ROLE OF CYANOBACTERIAL STRAINS ON *TRITICUM AESTIVUM* GROWTH UNDER CHROMIUM STRESSES IN LABORATORY

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ABSTRACT: Cyanobacterial strains isolated from different places near Lahore, Pakistan. They were proved to be efficiently promoting the growth of economically valuable crop Triticum aestivum, even under the stress of metal contaminant chromium in laboratory conditions, where petri plates were used. Seedling length of Triticum aestivum was severely affected by the application of chromate salt especially with the Cr (VI). About 18% and 61% reduction in seedling length respectively under 300 $\mu g\ mL^{-1}$ of $CrCl_3$ and K_2CrO_4 was observed. Hexavalent chromium had drastic effect on root numbers while trivalent chromium had no effect. Both trivalent (10.4%) and hexavalent (97.2%) chromium salts caused reduction in the leaf area. Fresh weight of seedlings was adversely affected with the application of chromium salts. With trivalent salt 15.1% and with hexavalent 59.3% reduction was observed. With chromium salts dry weight accumulation increased and under hexavalent salt dry weight accumulation was almost double than that of control. Protein content of seedlings increased with chromium salts. Cyanobacterial inoculations caused significant stimulation in auxin content in seedlings when compared to non-inoculated control. $CrCl_3$ (300 $\mu g\ mL^{-1}$) and K_2CrO_4 (300 $\mu g\ mL^{-1}$) treatments, stimulated more synthesis of auxin content in seedlings 142.8% and 285.7% increases respectively. About 45% and 230% increases in acid phosphatase activity, over control, were observed under 300 $\mu g\ mL^{-1}$ for each $CrCl_3$ and K_2CrO_4, respectively. Seedlings grown under 300 $\mu g\ mL^{-1}$ K_2CrO_4 uptake more chromium content as compared to the seedlings which were grown under 300 $\mu g\ mL^{-1}$ of $CrCl_3$.

Key word: Cyanobacteria, *Triticum aestivum*, chromium stress, plant growth promotion

INTRODUCTION:

Environmental conditions are under the constant influence of human activities that are causing harmful effects on all life forms on earth including plant. Chromium is naturally present in earth crust but industries are the main source of increasing its amount in soil, water and air above the appointed level which is approximately 1-300 mg/kg in soil and 9.7 µg/L in fresh water [1].Untreated Waste material from the various industries in which chromium is being used for the various purposes like Electroplating, leather tanning, dying and painting when exposed to environment, pollute the soil and water reservoir [2].

When plants are grown in Cr contaminated soil or irrigation of crops is done with Cr contaminated water, the plant start to accumulate the chromium within the root cells by up taking via carrier molecules that are usually use for essential metal ion e.g.; Fe, S, and P [3]. When Cr accumulates in roots it affects the cell elongation and division which ultimately retards the root length and growth as well as reduce the ability of seedlings to absorb water necessary for their germination [4, 5]. Due to this less amount of water, nutrients are not certainly transported to stem which results in the short stature of plant. Cr transportation to the stem and leaves cause the direct drastic effect on cells metabolism which also cause the shortening of plant height. It is also reported that Cr produces reactive oxygen species that cause the oxidative damage to cell organelles such as mitochondria and chloroplast which in result reduce the plant growth [6]. These ROS also damage the cell membrane transport mechanism for inorganic molecule and H^+ATPase which increases the uptake of Cr(VI) in the cell [7]. It also causes competition of Cr with Mn, Fe, Zn that are essential for growth and other enzyme activity [8] .Cr also affect the nitrate reduction property of plant by minimizing NR enzyme activity [9].

For the safe, unharmed and undamaged removal of metals from soil, Bioremediation is the effective way. For this purpose microbes are playing leading role. Microbes that are present in rhizosphere may play an important role in plant growth promotion either by competing with pathogen, by providing essential molecules or by reducing toxic metals. Among microbes, cyanobacteria belongs to very important photosynthetic organisms that diversify the universe from its most reducing state to the most oxygenic state and made life possible on earth by producing oxygen [10]. Current study indicates that not only bacteria are associated with plant growth promotion but cyanobacteria also play many important roles by reducing toxic metals. One way to positively affect nutrient content and soil structure is to add cyanobacteria [11-13]. Cyanobacteria can be incorporated into soil as organic matter and also as a source of enzymes as they produce acid and alkaline extracellular phosphatases that are active in solution or located in the periplasmatic space of the cell wall. The effects of *N. muscorum* mass on soil physical, chemical and biological properties indicate the possible benefits of cyanobacteria as soil inoculants [12] . Cyanobacteria are also known for fixing nitrogen and are also reported to establish association with plants and stimulate plant growth [14]. These properties of cyanobacteria enhance the plant growth by ensuring nutrient as

well as water availability to the plants.

