hormones and explained that auxin improves plant growth through different mechanism by combining with obigosaccharides, proteins, cell wall fragments and other biological components. Auxin content was also influenced by heavy metals stresses and it was reported that bacterial inoculation helped the plant to increase its auxin content under high stress of heavy metals (Hasnain and Sabri, 1997).

Based on these results we conclude that along with many other mechanisms involved for the seedling growth stimulation, it was obvious that bacterial strains promoted the plant growth by decreasing the availability of toxic Cr(VI), which was reduced by the bacterial strains into a less bioavailable Cr(III). Actually these bacterial strains give some alleviation to plants under Cr(VI) stress by decreasing the availability of toxic Cr(VI) to plants. Despite this factor many other factors such as IAA production, phosphate solubilization, siderophores production, enhancement in the uptake of essential plant nutrients might also be involved for the improvement of seedling growth.

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