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EFFECT OF BACTERIA CONTAINING ACC DEAMINASE ON GROWTH OF WHEAT SEEDLINGS GROWN WITH CHROMIUM CONTAMINATED WATER

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Abstract

Chromium (Cr) is considered as toxic environmental pollutant and causes harmful effects on growth and development of plants and human health. Usually, high level of ethylene (a plant hormone) is produced in plants in response to any biotic or abiotic stress. Negative effect of Cr-imposed stress on plants may be minimized by using bacteria possessing an enzyme 1-amino-cyclopropane-1- carboxylic acid (ACC)-deaminase. The aim of this study was to investigate the effect of plant growth-promoting bacteria containing ACC-deaminase on wheat under different levels of Cr applied to growth medium. Experiments were conducted under laboratory and lath house conditions. Results of this study demonstrated that inoculation of wheat seeds with two strains of bacteria *Pseudomonas fluorescens* (Q14) and *Bacillus thuringiensis* (KAP5) significantly increased the root length (up to 208%), shoot length (up to 67%), root dry weight (up to 140%) and shoot dry weight (up to 71%) respectively as compared to uninoculated control plants. Strain KAP5 possessing both ACC-deaminase as well as phosphate solubilizing activity was found to be the most effective in improving the plant growth compared to uninoculated control in both sand and soil experiments. Inoculation also significantly increased the accumulation of Cr in root and shoots compared to uninoculated control, where the Cr uptake 80.8µg/g dry mass and 69.9µg/g dry mass in root and shoot respectively, was observed. These findings indicated that ACC-deaminase producing bacterial strains could play vital role in improving the plant growth under metal-stress condition and they may enhance bioremediation process in Cr-contaminated environment. Moreover, presence of dual plant growth promoting trait such as ACC-deaminase and phosphate solubilizing activity could have more promising effect on plant growth and Cr removal than the single trait bacterium.

Introduction

Chromium pollution in the environment has significantly increased all over the world mainly through anthropogenic Cr sources (Lopez-Luna *et al*., 2009). Chromium exists in various oxidation states from Cr (II) to Cr (VI) but only the trivalent and hexavalent states are stable. Chromium has multifarious industrial uses as it is widely used in leather tanning, metal finishing, corrosion control, electroplating, pigment manufacture and nuclear weapon production (Srivastavaa & Thakur, 2006). However, high amount of Cr is released from tanneries which has higher tendency to convert into Cr (VI) in the effluent (Srivastavaa & Thakur, 2006; Mahmood *et al*., 2012). In this way, leather industry is one of the big sources that are polluting water resources.

Chromium disturbs normal functioning of soil ecosystems and development of plants when Cr enters into soil through irrigation system. In the surrounding of leather industrial area, accumulation of Cr in soil ecosystems occurred at higher rates than elimination via natural pathways (Somova & Pechurkin, 2009). In addition, Cr cannot be degraded into harmless products, hence persists in the environment for long time (Fernanda *et al*., 2007). High concentration of heavy metals results in the disruption of natural terrestrial ecosystem (Wei *et al*., 2007; Yadav *et al*., 2009). Heavy metals affect plant growth by reducing nutrient uptake and causing disorders in plant metabolisms reduction in chlorophyll contents and mineral nutrients and chlorosis (Broos *et al*., 2005; Dan *et al*., 2008; Ghani, 2011). Similarly, higher concentrations of Cr are very toxic at germination and early seedling growth (Akinci & Akinci, 2010). Cr toxicity in plants also results in inhibition of enzymatic activities, impairment of photosynthesis, nutrient and oxidative imbalances, and mutagenesis (Oliveira, 2012).

Phytoremediation is a low cost and environmental friendly technology for detoxification of soil (Ma *et al*., 2009; Nazir *et al.*, 2011; Shah *et al.*, 2011). Baker & Brooks, (1989) found that some plants in soil contaminated with heavy metals have potential to accumulate higher concentration of heavy metal without showing impact on growth and development. It is noticed that plants lacks the specific pathways for the Cr, it is transported by the carriers of essential ions such as sulphate and iron (Shanker *et al*., 2005). For toxicity assessment in industrial and aquatic ecosystems the US-EPA recommended several plants as biomarker (Anon., 1996). These plants include cucumber, lettuce, soybean, tomato, cabbage, oat, perennial ryegrass, maize, carrot, and onion (Lopez-Luna *et al*., 2009).

