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EFFECT OF MICROBIAL INOCULATION ON WHEAT GROWTH AND PHYTO-STABILIZATION OF CHROMIUM CONTAMINATED SOIL

MUHAMMAD YAHYA KHAN, HAFIZ NAEEM ASGHAR*, MUHAMMAD USMAN JAMSHAID, MUHAMMAD JAVED AKHTAR AND ZAHIR AHMAD ZAHIR

Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan
**Corresponding author: naeemasghar@yahoo.com*

Abstract

Higher concentration of Cr(VI) in the plant root zone affects many physiological processes and inhibits plant growth. Plant growth promoting rhizobacteria (PGPR) can improve plant health in contaminated soil as well as convert Cr(VI) to less toxic Cr(III). In this study, 180 Cr(VI) tolerant bacteria were isolated and after screening 10 efficient bacteria capable to work under chromium stress conditions were selected. Wheat (*Triticum aestivum* L.) seeds were inoculated with selected bacterial isolates and sown in Cr(VI) contaminated (20 mg kg^{-1}) pots. Results showed that Cr(VI) contamination significantly suppressed the plant growth and development. However, inoculation improved plant growth parameters significantly compared to un-inoculated plants. In inoculated pots Cr(VI) contents were decreased in soil upto 62% while plant analysis for Cr(VI) revealed that inoculation decreased uptake and translocation of Cr(VI) from soil to the aerial parts of plant. Concentration of Cr(VI) was upto 36% less in roots and 60% less in shoots as compared to un-inoculated plants grown in contaminated pots.

Introduction

Chromium (Cr), one of the heavy metals, is environmental pollutant and its compounds are widespread because of their application in many different industries, including metallurgical, electroplating, production of paints and pigments, tanning, wood preservation, chemicals production, pulp and paper production (Zayed & Terry, 2003). In soil chromium contamination is rising due to use of wastewater and industrial effluents as irrigation source for crop production mostly in the urban lands (Mushtaq & Khan, 2010). Different heavy metals suppress plant growth differently (Ahmad *et al*., 2012). The toxic effects of chromium are highly dependent on its oxidation state. Mainly in soils the chromium is present in most stable forms of either Cr(VI) and/or Cr(III). Cr(III) is considered to be relatively less toxic as compared to bio-available Cr(VI) compounds in the form of chromate (CrO_4^{-2}) and dichromate ($Cr_2O_7^{-2}$) that are highly toxic and have been shown to be mutagenic and carcinogenic (Srivastava *et al*., 1999; Messer *et al*., 2006). Cr(VI) can be toxic to plants upto concentration of 0.5mgL^{-1} in solution and 5 mg kg^{-1} in soil (Turner & Rust, 1971). Cr(VI) significantly decreases the seedling growth, root length, shoot length, total chlorophyll contents in shoots, rates of net photosynthesis, transpiration and of stomatal conductance in wheat (Dey *et al*., 2009). The activities of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase are also significantly affected by Cr(VI) treatment in wheat (Subrahmanyam, 2008). Interveinal chlorosis of young leaves occur which become necrotic at later growth stages due to low concentrations of Cr(VI). Chromium supply severely affected grain yield of wheat and even no seed formation was observed at 1.0 mM Cr(VI) (Sharma *et al*., 1995). However, the toxic effects of chromium on plants can be minimized by different physicochemical and biological approaches (Khan *et al*., 2012a & 2012b). Amongst biological approaches, one of the inexpensive and environment friendly way is use of plant growth promoting rhizobacteria to alleviate the chromium toxicity in plants (Khan *et al*., 2012b, Kang *et al*., 2012). Plant growth promoting rhizobacteria are the root colonizing bacteria (rhizobacteria) that exert beneficial effects on plant development via direct or indirect mechanisms (Nelson, 2004) and have potential to decrease the toxic effects of heavy metals on the plants through secretion of acids, proteins, phyto-antibiotics, and other chemicals (Bertrand *et al*., 2000). Plant growth promoting rhizobacteria having ACC-deaminase enzyme could improve the plant growth under stress conditions (Nadeem *et al*., 2006). Harms of chromium on plants could be minimized by plant growth promoting rhizbacteria *via* different mechanisms like biosorption and bioaccumulation, bioreduction to a less-toxic state, and chromate efflux (Nazir *et al*., 2011; Khan *et al*., 2012b). Keeping in view the possible role of PGPR to improve plant growth in chromium contaminated soil along with reduction of Cr(VI) into Cr(III). The present study was planned for isolation and screening of chromium tolerant PGPR and to evaluate the effect of PGPR on growth and yield of wheat under chromium stress conditions.