This study utilizes the Cr resistance property of cyanobacteria and effect of them on plants that are grown in Cr stress which can hinder the plant growth in the absence of cyanobacteria. Different growth parameters of *Triticum aestivum* var. Uqab-2000 are observed in the presence and absence of inoculum while Cr stress (trivalent ($CrCl_3$) and hexavalent (K_2CrO_4) salts (300 µg mL^{-1}) are provided in both cases. In laboratory conditions inoculated and un inoculated seeds were grown in petriplates for 10 days by providing Cr stress in its trivalent and hexavalent form in order to determine the difference in germination and growth pattern (biochemical analysis, chromium content of seedlings [15].

MATERIAL AND METHOD

Cyanobacterial strains and inoculum preparation

Strains of chromium resistant cyanobacteria were previously isolated , characterized and molecularly identified from local environment which were used in this study [16-18]. For preparation of Cyanobacterial suspensions, these strains were grown in liquid cultures; cells were then harvested, washed and resuspended in sterilized glass-distilled water. To ensure the equal cyanobacterial growth (cell population of cyanobacterial strains in the suspension for different experiments), chlorophyll-a concentration was measured.

Experimental plant and seed inoculation

Certified seeds of *Triticum aestivum var*.Uqab-2000 were procured from the National Agriculture Research Center - NARC, Islamabad, Pakistan. Healthy seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes, with continuous shaking followed by repeated washing with sterilized glass-distilled water, so that all traces of $HgCl_2$ were removed from the seeds. Surface sterilized

Seeds were dipped in cyanobacterial suspension for Fifteen minutes.

Seed germination and growth

Sterilized petriplates having filter paper were taken and 10 ml of 300 µg mL^{-1} of each trivalent ($CrCl_3$) and hexavalent (K_2CrO_4) chromium treatment was supplied to respective petriplates. Previously sterilized and inoculated seeds were uniformly spread on filter paper and All the plates were kept in dark at 22 ± 2°C for three days. Germination was noticed daily.

Addition of nutrient supplement

After germination, 10 mL of [19] nutrient solutions, containing the respective chromium salt concentration, were added to supplement the nutrient requirements of the seedlings and plates were shifted to light (195µE m^{-2} s^{-1} for 16 hours at 22 ± 2°C). Seedlings were grown for 10 days. Daily observations were made and general appearance of seedlings was noticed. The experiments were repeated four times and data was analyzed statistically [20]

Harvesting and growth parameters

Seedlings were removed from petriplates and excess of moisture was removed by gently pressing the seedlings between blotting papers and growth measurements such as percentage germination seedling length, no of roots, leaf area ,fresh and, dry weight g^{-1} were noted.**Determination of biochemical parameters**

For the estimation and extraction of Auxin produced by seedlings (µg g^{-1} fresh) [21] method was followed, Optical densities of the material was taken at 535nm on spectrophotometer and amount of auxin in the extract was measured and calculated as µg gm^{-1} fresh weight of cyanobacteria/ plants by using standard curve .

Extraction of Soluble protein content of seedlings (µg g^{-1} fresh) was done by following [22], whereas for soluble protein analysis method of [23] was adopted and for Acid phosphatase activity (unit g^{-1} fresh) [24] were followed. Optical density of the material was taken at 750 nm on spectrophotometer. Amount of soluble proteins was calculated using standard curve.

Chromium content in different part of plant was measured by digesting them according to the protocol followed by [25]. Cr content for both forms was analyzed with the help of UV-Vis spectrophotometer [26].

RESULTS:

Seed Germination

Germination of *Triticum aestivum* was hindered with $CrCl_3$ (11.3 %) and K_2CrO_4 (44.3%) but cyanobacterial inoculation lessen this adverse effect when compared with respective non-inoculated control and 3.5 to 33% increases in this parameter were observed with different strains (Table-1). Strains *Gloeocapsosis* sp and *Cyanosarcina* sp caused maximum 13.9% stimulation whereas with *Oscillatoria* "S.A" the least i.e. 4.6%, increase in seed germination was observed (Table-1).

Seedling Length (cm)

Seedling length of *Triticum aestivum* was severely affected by the application of chromate salt especially with the Cr (VI). About 18 and 61% reduction in seedling length respectively under 300 µg mL^{-1} of $CrCl_3$ and K_2CrO_4 was observed. All cyanobacterial inoculations stimulated seedlings length as compared to non-inoculated control. *Gloeocapsosis* sp caused maximum stimulation by increasing 39% and 51% seedling length under $CrCl_3$ and K_2CrO_4 stresses respectively (Table-1).

Number of Roots

Hexavalent chromium had drastic effect on root numbers while trivalent chromium had no effect on this parameter. Inoculation of cyanobacterial strains had no effect on number of roots and they remained same as that of control in $CrCl_3$ treatment, whereas under K_2CrO_4 stress inoculation of strain *Gloeocapsosis* sp caused increase (10%) in number of roots over respective non-inoculated control (Table-1). Inoculation of cyanobacterial strains *Arthrospira* sp, *Oscillatoria* "O", *Oscillatoria* "F.W" and *Cyanosarcina* sp caused increases (20%) in root numbers over non-inoculated control. Changes in the texture of roots as compared to control were also observed. Roots were stunted and become brownish black. Appearance of roots was much improved by the application of cyanobacterial strains.