However, remediation of soil contaminated with Cr can be speed up using biological potential of both plants and microorganisms, which have various biochemical capabilities. High concentration of nutrients exuded from roots attracts more bacteria. These bacteria increase plant growth, yield and crop quality by affecting plant growth through multifarious mechanisms such as nitrogen fixation, production of phytohormones and siderophores, solubilization of minerals and transformation of nutrient elements and enzyme products (Ma *et al*., 2009).

It has been reported that under Cr stress conditions ethylene is produced at substantially accelerated rates. Ethylene is essential requirement for plant growth, but at higher concentration it adversely affects the root growth, and consequently, the development of plants (Zhuang *et* *al*., 2007). In higher plants and microorganisms, methionine (MET) is a common biochemical precursor for ethylene biosynthesis through ACC; thus synthesis of ethylene is highly dependent on endogenous ACC levels. Along with other small molecules, ACC produced by plants is exuded from roots (Penrose *et al.,* 2001).

Certain plant growth promoting bacteria which colonize the rhizosphere of plant roots produce ACC-deaminase. ACC-deaminase plays a significant role in growth and development of plants by regulating the ethylene level (Arshad & Frankenberger, 2002). PGPR containing ACC-deaminase eliminates the ethylene mediated negative effects on plants by metabolizing ACC into ammonia and α–ketobutyrate (Glick *et al*., 1998). On the other hand, in soil many microorganisms are able to solubilize "unavailable" forms of heavy metal-bearing minerals by excreting organic acids. This phyto-bacterial technology may be very effective in improving plant growth in Cr-polluted soils and thus can be helpful to enhance bioremediation process. The present study was planned with the objective to study the influence of ACC-deaminase producing bacteria on growth of wheat under Cr stress conditions.

Materials and Methods

Two pre-isolated strains of PGPR containing ACC deaminase i.e., *Pseudomonas fluorescens* Q14 (Shaharoona *et al*., 2006) and *Bacillus thuringiensis* KAP5 (Baig *et al*., 2011) were used to study their effect on growth of wheat grown with Cr contaminated water. *Bacillus* strain KAP5 had also shown P-solubilizing activity. Cr solutions of different concentrations were used for irrigation purpose. The seeds of wheat (*Triticum aestivum*) variety NARC-2009 were obtained from National Agriculture Research Center (NARC), Islamabad, Pakistan.

Inoculum preparation: For the preparation of inocula, pre-isolated bacterial strains Q14 and KAP5 were cultured in liquid general purpose medium. Recipe of the medium was composed of (g/L) glucose (2), KH_2PO_4 (0.5), $(NH_4)_2SO_4$ (0.5), peptone (0.5), Mg $SO_4{\cdot}7H_2O$ and $FeSO_4{\cdot}7H_2O$ (traces). The medium was incubated at 30°C for 48 h. The population density of 10^7-10^8 cfu per ml was achieved and roots were inoculated by injecting 5 ml of inocula into the rhizosphere of wheat plant with the help of syringe.

Experimental setup: Experiments including jar and pot trials were conducted on wheat plant using completely randomized blocked design to determine the effect of different concentration of Cr solutions on growth of wheat.

Sand experiment: Experiments were performed in plastic jars (8.5 × 6.5 cm) filled with 250 g of sterilized sand. Pre-germinated seeds of wheat were transferred in jars. One time 10 ml Hoagland nutrient solution (1/2 strength) was applied in each treatment. Ten ml Cr solution of different concentrations (0, 20, 40, 60, 80, 100 mg/L) was applied on alternate days with irrigation water (10 ml) and total 70 ml solution of Cr was applied per jar. The jars were divided into three sets on the basis of inocula and subdivided into six groups on the basis of Cr-treatments. Each treatment was replicated three times. After two weeks, plants were harvested and data regarding root length, shoot length, root and shoot fresh and dry weights were recorded.