Materials and Methods

Isolation and preservation of Cr(VI) tolerant bacteria: Bacteria were isolated from wheat rhizosphere taken from the fields irrigated with tannery effluents, near the tannery industries of District Kasur by using Luria Bertani (LB) agar medium enriched with different (50, 75 and 100 mg L^{-1}) concentrations of Cr(VI) (Camargo *et al*., 2003). Further purification was done by streaking on glucose peptone agar medium. Bacterial isolates were preserved in Eppendorf by using glycerol at low temperature.

Characterization of bacteria: Selected bacteria isolated by using enrichment technique were characterized for reduction of Cr(VI) in Cr(III) (Campos *et al*., 1995) and plant growth promotion mechanisms like solubilization of inorganic phosphate (Mehta & Nautiyal, 2001), chitinase activity (Chernin *et al*., 1998), oxidase activity (Steel, 1961), catalase activity (Mac Faddin, 1980) and Gram staining (Holt *et al*., 1994).

Seed germination assay: Prior to pot trial an experiment was conducted to assess the effect of Cr(VI) on seed germination of wheat. Wheat seeds were surface sterilized by dipping in 95% ethanol for a moment and then in 5% Sodium hypochlorite for 10 minutes (Abd-Alla *et al*., 2012). Then seeds were washed six times with sterilized distilled water. After thorough washing seeds were sown on sterilized filter paper sheets placed in Petri plates. Ten seeds were sown in each Perti plate and 3 mL solution of different concentrations of Cr(VI) 10, 20, 50, 80 and 100 µg mL^{-1} respectively was poured in each Petri plate keeping control without Cr(VI) by pouring 3 mL of distilled water. The Petri plates were incubated at 28±1°C for 7 days. This experiment was conducted with three repeats following completely randomized design (CRD). Data regarding shoot length, root length, fresh weight and dry weight of 7 days old seedlings were recorded.

Pot trial: Soil having sandy clay loam texture with pHs; 7.5, saturation percentage; 35%,EC_e; 1.41 dS m^{-1}, CEC; 4.9 Cmolc kg^{-1}, organic matter; 0.62%, total nitrogen; 0.06%, available phosphorus; 7.34 mg kg^{-1}, extractable potassium; 131 mg kg^{-1} and non detectable Cr (VI) contents was taken. Soil was contaminated by using $K_2Cr_2O_7$ as source of Cr(VI) and finally 20 mg kg^{-1} Cr(VI) concentration was maintained. After contamination by addition of Cr(VI) solution, soil was equilibrated for a period of 15 days and used to fill the pots. Each pot was filled with 10 kg contaminated soil keeping one treatment without contamination. The inoculum for the pot trial was prepared by growing the selected isolates in glucose peptone broth medium. Broth medium was prepared by using the composition of glucose peptone medium except agar and was sterilized at 121°C temperature and 15 psi pressure for 20 minutes. Flasks containing glucose peptone broth were inoculated with selected isolates and incubated at 28 ± 1°C for 3 days. Uniform cell density (10^7-10^8 CFU mL^{-1}) was maintained by maintaining optical density of (OD=0.45) at 535 nm. The inoculum of each isolates was injected into sterile peat (100 ml kg^{-1}) and was incubated for 24 h at 28 ± 1°C before using it for seed coating. For seed inoculation, seed dressing was carried out with inoculated peat mixed with clay and 10% sugar solution. In case of the un-inoculated control, the seeds were coated with the same but autoclaved inoculum suspension. Inoculated wheat (*Triticum aestivum* L.) seeds with ten different bacterial isolates were sown in chromium contaminated soil, keeping control without inoculation, though having same level of contamination. Another treatment was included in this experiment where neither chromium nor PGPR were applied to segregate the effect of chromium stress on plant growth. The crop was harvested at maturity and data regarding plant growth parameters were recorded. After harvesting, Cr(VI) contents in soil and different plant parts (root, shoot and grains) were also determined by following the method described by Gheju *et al*., (2009). According to this, soil samples were digested by using aqua regia (HCl : HNO_3 = 3:1) at 85°C for 2 h. The digested mixture was allowed to cool, filtered, made the volume up to 50mL with HNO_3. While plant samples were oven dried at 105°C for two days so that constant weight may be attained. These plant samples were ashed in muffle furnace at 600°C for 6 h. The ashed samples were dissolved with a mixture of 2 M HCl and 1 M HNO_3, filtered and final volume made up to 50 mL. These soil and plant samples were analyzed for Cr(VI) contents by using 1,5-diphenylcarbazide as color developing reagent. Purple color was obtained after 15 minutes due to formation of complex by Cr(VI) in the presence of 1,5-diphenylcarbazide. The absorbance was measured at 540 nm by using spectrophotometer (Evolution 300 LC). The data were subjected to statistical analysis by following standard procedures (Steel *et al*., 1997), using Statistix 9 computer software.