Leaf Area (cm^2)

Both trivalent (10.4%) and hexavalent (97.2%) chromium salts caused reduction in the leaf area. The mean leaf area of

the inoculated seedlings was greater than those of control plants in chromium free treatment (Table-2). Under 300 μg mL^{-1} $CrCl_3$ treatment, strains *Cyanosarcina* sp and *Gloeocapsosis* sp exerted a substantial promoting effects on the leaf area of the *Triticum aestivum* (almost 14 and 12%, respectively), over control. In case of hexavalent chromium treatment, Seedlings inoculated with strain *Gloeocapsosis* sp enhanced the leaf area up to 21.4% as compared to non-inoculated control.

Fresh Weight of Seedlings

Fresh weight of seedlings was adversely affected with the application of chromium salts. With trivalent salt 15.1% and with hexavalent 59.3% reduction was observed. All cyanobacterial inoculations caused increases (3 to 36%) in fresh weight when compared to non-inoculated respective control. At 300 μg mL^{-1} of $CrCl_3$, cyanobacterial strains stimulated 18 to 44% increases over respective control in the fresh weight of seedlings. Inoculation of *Gloeocapsosis* strain caused maximum increase in this parameter in the presence of 300 μg mL^{-1} of K_2CrO_4, all strains also enhance the fresh weight of seedlings and 11 to 54% increases relative to respective non-inoculated control were observed with different strains (Table-2).

Dry Weight g^{-1} Fresh Weight

With chromium salts dry weight accumulation increased and under hexavalent salt dry weight accumulation was almost double than that of control. The dry weight of *Triticum aestivum* seedlings was increased with inoculations of 5 strains, *Oscillatoria* "F.W"(7%), *Anabaena* sp (7%), *Gloeocapsosis* sp (14%), *Cyanosarcina* sp (14%) and *Synechocystis* "MK(S)"(7%) over non inoculated chromium free control, while with other strains no change in this parameter was observed. Under chromium stresses all cyanobacterial inoculations caused decreases in the dry weight of seedlings relative to non-inoculated respective treatments. The decrease with cyanobacterial strains was more pronounced with hexavalent chromium, where 16 to 27% reductions in dry weight were observed (Table-2)

Protein Content

Protein content of *Triticum aestivum* seedlings increased with chromium salts. However, inoculation of strains *Gloeocapsosis* sp (17%), *Cyanosarcina* sp (13%) and *Anabaena* sp (13%) significantly increased the protein content of seedlings when compared with that of control. At 300 μg mL^{-1} $CrCl_3$, *Oscillatoria* "S.A" strain caused relatively low increase (0.2%) whereas strain *Gloeocapsosis* sp enhanced maximally (14%) over respective control (Table-3). Under K_2CrO_4 stress, inoculation with *Anabaena* treatment increased protein content up to 12.9% relative to respective non inoculated (Table-3).

Auxin Content (μg g^{-1} fresh weight)

Cyanobacterial inoculations caused significant stimulation in auxin content in wheat seedlings when compared to non-inoculated control. $CrCl_3$ (300 μg mL^{-1}) and K_2CrO_4 (300 μg mL^{-1}) treatments, stimulated more synthesis of auxin content in *Triticum aestivum* seedlings 142.8% and 285.7% increases respectively (Table-3). *Anabaena* sp inoculated seedlings in chromium free treatment showed 114 % increase in auxin content .Generally at 300 μg mL^{-1} $CrCl_3$, all cyanobacterial inoculations caused an increment in auxin content when compared to their respective control. At 300 μg mL^{-1} K_2CrO_4, High increases were observed with inoculations of strains *Anabaena* sp, *Arthrospira* sp, *Cyanosarcina* sp and *Gloeocapsosis* sp where 22.2%, 18.5%, 18.5% and 14.5% increases respectively, over non-inoculated respective treatment were observed (Table-3).

Acid Phosphatase (unit g^{-1} fresh weight)

About 45% and 230% increases in acid phosphatase activity, over control, were observed under 300 μgmL^{-1} of $CrCl_3$ and K_2CrO_4 respectively (Table-3). Under $CrCl_3$ stress cyanobacterial strains enhanced 2.8-24.7% activity of this enzyme over that of control. Under K_2CrO_4 stress, stimulation in the enzyme activity was more pronounced with inoculations of strains *Gloeocapsosis* sp, *Cyanosarcina* sp and *Arthrospira* sp, which caused 18.9%, 13 % and 12.6 % increases respectively, in acid phosphatase activity of wheat seedlings over their respective un-inoculated treatment.

Chromium Content (mg Cr g^{-1} d.w)

Seedlings which were grown under 300 μg mL^{-1} K_2CrO_4 uptake more chromium content as compared to the seedlings which were grown under 300 μg mL^{-1} of $CrCl_3$ (Table-3). In general there was a reduction in chromium uptake with the cyanobacterial inoculation. In case of 300 μg mL^{-1} K_2CrO_4 treatment, except strain *Cyanosarcina* sp where some increase in chromium content of wheat seedlings was observed, all other cyanobacterial strains manifested a reduction or no change in the chromium content of seedlings when compared to respective non-inoculated control treatment (Table-3).