Pot experiment: Plastic pots (25.3×13.6 cm) were filled with 5.5 kg of air dried sieved soil. Soil was thoroughly mixed with N and P fertilizers i.e. urea (100 mg/kg of soil) and single super phosphate (50 mg/kg of soil) before adding into pots. Six surface sterilized seeds were placed in each pot. After germination, plants were thinned to four plants per pot. Five ml of inoculum was applied into the rhizosphere through a syringe. Plants were irrigated with 100 ml of Cr solution of different concentrations (0, 100, 250, 500 mg/L) according to water requirement. The 800 ml total Cr solution was applied per pot. There were three treatments on the basis of inoculum applied to plants under four levels of Cr solutions. Each treatment was performed in four replications. Plants were harvested after 45 days and data regarding root length, shoot length, root and shoot fresh and dry weights were recorded.

Soil analysis: Soil used for pot experiment was analyzed for physiochemical parameters like soil texture, soil moisture content, pH and electrical conductivity (Table 1) by following standard procedures as described by Moodie *et al*., (1959) & Rhoades *et al*., (1982).

Table 1. Physiochemical characteristics of soil.

S #	Soil parameters	Values
1.	Particle size distribution	
	Clay %	15.2
	Silt %	6.8
	Sand %	78
	Textural class	Sandy loam
2.	Soil moisture contents (%)	3.2
3.	Electrical conductivity (µS/cm)	640
4.	pH	7.2

Chromium analysis in plants: Plants were air-dried and then ground using a pestle and motar. Each sample (1g) was taken in conical flask and 20 ml of tri acid mixture (HNO_3; H_2SO_4; $HClO_3$ 10:1:3) was added (Srivastava & Thakur, 2006). The contents of flasks were mixed thoroughly. Initially, the flasks were placed on slow heating hot plate in a digestion chamber and then the flasks were heated at high temperature (150°C) until the production of red fumes ceased. The contents were further evaporated till the volume reduced to about 3-5 ml but not to dryness. The completion of digestion was confirmed when the liquid became colorless. Then digested samples were transferred into 50 ml volumetric flask and the volume was made up to 50 ml by adding distilled water. The extract was filtered with filter paper (Whatman 42) and collected in plastic bottles. Cr was determined by using atomic absorption spectrometer.

Measurement of impact on wheat seedling growth in response to chromium and ACC-deaminase containing bacteria: In order to determine the effect of chromium applied and ACC-containing plant growth promoters on wheat seedling growth parameters like root length, shoot length, fresh weight of root, dry weight of root, fresh weight of shoot and dry weight of shoot were measured. Bioaccumulation of Cr was measured by calculating the bioaccumulation factors (BAF) for root and shoot by following formula.

$$\text{BAF (soil-root)} = \frac{\text{Cr concentration in root (µg/g)}}{\text{Cr concentration in soil (µg/g)}}$$

Statistical analysis: The data were subjected to analysis of variance using 2 factorial completely randomized design. Treatment means were compared by using LSD test at 5% level of significance. All the statistical analyses were performed by using M-STAT software.

Results

Sand experiment: The inoculated and uninoculated plants exposed to different levels of Cr in sand for two weeks responded differently in terms of root length (Table 2). Mean results showed a significant decrease in root length of wheat seedlings with the increase in Cr concentration. However, inoculation either with Q14 or KAP5 significantly improved the root length at different levels of Cr compared to uninoculated treatments. Strain Q14 showed a significant increase in root length at all concentration of Cr (ranging from 0-100 mg/L) compared to respective uninoculated Cr treated wheat seedlings. Maximum increase in root length was observed at 100 mg/L Cr which was 99 % greater than uninoculated treatment. Strain KAP5 also significantly increased the root length at all levels of Cr, compared to uninoculated treatments. At different Cr levels ranging from 0-100 mg/L, the increase in root length by strain KAP5 was between 0.8 to 209%, compared to uninoculated control.

Table 2. Effect of bacteria containing ACC-deaminase on root growth (root length and root weight) of wheat grown under different Cr concentrations. (Average of three replicates).