Results

The present study consisted of a laboratory experiments to isolate and characterize Cr(VI) tolerant PGPR and to assess their effect on wheat (*Triticum aestivum* L.) in Cr(VI) contaminated soil. Here in this experiment different ten Cr(VI) tolerant bacterial isolates were characterized for their plant growth promoting activities. A laboratory experiment was conducted to assess the effect of Cr(VI) on seed germination and seedling growth of wheat (*Triticum aestivum* L.). The purpose of study was to evaluate the harmful effect of Cr(VI) on growth and yield of wheat (*Triticum aestivum* L.) and role of PGPR to decrease this detrimental effect by their plant growth promoting activities.

Isolation and characterization of Cr(VI) rhizobacterial isolates: About 180 rhizobacterial strains were isolated by enrichment technique. Out of 180 isolates, 10 rhizobacterial isolates K-10, K-20, K-23, E-25, E-30, K-11, E-20, E-27, K-8 and K-13 were able to grow efficiently on enriched media. These ten isolates were selected for further characterization. Results regarding chromate reduction (Fig. 1) showed that all bacterial isolates had chromate reduction capability with variable rates. Maximum chromate reduction (90%) was observed with bacterial isolate K-13 and minimum chromate reduction (60%) was observed with isolate K-10 which was statistically non-significant with bacterial isolate K-20 which reduced 61% chromate as compared to control (no bacterial isolates). Data given in Table 1 showed that out of ten selected rhizobacterial isolates nine had the ability to solubilize inorganic phosphate while only one isolate K-20 was not able to solubilize inorganic phosphate. While seven isolates were positive for oxidase activity and only three isolates (K-20, K-8 and K-13) were negative regarding production of cytochrome oxidase enzyme. Results of catalase test showed that eight rhizobacterial isolates were positive regarding production of catalase enzyme and isolate K-8 and K-13 were negative for catalase acitivity. Chitinase activity of selected isolates was measured and results showed that out of ten rhizobacterial isolates seven isolates (K-23, E-25, E-30, K-11, E-20, K-8 and K-13) were positive while rest of isolate were negative regarding chitinase activity. Results of Gram's staining showed that seven isolates were negative and three isolates (K-11, K-8 and K-13) were positive.

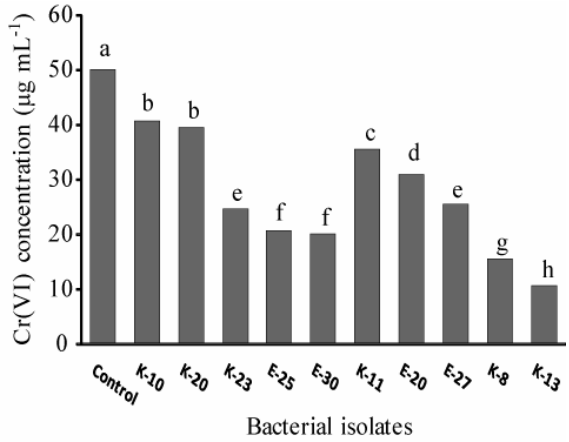

Fig. 1. *In vitro* chromate reduction by bacterial isolates. Bars sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$).