DISCUSSION:

In the present study ten filamentous chromium resistant cyanobacterial strains that exhibited greater reduction potential were used for inoculating seeds of *Triticum aestivum* var. Uqab-2000, which were subsequently germinated and grown under different chromium salts (300 μg mL^{-1} of $CrCl_3$ and K_2CrO_4) in both laboratory and pots condition. The effects of hexavalent salt were more severe than that of trivalent salt. Hexavalent chromium is highly soluble and easily available to seeds, hence is different valence state of chromium when compared with control. Without cyanobacterial inoculation, about 11 and 44.3% more toxic. Higher uptake of metallic salt disturbs nuclear division and hinders cytokinesis, which causes adverse effects on seed germination [27]. Cyanobacterial strains not only enhanced the plant growth but also provided resistance to crop against reduction in seed germination was observed in *T. aestivum* under 300μg $mL^{-1}$of $CrCl_3$ and K_2CrO_4, respectively. In fact these cyanobacterial strains gave some alleviation to plants under Cr (VI) stress by decreasing the availability of toxic Cr (VI) to plants. One possible explanation for stimulation in germination and growth parameters might be that cyanobacteria take up chromium in

Table-2: Effect of Inoculation of Chromium Resistant Cyanobacterial Strains on Leaf Area cm^2, Fresh Weight and Dry Weight g^{-1} Fresh Weight of *Triticum aestivum* var. Uqab-2000 Seedlings Grown at 0 and 300 μg mL^{-1} of $CrCl_3$ and K_2CrO_4

Strains	% Germination			Seedling length (cm)			No. of roots		
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	97±4	86±4.3	54±3.2	25.4±2.5	20.6±1.8	9.7±0.8	5±0.01	5±0.05	9±0.1
Arthrospira sp	100±0	97±6.2	66±4.2	30.8±2.9	24.6±2.2	12.6±1.1	6±0.2	5±0.05	9±0.2
Lyngbya sp	100±0	93±5.0	58±3.7	28.2±2.7	21.4±2.0	11.0±0.9	5±0.1	5±0.05	9±0.1
Oscillatoria "G"	100±0	92±4.9	60±3.8	27.2±2.6	21.5±2.0	10.0±0.8	5±0.2	5±0.05	9±0.3
Oscillatoria "BJ2"	100±0	91±4.8	59±3.7	28.3±2.7	21.7±2.1	9.8±0.8	5±0.1	5±0.05	9±0.4
Oscillatoria "BJ1"	100±0	93±5.0	56±3.6	28.9±2.8	21.3±2.1	11.1±1.0	5±0.1	5±0.05	9±0.3
Oscillatoria "A"	100±0	94±5.2	59±3.7	28.5±2.7	21.9±2.1	12.2±1.1	5±0.1	5±0.05	9±0.2
Oscillatoria "O"	100±0	91±4.8	60±3.9	30.5±3.0	21.8±2.0	11.1±0.9	6±0.1	5±0.05	9±0.2
Oscillatoria "F.W"	100±0	97±6.0	65±4.0	32.0±3.1	25.3±2.3	12.3±1.3	6±0.2	5±0.05	9±0.1
Anabaena sp	100±0	96±4.8	58±3.3	33.0±3.3	24.2±2.2	11.1±1.0	5±0.05	5±0.05	9±0.1
Gloeocapsosis sp	100±0	98±6.2	69±3.7	39.4±3.5	28.7±2.8	14.8±1.3	6±0.2	5±0.05	10±0.5
Cyanosarcina sp	100±0	98±6.2	72±3.7	30.2±3.1	25.31±3	12.7±1.2	5±0.05	5±0.05	9±0.2
Synechocystis "AHZ-HB-MK"	100±0	92±4.9	60±3.6	26.7±2.4	24.58±2	10.1±0.9	5±0.05	5±0.05	9±0.3
Synechocystis "AHZ-HB-P2A"	100±0	91±4.6	59±3.5	27.2±2.6	21.7±2.0	10.7±0.8	5±0.1	5±0.05	9±0.1
Oscillatoria "S.A"	100±0	90±4.8	56±3.4	27.2±2.6	20.8±1.9	11.1±1.0	5±0.05	5±0.05	9±0.3
L. S. D ($p_=$ 0.05)									
For treatments		2.05			2.20			0.20	
For strains		4.59			4.9			0.46	

Table ! **Table-1: Effect of Inoculation of Chromium Resistant Cyanobacterial Strains on Seed Germination, Seedling Length and Number of Roots of *Triticum aestivum* var. Uqab-2000 Grown at 0 and 300 μg mL^{-1} of $CrCl_3$ and K_2CrO_4 Concentrations (Means of Four Replicates)**