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Root length (cm)			Root weight (mg/plant)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	13.1c[a]	14.9a	14.1b	5.5a[b]	5.8a	3.5b
20	5.6	12.4d	13.3c	12.5d	3.4bc	3.5b	2.8b-e
40	11.2	11.5e	12.1d	12.1d	2.7b-e	5.1a	3.4b-d
60	16.8	10.3g	11.1f	11.3ef	2.4b-f	1.9c-f	3.1b-e
80	22.4	8.2j	9.7h	10.3g	1.6ef	1.8d-f	2.5b-f
100	28	3.0l	6.0k	9.3i	1.0f	1.5ef	2.4b-f

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test
a= Interaction effects of chromium and strains (LSD value=0.43)
b= Interaction effects of chromium and strains (LSD value=1.59)

The results of this study indicated that root biomass was significantly reduced with increase in Cr concentration up to 100 mg/L (Table 2). It was observed that that the wheat seeds inoculated with strains Q14 and KAP5 showed 74.1 to 31.4% decreases in root biomass than uninoculated wheat seedlings at 100 mg/L Cr. In case of strain Q14, maximum increase in root dry weight up to 88.9% at 40 mg/L of Cr concentration was observed compared to uninoculated Cr treated plants at same concentration. Inoculation with KAP5 of Cr treated plant showed increase in root dry weight up to 25.9, 29.2, 56.3 and 140% at 20, 40, 60, 80 and 100 mg/L Cr, respectively.

Application of different concentration of Cr had significant effect on shoot length of wheat and increase in Cr concentration from 0 to 100 mg/L decreased the shoot length of inoculated and uninoculated seedlings significantly (Table 3). In general, plants inoculated with strain Q14 had non-significant difference in shoot length with uninoculated control plants. At 60, 80 and 100 mg/L Cr, inoculation with strain Q14 showed significant increase in shoot length compared to uninoculated plants at these levels. Maximum increase up to 34% was observed in case of inoculation with strain Q14 at 100 mg/L Cr concentration compared to uninoculated treatment and KAP5 showed non-significant difference in shoot length with uninoculated control plants. Overall, there was a significant difference in shoot length with the increase in Cr concentration, except at 40 and 80 mg/L of Cr concentration.

The data regarding the effect of inoculation with PGPR containing ACC-deaminase on shoot dry weight at various levels of Cr concentration are summarized in Table 3. The results showed that Cr stress affected the shoot dry weight of wheat seedlings. Shoot dry weight was decreased by 62.2% at 100 mg/L Cr. But the wheat plants inoculated with Q14 and KAP5 under Cr stress significantly accumulated more dry weight in shoot than those not inoculated with bacterial strains. Shoot dry weight of wheat seedlings inoculated with Q14 and KAP5 was increased by 26.2 and 71.4% respectively as compared to uninoculated treatments at 100 mg/L Cr. The strain KAP5 showed significant improvement in the shoot dry weight of wheat seedlings. The results also demonstrated that bacterial strains (Q14 and KAP5) had tremendous ability of relieving the negative effect of Cr on shoot dry weight of wheat. Plants inoculated with strain KAP5 under Cr stress accumulated dry weight in shoot almost to the same extent as uninoculated plants grown in Cr untreated sand. It is obvious from the data that the wheat plants inoculated with strain KAP5 had significant increase in shoot dry weight at 40, 60, 80 and 100 mg/L of Cr concentration except at 0 and 20 mg/L Cr when compared with uninoculated control plants. Strain Q14 inoculated plants showed a significant difference in shoot dry weight with uninoculated control plants.

Table 3. Effect of bacteria containing ACC-deaminase on shoot growth (shoot length and shoot weight) of wheat grown under different Cr concentrations. (Average of three replicates).

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Shoot length (cm)			Shoot weight (mg/plant)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	12.6a[a]	14.1a	13.9a	11.4c[b]	13.5a	11.4c
20	5.6	12.1b	11.7c	10.6ef	10.9d	12.5b	10.6d
40	11.2	10.9de	10.3fg	10.3fg	8.5f	9.2e	8.9e
60	16.8	8.1k	9.2i	9.9gh	7.7g	8.4f	8.4f
80	22.4	9.9h	11.1d	9.9h	4.5j	5.4i	7.6g
100	28	5.2m	7.0l	8.7j	4.2k	5.3i	7.2h

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test

a= Interaction effects of chromium and strains (LSD value=0.45)

b= Interaction effects of chromium and strains (LSD value=0.31)