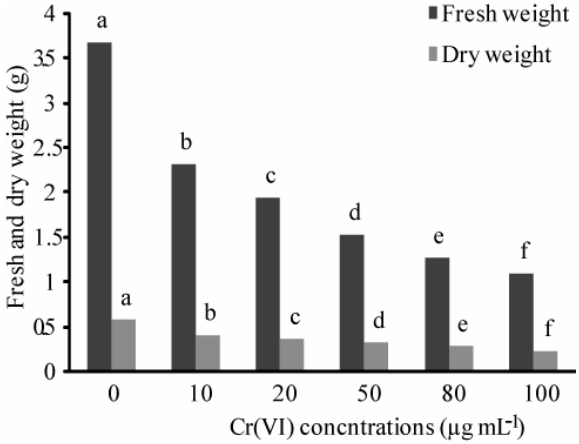

Fig. 2. Effect of different concentrations of Cr(VI) on fresh and dry weight of wheat (*Triticum aestivum* L.) seedlings. Bars sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$)

Table 1. Characterization of selected Cr(VI) tolerant bacterial isolates.

Isolate Identity	Phosphate Solubilization	Catalase Activity	Oxidase Activity	Gram's Staining	Chitinase Activity
K-10	+ve	+ve	+ve	-ve	-ve
K-20	-ve	+ve	-ve	-ve	-ve
K-23	+ve	+ve	+ve	-ve	+ve
E-25	+ve	+ve	+ve	-ve	+ve
E-30	+ve	+ve	+ve	-ve	+ve
K-11	+ve	+ve	+ve	+ve	+ve
E-20	+ve	+ve	+ve	-ve	+ve
E-27	+ve	+ve	+ve	-ve	-ve
K-8	+ve	-ve	-ve	+ve	+ve
K-13	+ve	-ve	-ve	+ve	+ve

Effect of Cr(VI) on seed germination and seedling growth of wheat (*Triticum aestivum* L.): Results regarding effect of Cr(VI) on seed germination showed that wheat variety FSD-2008 was highly tolerant to Cr(VI) stress at germination stage and showed 100% seed germination up to 100 µg mL^{-1} Cr(VI) concentration. But, increase in concentration of Cr(VI) from 0 to 100 µg mL^{-1} significantly decreased the shoot length, root length, seedling fresh weight and seedling dry weight. Data presented in Figs. 2 & 3 showed that maximum reduction in shoot length (75%), root length (90%), seedling fresh weight (70%) and seedling dry weight (61%) was observed at 100 µg mL^{-1} Cr(VI) concentration while minimum reduction in shoot length (20%), root length (48%), seedling fresh weight (37%) and seedling dry weight (29%) was observed at 10 µg mL^{-1} Cr(VI) concentration as compared to seedling grown without Cr(VI).

Pot experiment: Results regarding growth and yield parameters indicated that Cr(VI) significantly decreased the shoot length, shoot fresh and dry weight, root fresh and dry weight, spike length and grain yield as compared to un-inoculated plants grown in non-contaminated pots (Table 2). However, the inoculation of wheat plants with Cr(VI) tolerant PGPR isolates improved the all growth and yield parameters under same level of Cr(VI) stress as compared to un-inoculated plants grown in contaminated pots. It was observed that un-inoculated plants in contaminated pots died off after 15 days of germination while some inoculated plants remained alive up to maturity. No tillers were observed in all contaminated pots either these were inoculated and/or un-inoculated except the pots where neither contamination nor inoculation was applied. Maximum reduction (82%) in shoot length was observed in un-inoculated plants grown in contaminated pots as compared to un-inoculated plants grown in non-contaminated pots. Compared to un-inoculated plants grown under Cr(VI) stress, inoculation with Cr(VI) tolerant rhizobacterial isolate (K-13) enhanced the shoot length up to 3 folds. Plants inoculated with isolate K-8 showed best results regarding shoot fresh weight where 26.38 folds more shoot fresh weight was