Strains	Leaf area (cm^2)			Fresh wt (g)			Dry wt g^{-1} fresh wt (g)		
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4
Control	4.8±0.5	4.3±0.01	0.14±0.01	8.6±0.2	7.3±0.1	3.5±0.2	0.14±0.01	0.17±0.02	0.29±0.01
Arthrospira sp	5.3±0.6	4.7±0.1	0.14±0.02	10.8±0.1	9.6±0.5	5.2±0.2	0.14±0.02	0.14±0.02	0.21±0.02
Lyngbya sp	5.1±0.6	4.4±0.1	0.14±0.02	9.7±0.4	8.6±0.4	3.9±0.2	0.14±0.02	0.15±0.03	0.22±0.01
Oscillatoria "G"	4.9±0.5	4.4±0.14	0.14±0.01	10.4±0.1	9.7±0.5	4.7±0.2	0.14±0.01	0.14±0.01	0.23±0.01
Oscillatoria "BJ2"	4.9±0.5	4.5±0.14	0.14±0.01	9.3±0.2	8.9±0.4	4.3±0.3	0.14±0.01	0.15±0.02	0.23±0.01
Oscillatoria "BJ1"	4.9±0.5	4.3±0.11	0.14±0.02	9.6±0.4	8.6±0.2	4.8±0.1	0.14±0.02	0.15±0.02	0.23±0.01
Oscillatoria "A"	5.0±0.6	4.5±0.10	0.14±0.02	9.7±0.1	9.1±0.3	4.2±0.2	0.14±0.02	0.13±0.01	0.25±0.02
Oscillatoria "O"	5.0±0.5	4.5±0.1	0.14±0.01	8.9±0.03	9.2±0.4	4.5±0.1	0.14±0.01	0.14±0.01	0.24±0.02
Oscillatoria "F.W"	5.2±0.6	4.6±0.20	0.15±0.02	10.4±0.1	9.4±0.1	4.8±0.1	0.15±0.02	0.14±0.02	0.21±0.01
Anabaena sp	4.9±0.5	4.5±0.11	0.15±0.01	10.7±0.9	9.6±0.2	4.9±0.2	0.15±0.01	0.17±0.02	0.25±0.02
Gloeocapsosis sp	5.4±0.6	4.8±0.12	0.17±0.01	11.2±0.1	10.5±0.3	5.4±0.2	0.16±0.01	0.15±0.01	0.21±0.01
Cyanosarcina sp	5.3±0.6	4.9±0.14	0.16±0.02	11.7±0.2	10.3±1.0	5.3±0.1	0.16±0.02	0.16±0.01	0.22±0.02
Synechocystis "AHZ-HB-MK(S)"	5.0±0.6	4.4±0.01	0.15±0.01	10.8±0.0	9.7±0.4	4.3±0.4	0.15±0.01	0.13±0.01	0.23±0.02
Synechocystis "AHZ-HB-P2A"	5.1±0.6	4.3±0.12	0.14±0.03	9.7±0.04	9.5±0.2	4.5±0.2	0.14±0.03	0.16±0.02	0.23±0.02
Oscillatoria "S.A"	4.9±0.5	4.4±0.23	0.14±0.02	9.9±0.05	9.2±0.6	3.9±0.3	0.14±0.02	0.14±0.01	0.24±0.04
L. S. D ($p_=$ 0.05)									
For treatments		0.06			0.27			0.00	
For strains		0.16			0.60			0.00	

Table-3: Effect of Inoculation of Chromium Resistant Cyanobacterial Strains on Protein Content, Auxin Content, Acid Phosphatase and Chromium Content of *Triticum aestivum* var. Uqab-2000 Seedlings Grown at 0 and 300 μg mL^{-1} of $CrCl_3$ and $KCrO_4$ Concentrations (Mean of Four Replicates)