Chromium accumulation and transport in root: Accumulation of Cr in the root increased with the increase in the initial concentration of Cr in sand (up to 28 µg/g). Further, data exhibited significantly higher Cr accumulation in tissues of inoculated wheat plants compared with uninoculated control plants (Table 4). In this study, the maximum accumulation of Cr in the root was determined as 20.5µg/g in the presence of bacterial strain Q14, at 20 mg/L concentration of Cr in sand. However, in uninoculated plants the Cr accumulation in the root was assessed as 8.7µg/g. For wheat plants inoculated with strain KAP5, Cr concentration was increased up to 136% as compared to the uninoculated control. With the increase in Cr concentration in sand more accumulation of Cr in root was observed in root whether inoculated with bacterial strains or uninoculated state. Overall, strain Q14 caused the more accumulation of Cr in root about 31.9% than strain KAP5 which accumulated only 13.9%, compared to uninoculated plants. Bioaccumulation of Cr increased with the increase in Cr concentration and inoculation with bacterial strains containing ACC-deaminase also responsible for transfer of higher concentration of Cr from soil to root. In case of KAP5 inoculation more translocation (BAFs=3.68) of Cr from soil to root was observed at 20 mg/L concentration of Cr. On the other hand, strain Q14 inoculation translocated only 2.95 BAFs Cr from soil-root at 20 mg/L of Cr concentration.

Table 4. Cr accumulation and transport in root of wheat seedlings. (Average of three replicates).

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Cr concentration in root (µg/g) DW			BAF (soil-root)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	Nd	Nd	Nd	0.0	0.0	0.0
20	5.6	8.7de[a]	16.5cd	20.6b-d	1.6	2.9	3.7
40	11.2	22.3b-d	24.9bc	17.5cd	1.9	2.2	1.6
60	16.8	22.7b-d	30.0bc	24.2bc	1.4	1.8	1.4
80	22.4	29.5bc	33.5ab	35.2ab	1.3	1.5	1.6
100	28	33.3ab	48.4a	34.7ab	1.2	1.7	1.2

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test

a= Interaction effects of chromium and strains (LSD value=0.51) DW, dry weight, Nd, not detected

Chromium accumulation and transport in shoot: Effect of inoculation on Cr accumulation and transport in shoots of inoculated and uninoculated control wheat plants is represented in Table 5. There were significant differences in Cr accumulation of wheat plants inoculated with strains Q14 and KAP5 and uninoculated control plants at all levels of Cr. Plants inoculated with strain KAP5 showed maximum concentration of Cr up to 277% in shoot at 0 mg/L concentration of Cr. Inoculation significantly increased the Cr accumulation in shoot compared to uninoculated control at all levels of Cr. Higher translocation of Cr from root to shoot was observed with strain KAP5 which was up to 1.6 BAFs at 100 mg/L concentration of Cr. However, strain Q14 inoculation showed maximum Cr translocation from root to shoot about 0.92 BAFs at 40 mg/L concentration of Cr. Results demonstrated that strain KAP5 inoculation accumulated maximum concentration of Cr in shoot than uninoculated control wheat seedlings.

Table 5. Cr accumulation and transport in shoot of wheat seedlings. (Average of three replicates)

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Cr concentration in shoot (µg/g) DW			BAF (root-shoot)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	Nd	Nd	Nd	0.0	0.0	0.0
20	506	2.20[a]	9.4m	8.3n	0.25	0.57	0.40
40	11.2	11.31	23.0h	15.6k	0.51	0.92	0.89
60	16.8	16.4j	25.2f	24.6g	0.72	0.84	1.0
80	22.4	18.6i	27.6e	35.2c	0.63	0.82	1.0
100	28	32.3d	41.7b	56.5a	0.97	0.86	1.6

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test

a= Interaction effects of chromium and strains (LSD value=0.51) DW, dry weight, Nd, not detected