observed over un-inoculated plants grown in contaminated pots. Comparison with un-inoculated plants grown in non-contaminated pots also showed that plants inoculated with isolate K-8 were best by showing minimum reduction (46%) in fresh shoot weight. Inoculation with Cr(VI) tolerant rhizobacterial isolates improved the plant growth and shoot dry weight under Cr(VI) contaminated conditions. Plants inoculated with isolate K-8 also performed best regarding shoot dry weight and resulted in 27.19 folds increase in shoot dry weight compared to un-inoculated plants grown in contaminated pots but it was 51% less as compared to un-inoculated plants grown in non-contaminated pots. Maximum reduction (92%) in fresh root weight was recorded in un-inoculated plants grown in contaminated pots which was severely affected by Cr(VI) contamination. However, inoculation of seeds with Cr(VI) rhizobacterial isolates enhanced the root growth under contaminated conditions. Plants inoculated with isolates K-8 and K-13 showed better results compared to all other isolates and helped the plants to cope with contamination by decreasing fresh root weight reduction effect of Cr(VI) from 92% (un-inoculated plants grown in contaminated pots) to 35% and 39% respectively as compared to un-inoculated plants grown in non-contaminated pots. Contamination decreased root dry weight upto 93% but inoculation with PGPR isolates improved the root dry weight upto7.76 folds (37% reduction compared to un-inoculated plants grown without Cr(VI) contamination) with plants grown in contaminated soil without inoculation. Data regarding spike length revealed that Cr(VI) significantly affected the spike length and even no spikes were formed in un-inoculated plants grown in contaminated pots. However, in all inoculated plants with Cr(VI) tolerant rhizobacterial isolates spikes were observed under Cr(VI) stress conditions except inoculation with isolate K-10. In those treatments where spikes were formed, plants in which Cr(VI) tolerant rhizobacterial isolate K-8 was used showed better results by showing minimum reduction (20%) in spike length as compared to un-inoculated plants grown without Cr(VI) contamination. Results showed that out of ten isolates only five isolates viz., K-8, E-25, K-13, E-30 and K-23 supported the plants to give yield response under Cr(VI) stress. Minimum reduction (85%) in yield was observed with isolate K-8 which was statistically non-significant with isolate E-25. The remaining plants inoculated with isolates (E-20, E-27, K-10, K-11 and K-20) were statistically non-significant with un-inoculated and contaminated control where no grains were observed. No doubt, all growth and yield parameters were increased by inoculation under Cr(VI) stress but recovery was always less than 100%.

As far as uptake and translocation of Cr(VI) to the upper parts of plant is concerned, plant shoot analysis showed that there was significant concentration of Cr(VI) in un-inoculated plants grown in contaminated pots (Table 3) . Inoculation of plants with Cr(VI) tolerant rhizobacterial isolates decreased the Cr(VI) concentration in shoots compared to un-inoculated plants grown in contaminated pots. Cr(VI) was not detectable in grains of plants grown in contaminated pots. Compared to un-inoculated plants grown in Cr(VI) stress, maximum decrease (60%) in Cr(VI) concentration in shoots was observed with isolate K-8 and minimum decrease (4%) in Cr(VI) concentration in shoots was observed with isolate K-10. Inoculation of plants with Cr(VI) tolerant rhizobacterial isolates also decreased the Cr(V) concentration in roots. Maximum decrease (36%) in Cr(VI) concentration in roots was observed with isolate K-8 and minimum decrease (3%) in Cr(VI) concentration in roots was recorded with isolate K-11 compared to un-inoculated plants grown in contaminated pots.

It is clearly obvious from results (Fig. 4) that Cr(VI) concentration in soil significantly decreased by plant-microbes interactions. Isolate K-13 decreased Cr(VI) concentration in soil by 62% as compared to un-inoculated and contaminated control pots. Isolates K-8 and E-25 also effective by decreasing Cr(VI) concentration in soil upto50% and 40% respectively as compared to un-inoculated and contaminated control. Minimum decrease (12%) in Cr(VI) concentration in soil was observed with isolate K-10.

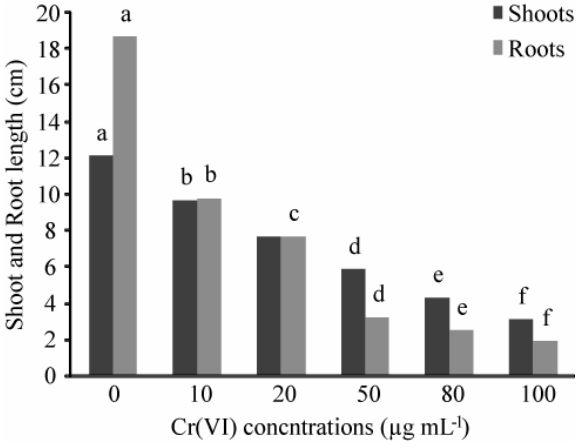

Fig. 3. Effect of different concentrations of Cr(VI) on shoot and root length of wheat (*Triticum aestivum* L.) seedling. Bars sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$).