Strains	Protein content (μg g^{-1} fresh weight)			Auxin content (μg g^{-1})			Acid phosphates (unit g^{-1} fresh weight)			Chromium content (mg g^{-1})	
	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	0	$CrCl_3$	K_2CrO_4	$CrCl_3$	K_2CrO_4
Control	382±32	424±36	581±16.2	0.7±0.06	1.7±0.06	2.7±0.4	72±2.2	105±2.4	238±10.1	0.37±0.02	2.9±0.02
Arthrospira sp	421±38	446±35	626±18.4	0.9±0.08	1.9±0.07	3.2±0.05	85±4.3	130±3.6	268±12.5	0.36±0.02	2.5±0.98
Lyngbya sp	384±33	430±35	593±26.9	0.7±0.02	1.8±0.06	3.0±0.0	76±3.5	113±4.5	262±10.4	0.37±0.02	2.6±0.99
Oscillatoria "G"	402±36	445±37	590±26.7	0.8±0.05	1.8±0.07	2.9±0.09	73±2.3	108±5.7	248±11.8	0.37±0.02	2.9±0.68
Oscillatoria "BJ2"	406±36	454±38	610±47.6	0.8±0.06	1.8±0.06	3.1±0.02	77±2.8	109±3.6	231±10.9	0.36±0.01	2.3±0.95
Oscillatoria "BJ1"	417±36	450±38	625±17.9	0.7±0.05	1.8±0.05	2.8±0.08	76±2.7	111±4.9	249±12.1	0.35±0.02	2.4±0.96
Oscillatoria "A"	397±35	465±39	616±37.4	0.9±0.07	1.8±0.04	3.1±0.0	84±2.2	117±5.0	240±19.9	0.37±0.02	2.8±0.01
Oscillatoria "O"	396±35	430±37	621±27.7	0.8±0.06	1.8±0.06	2.8±0.09	73±3.4	121±6.3	252±12.1	0.35±0.01	2.6±0.0
Oscillatoria "F.W"	420±37	481±39	622±48.4	0.9±0.01	1.8±0.05	3.0±0.03	74±1.9	127±3.5	245±13.8	0.34±0.01	2.0±0.07
Anabaena sp	432±38	475±37	656±46.7	1.5±0.0	2.2±0.0	3.3±0.09	79±2.6	110±2.9	247±12.6	0.37±0.01	2.3±0.01
Gloeocapsosis sp	447±39	485±39	654±37.8	1.4±0.04	2.1±0.9	3.1±0.03	101±2	131±3.6	283±16.1	0.37±0.02	2.9±0.76
Cyanosarcina sp	432±38	480±38	653±28.2	1.3±0.02	2.1±0.8	3.2±0.03	105±3	129±4.0	269±10.8	0.39±0.02	2.8±0.01
Synechocystis "AHZ-HB-MK(S)"	412±36	435±35	612±47.5	0.9±0.06	2.0±0.06	3.0±0.01	81±2.6	123±4.4	250±11.6	0.35±0.01	2.4±0.02
Synechocystis "AHZ-HB-P2A"	406±35	430±35	601±45.8	0.9±0.06	1.9±0.09	2.9±00.9	75±6.9	115±4.9	246±15.4	0.37±0.01	2.3±0.03
Oscillatoria "S.A"	400±33	425±37	590±38.6	0.9±0.05	1.9±0.0	2.8±0.08	78±7.9	108±3.9	248±13.4	0.37±0.02	2.4±0.07
L. S. D ($p_=$ 0.05)											
For treatments		7.33			0.07			4.90			0.14

their cells and may be capable of reducing the availability to seeds. Thus cyanobacteria by lessening the harmful effects of chromium are provoking germination and subsequently stimulating seedling growth

Under chromium stress, different growth parameters of crop *T. aestivum* were also severely affected. Actually under hexavalent chromium stress different vital functions such as electron transport chain and photosynthesis [28, 29] severely affected, which ultimately lead to poor growth of seedlings in comparison to respective control plants Majority of cyanobacterial inoculations caused an increment in the seedling length both under chromate stress and unstressed medium. In *T. aestivum,* strains *Anabaena* sp and *Arthrospira* sp posed relatively more stimulatory effects both in chromium stressed and unstressed conditions. It may be due to the fact that growth stimulatory cyanobacteria released some chemotaxis to root exudates, metabolism which helps for the suppression of competitor microorganism and most importantly the ability to bind with plant root surface [12, 30-32] which helps plant for better growth. Chromium stress also caused reduction in the number of leaves. In *Triticum aestivum* 300 µg g^{-1} of K_2CrO_4 caused much reduction in the number of leaves as compared to chromium free control. Cyanobacterial inoculation caused increment in the number of leaves not only in chromium free but also in the presence of trivalent and hexavalent chromium in *T. aestivum*. A major effect of chromium stress on roots was shortening and thickening, which may be due to decrease in the rate of cell elongation and growth. This morphological adaptation of roots is a typical change under stress environment [33]. Plant growth promoting microorganisms / rhizobacteria in addition to stimulating plant growth (length and weight parameters) and nutrient uptake, also enhance number and length of root hairs [34].

In the presence of chromate, weight parameters of *T. aestivum* were severely affected while cyanobacterial strains caused increase in fresh weight of this crop both under normal and chromium stress conditions, when compared with non-inoculated respective control. The response of wheat seedlings upon *Azospirillum* inoculation, particularly the stimulation of root differentiation, in addition to the promotion and elongation of the primary root has been attributed to compounds presenting auxin activity, such as 1AA and nitrate [35, 36]. The reduction in fresh weight of *Triticum aestivum* seedling under hexavalent chromium stress might be due to reduced uptake of water and probably cell expansion as indicated by stunted growth was also inhibited. Heavy metal toxicity hampered cell division and decrease turgor pressure of plant cell [37].

With application of 300µg mL^{-1} of K_2CrO_4, dry weight and dry mass accumulation increased significantly. Enhanced dry weight could be attributed to the uptake and increase level of organic salts in cytoplasm [38]. In the presence of hexavalent chromium stress, inoculation with all strains caused reduction in dry weight of *Triticum aestivum* seedlings. The decrease in dry weight and dry mass accumulation in inoculated seedlings might be due to the alleviation of stressed conditions by the cyanobacterial strains. Several others workers also reported increased fresh weight, plant height and leaf size of plants with microbial inoculation [39].