Pot experiment: Uninoculated control plants under no Cr treatment indicated maximum root length as compared to uninoculated plants under different Cr stress levels (Table 6). Inoculation with strain KAP5 in the presence of Cr significantly increased the root length up to 17.8 % when compared to root length of uninoculated control seedlings. Maximum increase in root length was observed when plants were inoculated with strain KAP5 compared to uninoculated control. Inoculation with strain Q14 showed a non-significant difference in root length at 100 mg/L Cr concentration, while at 250 and 500 mg/L concentration of Cr significant difference was observed with uninoculated untreated control. Overall, it was observed a non-significant difference in root length at 250 and 500 mg/L of Cr concentration. Dry weight of roots increased at all concentrations of Cr compared to uninoculated control but not significantly among treatments (Table 6). Plants grown with strain Q14 showed non-significant difference in root dry weight with strain KAP5 inoculated plants at 250 and 500 mg/L of Cr concentration, while at 0 and 100 mg/L Cr concentration significant difference was observed. Plants inoculated with strain Q14 also showed a non-significant increase in root dry weights than uninoculated control. Strain KAP5 increased the root dry weight up to 4.3% compared to uninoculated control. It was also observed that increasing concentration of Cr significantly decreased the root dry weights ranging from up to 67.8 % and up to 68.7 % respectively in case of strains Q14 and KAP5. Non-significant difference in root dry weight was observed among the uninoculated plants and at 250 and 500 mg/l of the Cr concentration.

Table 6. Effect of bacteria containing ACC-deaminase on root growth (root length and root weight) of wheat grown under different Cr concentrations. (Average of three replicates).

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Root length (cm)			Root weight (mg/plant)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	11.8b[a]	13.6a	13.9a	87.4c[b]	90.0b	91.1a
100	14.6	10.0de	11.6b	10.7c	39.7e	39.5e	41.1d
250	36.4	9.6e	9.9de	10.3cd	30.2g	32.2f	3.3f
500	72.7	9.0f	9.6e	9.9de	29.2gh	29.0hi	28.5i

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test.

a= Interaction effects of chromium and strains (LSD value=0.51)

b= Interaction effects of chromium and strains (LSD value=0.99)

Plants inoculated with strain KAP5 showed better results than plants inoculated with strain Q14 (Table 7). Plants inoculated with strain Q14 significantly decreased the shoot length (up to 10.3%) compared to uninoculated unstressed control plants. On the other hand, uninoculated controls also decreased the shoot length up to 13.7% with the increase in Cr concentration. A significant difference was observed between inoculated and uninoculated control plants at all Cr concentrations. Strain KAP5 inoculated plants increased shoot length up to 14.8% than strain Q14 inoculated plants up to 7.8% in control conditions. Strain KAP5 significantly increased the shoot dry weight up to 59.3% compared to inoculated control, while strain KAP5 increased the shoot dry weight up to 18.8% compared to those not treated with strains. At 250 mg/L concentration, shoot dry weight with strain KAP5 was 14.8%. On the other hand, strain Q14 increased the shoot dry weight by 11.9% compared to uninoculated plants. Strain Q14 inoculated plants significantly increased the shoot dry weights ranging from 0.75 to 18.8% than uninoculated control. When Cr concentration increased up to 500 mg/L, inoculation with bacterial strains decreased the shoot dry weight up to 44% compared to uninoculated control plants.

Table 7. Effect of bacteria containing ACC-deaminase on shoot growth (shoot length and shoot weight) of wheat grown under different Cr concentrations. (Average of three replicates).

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Shoot length (cm)			Shoot weight (mg/plant)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	35.8c[a]	38.6b	41.1a	507.0e[b]	567.3c	706.8a
100	14.6	32.6e	32.2d	38.8b	416.9g	454.5f	592.3b
250	36.4	32.3e	33.9d	34.9cd	342.5j	354.2i	545.6d
500	72.7	30.9f	32.1e	35.2d	322.81	332.0k	394.9h

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test

a= Interaction effects of chromium and strains (LSD value=1.14)

b= Interaction effects of chromium and strains (LSD value=0.99)

Chromium accumulation and transport in root: Concentration of Cr in root of wheat was significantly higher for plants grown in inoculated soil as compared to those grown in uninoculated control soil (Table 8). Highest concentration of Cr was recorded up to 102% at 25 mg/L level of Cr concentration. Increments in Cr concentration were 42.5 and 102% for strain Q14 inoculation and 30 and 130% for strain KAP5 inoculated plants with the increase in Cr concentration, compared to uninoculated control. Plants inoculated with strain KAP5 showed the highest translocation of Cr from soil to root of about 1.79 BAF than uninoculated control at 100 mg/L concentration of Cr. Strain KAP5 significantly increased Cr content of root from 26.0 to 73.7 µg/g dry mass compared to uninoculated control plants. In addition, KAP5 inoculation significantly increased the Cr concentration in root at 100 and 250 mg/L of Cr but at 500 mg/L of Cr concentration a significant decrease in Cr concentration was observed in root as compared to strain Q14 inoculated wheat plants.