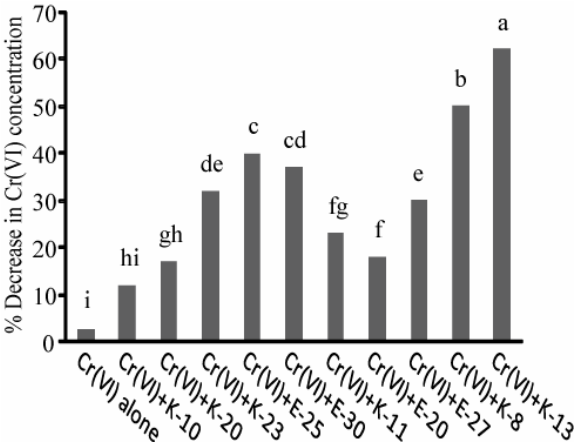

Fig. 4. Effect of Cr(VI) tolerant PGPR on reduction of Cr(VI) in soil. Bars sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$).

Table 2. Effect of Cr(VI) tolerant PGPR on different growth and yield parameters of wheat (*Triticum aestivum* L.) in chromium contaminated soil.

Treatments	Shoot Length (cm)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Fresh Root Weight (g)	Dry Root Weight (g)	Spike Length (cm)	Yield Plant^{-1} (g)
No Cr(VI), no inoculation	55.52±0.28 a	17.59±0.13 a	16.11±0.52 a	5.45±0.15 a	5.05±0.07 a	9.50±0.28 a	5.68±0.22 a
Cr(VI) alone	10.16±0.44 j	0.34±0.02 h	0.28±0.03 h	0.44±0.03i	0.36±0.03 k	0.00±0.00 f	0.00±0.00 d
Cr(VI)+K-10	17.20±0.41i	1.23±0.02 g	1.03±0.05 g	1.29±0.02 g	1.16±0.02gh	0.00±0.00 f	0.00±0.00 d
Cr(VI)+K-20	19.16±0.44 h	0.34±0.02 h	0.29±0.02 h	0.91±0.02 h	0.66±0.03 j	3.96±0.28 e	0.00±0.00 d
Cr(VI)+K-23	25.83±0.44 e	4.66±0.02 d	3.66±0.18 e	2.37±0.09 d	2.17±0.06 d	5.16±0.27 c	0.54±0.08 c
Cr(VI)+E-25	33.16±0.44 c	5.14±0.01 d	4.58±0.17 d	1.16±0.02 g	1.05±0.02 hi	5.03±0.17 cd	0.71±0.09bc
Cr(VI)+E-30	30.86±0.46 d	6.26±0.02 c	5.45±0.07c	1.90±0.02 e	1.70±0.02 e	5.56±0.23 c	0.59±0.01 c
Cr(VI)+K-11	21.16±0.44 g	1.58±0.02 g	1.23±0.7fg	1.32±0.02fg	1.19±0.02g	4.00±0.17 e	0.00±0.00 d
Cr(VI)+E-20	21.00±0.57 g	2.71±0.03 f	1.71±0.11 f	1.17±0.01 g	1.02±0.01i	4.50±0.20 de	0.00±0.00 d
Cr(VI)+E-27	22.83±0.44 f	1.47±0.02 g	1.27±0.02fg	1.50±0.01 f	1.38±0.01 f	4.00±0.28 e	0.00±0.00 d
Cr(VI)+K-8	40.50±0.76 b	9.40±0.02 b	7.89±0.20 b	3.54±0.05 b	3.15±0.02 b	7.51±0.15 b	0.83±0.05 b
Cr(VI)+K-13	41.16±0.60 b	4.14±0.03 e	3.60±0.03 e	3.29±014 c	2.99±0.05c	7.03±0.14 b	0.62±0.03 c

Means ± S.E. sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$).

Table 3. Effect of Cr(VI) tolerant PGPR on Cr(VI) concentration in roots of wheat (*Triticum aestivum* L.) in chromium contaminated soil.