Cyanobacterial strains also showed production of plant growth regulators such as auxin, gibberellins, ethylene or abscisic acid [40-43]. In the present study amount of auxin content increased in *Triticum aestivum*, seedling under chromium stress by the help of cyanobacterial inoculations that exhibited some increment in auxin content both under chromium stress and normal conditions. According to researchers [44], cyanobacterial strains stimulated plant growth by synthesizing and liberating growth hormones. Chromium stress also caused some increment in soluble protein content in the seedlings as compared to respective chromium free control. Majority of cyanobacterial inoculations enhanced the protein content of seedlings relative to respective non-inoculated treatment. In view of the above results it can be believed that along with many other mechanisms involved for the growth promotion of seedling of this important cash crops, it is apparent that strains supported the plant growth by lessening the availability of toxic Cr (VI), which was reduced by the cyanobacterial strains into a less soluble Cr (III).

Chromium content of *Triticum aestivum* seedlings increased in case of hexavalent chromium stress as compared to trivalent chromium. In the present study under 300 µg mL^{-1} of Cr (VI), majority of cyanobacterial inoculation resulted a decrease in chromate uptake in *Triticum aestivum* seedlings as compared to their respective non-inoculated control. In fact majority of the cyanobacterial strains were also capable of reducing more toxic and mobile Cr(VI) in to Cr(III). Hence majority of the strains reduced this Cr(VI) in the vicinity of root, thus limiting the entry of chromium into the plant roots. In the present study majority of cyanobacterial inoculations caused an increment in the acid phosphatase activity as compared to their respective non-inoculated control. The activity of acid phosphatase is related with metal accumulation by the cell [45]. In *Triticum aestivum* The enhanced activity was much higher with inoculations of *Arthrospira* sp.as compared to other strains, which are also showing good chromium uptake, chromium reduction and auxin production.

These findings urge us for the safe and long term use of cyanobacteria in fields to enhance the growth of plant by acting as bioremediation and biofretilitation.

REFERENCES

1. Shanker, A.K., et al., chromium toxicity in plants *Environment International*. **31:** 739–753 (2005).
2. Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man* 8th ed2008: Biomedical Publications.
3. Sharmin, S.A., et al., Chromium-induced physiological and proteomic alterations in roots of Miscanthus sinensis. *Plant Science*. **187(0):** 113-126 (2012).
4. Volland, S., et al., Intracellular chromium localization and cell physiological response in the unicellular alga Micrasterias. *Aquatic Toxicology*. **109(0):** 59-69 (2012).