Table 8. Cr accumulation and transport in root of wheat seedlings. (Average of three replicates).

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Cr concentration in root (µg/g) DW			BAF (soil-root)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	Nd	Nd	Nd	0.0	0.0	0.0
100	14.6	13.6i[a]	21.3h	26.0g	0.92	1.47	1.79
250	36.4	27.5f	55.5e	63.3c	0.76	1.53	1.74
500	72.7	56.7d	80.8a	73.7b	0.78	1.11	0.97
0	0.0	Nd	Nd	Nd	0.0	0.0	0.0
100	14.6	13.6i[a]	21.3h	26.0g	0.92	1.47	1.79

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test

a= Interaction effects of chromium and strains (LSD value=0.52)

DW, dry weight, Nd, not detected

Chromium accumulation and transport in shoot: Strains Q14 and KAP5 significantly increased the accumulation of Cr in shoot than un-inoculated plants treated with Cr (Table 9). Strain KAP5 showed better results than strain Q14 and up to 69.9 µg Cr/g dry mass was recorded in case of KAP5 at 500 mg/L concentration of Cr in soil. Results also showed maximum transport of Cr from root to shoot in case of strain KAP5 compared to strain Q14 and uninoculated control, at different levels of Cr applied to soil. However, strain Q14 translocated only 0.81 BAF of Cr from root to shoot at 500 mg/L concentration of Cr as compared to uninoculated control wheat seedlings.

Table 9. Cr accumulation and transport in shoot of wheat seedlings. (Average of three replicates).

Cr concentration in solution (mg/L)	Total Cr concentration in sand (µg/g)	Cr concentration in shoot (µg/g) DW			BAF (root-shoot)		
		Uninoculated	Q14	KAP5	Uninoculated	Q14	KAP5
0	0.0	Nd	Nd	Nd	0.0	0.0	0.0
100	14.6	4.6i[a]	5.4h	8.3g	0.34	0.25	0.32
250	36.4	23.3f	33.6c	49.7c	0.85	0.61	0.79
500	72.7	40.5d	65.5b	69.9a	0.71	0.81	0.95

Means followed by same uppercase or lowercase letters are not significantly different (p=0.05) in their main and interaction effects, respectively according to Fisher's protected LSD test.

a= Interaction effects of chromium and strains (LSD value=0.25)

DW, dry weight, Nd, not detected

Discussion

This study demonstrate the effect of plant growth promoting bacteria containing ACC-deaminase on growth and Cr accumulation in wheat plants irrigated with different concentration of Cr. Inoculation with ACC-deaminase producing bacterial strains significantly increased the growth of wheat compared to uninoculated control in both sand and pot experiments. Furthermore, inoculation with strain Q14 and KAP5 enhanced the Cr uptake by root and shoot when irrigated with Cr contaminated water. In the present study, plants inoculated with bacterial strains significantly increased the root elongation and root biomass. Increasing concentrations of Cr had inhibitory effect on root growth; however, in most of the cases inoculation with bacteria containing ACC-deaminase significantly enhanced the root elongation at varying levels of Cr applied with irrigation water compared to uninoculated control. In an earlier study, Ahmad *et al.* (2011) reported the inhibiting effects of Cr on root length of rice seedlings at 100 µM concentration. In metal contaminated environment reduced root growth may be due to synthesis of ethylene under stress conditions (Glick, 2005) and bacteria exhibiting ACC-deaminase activity can enhance root elongation by hydrolytic modulation of ethylene level produced under metal-stress by ACC-deaminase enzyme. Inoculation with *Pseudomonas* sp. A4 and *Bacillus* sp. 32 increased the root length of Indian mustard under Cr stress conditions (Rajkumar *et al.,* 2006). Ma *et al.,* (2009) reported that inoculation with *Achromobacter xylosoxidans* A×10 increased the root of *Brassica juncea* under Cu contamination. Results of this study further revealed significant increases in root biomass when soil was inoculated with bacterial strains containing ACC-deaminase. Studies have indicated that PGPR containing ACC-deaminase can enhanced the root dry weights up to 37 mg/plant of rape under lead contamination (Sheng *et al*., 2008). Ability of bacterial strains to increase the dry weight may be because of their ACC-deaminase activity as has been shown by other many PGPR (Burd *et al*., 2000). Similarly, inoculation with bacterial stains producing ACC-deaminase significantly increased the shoot length which could be attributed to an increase in root elongation, as improved root growth can transport more nutrients from the soil and it can also support increase shoot weight. Rajkumar *et al.,* (2006) reported that inoculation with *Pseudomonas* spp. increased the shoot length under Cr stress. In microbe-plant association ACC-deaminase has been proposed to play an important role (Hontzeas *et al*., 2004). Under the effects of different metals (lead, nickel and zinc), PGPR significantly increased the growth of plants (Burd *et al.,* 2000).