Treatments	Cr (VI) Concentration (µg g^{-1})	
	Shoots	Roots
No Cr(VI), no inoculation	ND*	ND*
Cr(VI) alone	12.65 ± 0.32 a	35.75 ± 0.38 a
Cr(VI) + K-10	12.20 ± 0.22 b	33.90 ± 0.20 cd
Cr(VI) + K-20	11.66 ± 0.05 c	34.00 ± 0.28 c
Cr(VI) + K-23	6.24 ± 0.14 ef	24.90 ± 0.07 f
Cr(VI) + E-25	6.52 ± 0.19 e	23.90 ± 0.07gh
Cr(VI) + E-30	5.91 ± 0.06 f	24.25 ± 0.12 g
Cr(VI) + K-11	11.67 ± 0.12 c	34.67 ± 0.10 b
Cr(VI) + E-20	9.83 ± 0.08 d	33.34 ± 0.16 de
Cr(VI) + E-27	10.05 ± 0.08 d	32.83 ± 0.44 e
Cr(VI) + K-8	5.00 ± 0.76 g	22.78 ± 0.06i
Cr(VI) + K-13	5.08 ± 0.4 g	23.56 ± 0.09 h

*ND= Not detectable

Means ± S.E. sharing the same letter (s) are statistically non-significant according to Duncan's multiple range test ($p<0.05$)

Discussion

Chromium is potentially a toxic heavy metal and can cause severe damage to plants and animals in Cr(VI) form. In present study large numbers of bacterial strains were isolated from the rhizosphere soil. Ten highly Cr(VI) tolerant bacterial isolates were selected for pot experiment. Wheat seeds inoculated with these selected bacterial isolates were sown in Cr(VI) contaminated soil for screening of their plant growth promoting capabilities under Cr(VI) stress.

Chromate reduction assay showed that all the bacterial isolates reduced the Cr(VI) into Cr(III) with respect to un-inoculated control treatment at varying level. Exact mechanism by which bacteria reduce Cr(VI) in to Cr(III) is not known. This Cr(VI) reduction may be due to several reasons i.e. intracellular mechanisms involved in detoxification of Cr(VI), involvement of chromate in intracellular metabolism as terminal electron acceptor for gaining energy (Wani *et al.*, 2007) and may be due to excretion of waste products by bacteria which reduce Cr(VI) into Cr(III) like H_2S (Fude *et al*., 1994). This reduction may also be due to enzymatic activity of bacterial isolates (Cheung & Gu, 2003). Mistry *et al*., (2009) reported that chromium resistance bacterial strain *Pseudomonas olevorans* had ability to reduce the Cr(VI) into Cr(III). They further concluded that this bacterium had ability to bioremediate Cr(VI) containing waste.

Morales *et al*., (2007) isolated *Streptomyces sp.*, CG252 which was highly tolerant to Cr(VI) and have ability to reduce Cr(VI) into Cr(III).

In our experiment Cr(VI) contamination did not show any negative effect on seed germination upto 100 µg mL^{-1} concentration. These results correspond the findings of Datta *et al*., (2011), they reported Cr(VI) tolerance capability of different varieties of wheat in their study. It showed that wheat variety FSD-2008 used in our study might be Cr(VI) tolerant. The reduction in root and shoot length with increasing concentration of Cr(VI) might be due to increasing toxic effect of Cr(VI) on plant physiological processes, nutrient and water transport to shoots by roots. It might also be due to direct toxic effect of Cr(VI) on cellular metabolism of shoots as observed by Panda & Chaudhury (2005). Root length was more severely affected by Cr(VI) as compared to all other parameters. This may be due to restriction of division and/or elongation of root cells by increasing Cr(VI) concentration in roots (Woolhouse, 1983; Shanker *et al.*, 2004; Zou *et al.*, 2006). Fresh weight and dry weight of wheat seedling was also severely affected by Cr(VI) toxicity and decreased by increasing Cr(VI) concentration. This decrease in fresh and dry weight of seedlings might be due to decrease in chlorophyll contents due to Cr(VI) toxicity which effect the photosynthetic activity (Subrahmanyam, 2008; Sharma & Sharma 1993, Chatterjee & Chatterjee, 2000; Nichols *et al*., 2000) of plants ultimately reduced the biomass production. This decrease in chlorophyll might be due to deterioration and degradation of the proteins contents of antenna complex (Shanker, 2003).

Results of pot trial showed that Cr(VI) contamination significantly decreased growth and development of wheat. The yield of wheat was also severely affected by Cr(VI) stress and even no spikes were observed in un-inoculated plants grown in contaminated pots. The decrease in growth parameters and yield of wheat might be due to several possible reasons. Excess chromium in soil retard the plant growth due to chromium toxicity and this growth retardation with chromium has been observed in several plant species (Bishnoi *et al*., 1993; Sharma & Sharma, 1996). Root growth and functions severely affected by higher concentration of Cr(VI) result in root damage, decrease fresh and dry weight, and reduced uptake of water and nutrients (Terry & Banuelos, 2000). However, inoculation of wheat seeds with Cr(VI) resistance bacterial isolates improved the plant health under Cr(VI) stress and improved the all growth parameters of wheat as compared to un-inoculated plants grown in contaminated pots. Inoculation of seeds with Cr(VI) resistant rhizobacterial isolates improved the plant health and yield as compared to un-inoculated plants grown in contaminated pots. Several studies showed that rhizosphere bacteria stimulate plant growth and development under stress conditions (Jacobson *et al*., 1994; Glick *et al*., 1998; Gupta *et al*., 2002). These changes in growth and development of wheat by Cr(VI) resistant rhizobacterial isolates might be due to involvement of single or multiple possible mechanisms of actions i.e. solubilization of insoluble phosphate (Yasmin & Bano 2011; Gupta *et al*., 2002; Pena & Reyes, 2007), production of siderophore (Glick *et al*., 1999; Meyer, 2000), production of phytohormones (Glick *et al*., 1998; Asghar *et al.*, 2004; Humphry *et al*., 2007) and indirect mechanisms of action i.e. reduction of Cr(VI) to Cr(III) by which it decreases the harmful effects of Cr(VI) to the plants (Salunkhe *et al*., 1998), biocontrol (Chandra *et al*., 2007) or induces systemic resistance in plants (Mishra *et al*., 2006) against phytotoxicity of Cr(VI). This may involve the production of different metabolites like phytohormones, siderophore, enzymes and organic acids in the rhizosphere by PGPR, which resulted in plant growth promotion (Zahir *et al.*, 2001). Plant growth promoting rhizobacteria may also enhance nutrient availability by recycling of organic waste (Asghar *et al.*, 2006). Kumar *et al*., (2009) suggested that the plant growth promoting bacteria (*Enterobacter aerogenes* and *Rahnella aquatilis*) reduce the toxicity of Ni and Cr in *Brassica juncea* (Indian mustard) and promoted plant growth under pot culture experiments. Growth promotion by bacteria in Cr(VI) contaminated soil might be due to auxin production and stress specific protein formation**(**Hasnain & Sabri, 1997). Such inline findings by other researchers support our results.

Results regarding Cr(VI) concentration in shoot and roots of wheat plants showed that there was more Cr(VI) concentration in roots as compared to shoot of wheat in Cr(VI) contaminated soil. This may be due to poor translocation of chromium from root to shoot system (Huffman & Allaway, 1973; Zayad *et al*., 1998). This might be due to immobilization of Cr(VI) in vacuoles of root cells hence more accumulation in roots, this might be a natural toxicity response of plants (Shanker *et al*., 2004). Inoculation of wheat seeds with Cr(VI) tolerant bacterial isolates decreased the uptake and translocation of Cr(VI) from soil to root and aerial parts of plant. This decreased in Cr(VI) concentration in roots and shoots of wheat plants may due to reduction of Cr(VI) in to Cr(III) by bacterial isolates (Salunkhe *et al*., 1998) which ultimately decreased the Cr(VI) contents in soil. Hasnain & Sabri (1997) also reported similar remarks that inoculation of seeds with *Pseudomonas sp*. decreased the uptake and accumulation of chromium contents in root and shoot system of *Triticum aestivum.*

Results regarding decrease in Cr(VI) concentration in soil after harvesting of wheat crop showed that Cr(VI) contents decreased significantly from the initial Cr(VI) concentration maintained in each pot. Minimum decrease in Cr(VI) concentration in soil was observed in un-inoculated and contaminated soil. This decrease might be due to uptake and accumulation of Cr(VI) contents in to root and shoot system (Mishra *et al*., 1997) of wheat plant and may be due to reduction of Cr(VI) in to Cr(III) by bacterial isolates (Cheung & Gu, 2003; Salunkhe *et al*., 1998).

Conclusion

Inoculation with bacteria having capabilities to survive in Cr(VI) stress and to reduce Cr(VI) to Cr(III) could be very helpful to improve plant growth in chromium contaminated soil, possibly by using different direct and indirect mechanisms of plant growth promotion especially growth regulator production and regulating stress induced physiological mechanisms of plants.

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