This study also revealed that application of higher concentration of Cr had strong inhibiting effect on the growth of wheat. In another experiment (data not shown), higher concentrations of Cr (500, 1000 and 1500 mg/L) suppressed germination of wheat seeds dramatically even inoculated with bacterial strains possessing ACC-deaminase activity. This indicated that bacterial strains can perform up to a certain limit and high concentration had very deleterious effect not only on germination, growth and development of wheat but also on bacterial activities. Parr, (1992) concluded in his study that inhibitory effects of Cr on seed germination could be due to its depressive effects on amylases activity and ceased transport of sugars to the embryo axes (Parr, 1992). Secondly Cr stress also increases the protease activity which reduces the germination of Cr treated seeds. In addition, cellular metabolism of shoot may be directly affected by transport of Cr to the shoot (Shanker *et al*., 2005). Inhibitory effects of Cr on plant growth may also be due to a greater accumulation of ACC and ethylene in plant roots under contamination of soil with heavy metals (Pennasio & Roggero, 1992). Increase in concentration of ACC and ethylene in rhizosphere depresses growth of plants (Shahzad *et al*., 2010). *Bacillus thuringiensis* strain KAP5 performed better than *Pseudomonas fluorescens* strain Q14 under Cr contamination. Better effect of strain KAP5 on plant growth and development may be attributed to its dual plant growth promoting trait such as ACC-deaminase activity and phosphate solubilizing activity (Baig *et al.,* 2011).

In the present study, more accumulation of Cr was observed in root inoculated with strains Q14 than KAP5. Accumulation of Cr in plants tissues was increased with the increase in Cr concentrations. Jamali *et al.,* (2009) studied the relationship of bacterial Cr mobilization in soil with total Cr accumulation in wheat plant. He found a positive correlation between Cr mobilization of bacteria and Cr concentration in wheat (R^2=0.90). It was also observed the maximum quantity of Cr in roots and a minimum in the vegetation and reproductive organs (Shanker *et al*., 2005). The study carried out on bean under Cr contamination showed 0.1% of the Cr accumulation in the seeds against 98% in the root. Maximum concentration of Cr in root may be result of immobilization of Cr in vacuole of root cell which is natural response of plants against metal stress (Shanker *et al.,* 2004). The data obtained from sand experiment revealed significantly higher concentration of Cr in the root and shoot inoculated with bacteria containing ACC-deaminase. Maximum transport of Cr was observed from soil to root but less was transported from root to shoot. ACC-deaminase inoculation showed higher transport of Cr about 3.68 BAFs from soil to root, while low concentration of Cr was transported from root to shoot (0.95 BAFs). Since root growth was improved in response to inoculation with bacteria containing ACC deaminase, so improved soot system might have helped plant better uptake of Cr. Moreover, improved phytoremediation is related to plant itself and association of plant root with bacteria and on the bioavailability of heavy metals in the soil (Jiang *et al*., 2008).

In conclusion, this study revealed that Cr contamination significantly affected the growth of wheat plant. Inoculation with bacterial strains possessing ACC-deaminase activity or multiple traits could be used to eliminate, to some extent, stress of Cr. Such bacteria could be very effective for plant growth in metal -contaminated soil.

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