5. Wani, P.A. and M.S. Khan, Bacillus species enhance growth parameters of chickpea (Cicer arietinum L.) in chromium stressed soils. *Food and Chemical Toxicology*. **48(11):** 3262-3267 (2010).
6. Panda, S.K., Chromium-mediated oxidative stress and ultrastructural changes in root cells of developing rice seedlings. *Journal of Plant Physiology*. **164(11):** 1419-1428 (2007).
7. Ali, S., et al., The ecotoxicological and interactive effects of chromium and aluminum on growth, oxidative damage and antioxidant enzymes on two barley genotypes differing in Al tolerance. *Environmental and Experimental Botany*. **70(2–3):** 185-191 (2011).
8. Liu, D., et al., Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in Amaranthus viridis L. *Bioresource Technology*. **99(7):** 2628-2636 (2008).
9. Gangwar, S. and V.P. Singh, Indole acetic acid differently changes growth and nitrogen metabolism in Pisum sativum L. seedlings under chromium (VI) phytotoxicity: Implication of oxidative stress. *Scientia Horticulturae*. **129(2):** 321-328 (2011).
10. Quintana, N., et al., Renewable energy from Cyanobacteria: energy production optimization by metabolic pathway engineering. *Appl Microbiol Biotechnol*. **91(3):** 471-90 (2011).
11. Gupta, V., et al., New insights into the biodiversity and applications of cyanobacteria (blue-green algae)—Prospects and challenges. *Algal Research*. **2(2):** 79-97 (2013).
12. Rogers, S.L. and R.G. Burns, Changes in aggregate stability, nutrient status, indigenous microbial populations, and seedling emergence, following inoculation of soil withNostoc muscorum. *Biology and Fertility of Soils*. **18(3):** 209-215 (1994).
13. Faisal, M., A. Hameed, and S. Hasnain, Chromium-resistant bacteria and cyanobacteria: impact on Cr (VI) reduction potential and plant growth. *Journal of Industrial Microbiology and Biotechnology*. **32(11-12):** 615-621 (2005).
14. Karthikeyan, N., et al., Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *European journal of soil biology*. **43(1):** 23-30 (2007).
15. Santana, K.B., et al., Physiological analyses of Genipa americana L. reveals a tree with ability as phytostabilizer and rhizofilterer of chromium ions for phytoremediation of polluted watersheds. *Environmental and Experimental Botany*. **80:** 35-42 (2012).
16. Hameed, A. and S. Hasnain, Cultural characteristics of chromium resistant unicellular cyanobacteria isolated from local environment in Pakistan. *Chinese Journal of Oceanology and Limnology*. **23(4):** 433-441 (2005).
17. Hameed, A. and S. Hasnain. *Cultural characteristics of chromium resistant filamentous cyanobacteria isolated from local environment in pakistan*. in *Proceedings of the first international conference on environmentally sustainable development v. 1-3*. 2005.
18. Hameed, A. and Hasnain. S, Isolation and molecular identification of metal resistant Synechocystis from polluted areas. *African Journal of Microbiology Research*. **6(3):** 648-652 (2012).
19. Hewitt, E., *Minerals nutrition of plants in culture media*, in *Plant Physiology*1963, Academic Press: New York. p. 99-137.
20. Steel, R.G.D. and J.H. Torrie, *Principal and Procedures of Statistics, a Biometrical Approach*. Vol. 2nd edition. 1981: McGraw Hill Int. Book Company.
21. Mahadevan, A., *Growth regulators, Microorganisms And Diseased Plants*1984, india: Oxford and IBH Publishing company.
22. Bhatti, G.A., et al., Studies on heat shock response of wheat seedlings using E. coli GroEL antibodies. *Pakphyton*. **5:** 157-166 (1993).
23. Lowery, O., et al., Protein measurement with folin phenol reagent. *J. Biol. Chem*. **193:** 265-275 (1951).
24. Iqbal, J. and N. Rafique, Toxic effects of BaCl2 on germination, early seedling growth, soluble proteins and acid phosphatase in Zea mays *L. Pak. J. Bot*. **19:** 1-8 (1987).
25. Humphries, E.C., *Mineral component and ash analysis.in: Modern Methods of Plant Analysis*1956: Springer-Verlag Berlin, Goltingen, Heidelberg.
26. Rand, M.C., E. Arnod, and J. Michel, Standard Methods for the Examination of Water and Wastewater. *American Public Health Association*. (1976).
27. Prado, C., et al., Uptake of chromium by< i> Salvinia minima</i>: Effect on plant growth, leaf respiration and carbohydrate metabolism. *Journal of hazardous materials*. **177(1):** 546-553 (2010).
28. Larcher, W., *Ecophysiology and Stress Physiology of Functional groups*, in *Physiological Plant Ecology*1995, Springer: Germany. p. 425.
29. Gardea-Torresdey, J.L., et al., Differential uptake and transport of trivalent and hexavalent chromium by tumbleweed (Salsola kali). *Arch. Environ. Contam. Toxicol*. **48:** 225-232 (2005).
30. Motavalli, P.P., et al., Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. *J. Environ. Qual*. **33:** 816-824 (2004).
31. de Caire, G.Z., et al., Exopolysaccharide of Nostoc muscorum (Cyanobacteria) in the aggregation of soil particles. *Journal of applied phycology*. **9(3):** 249-253 (1997).
32. Bashan, Y. and G. Holguin, Root to root travel of the beneficial Azospirillum brasilense. *Can. J. Microbiol*. **6:** 2120-2131 (1994).
33. Degenhardt, B. and H. Gimmler, Cell wall adaptations to multiple environmental stresses in maize roots. *J. Exp. Bot.*, **51:** 595-603 (2000).
34. Bertrand, H., et al., Isolation and identification of the most efficient plant growth-promoting bacteria

associated with canola (Brassica napus). *Biol. Fertil. Soils*. **33:** 152-156 (2001).

35. Didonet, A.D. and A.C.A. Magalhaes, *The role of auxin like compounds in plant growth promoting rhizobacteria*, in *The wheat-azospirillum association*1993, R. Bras. Fisiol. Veg. p. 179-183.
36. Watt, C.A., et al., Selenate reduction by Enterobacter cloacae SLD1a-1 is catalysed by a molybdenum-dependent membrane-bound enzyme that is distinct from the membrane-bound nitrate reductase. *FEMS Microbiol. Lett*. **228:** 273-279 (2003).
37. Nagajyoti, P., K. Lee, and T.V. Sreekanth, Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*. **8(3):** 199-216 (2010).
38. Sudhakar, C., P.S. Reddy, and K. Veeranjaneyulu, Effect of salt stress on the enzymes of proline synthesis and oxidation in greengram (Phaseolus aureus Roxb.) seedlings. *J. Plant Physiol*. **(141):** 621-623. (1993).
39. Nejad, P. and P.A. Johnson, Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rapeand tomato. *Biological Control*. **(18):** 208–215 (2000).
40. Ordog, V. *Beneficial effects of microalgale and cyanobacteria in plant/ soils-systems, with special regard to their auxin- and cytokinin-like activity. International workshop and training course on microalgal biology and biotechnology Mosonmagyarovar*. 1999. 13-26.
41. Stirk, W.A., et al., Cytokinin- and auxin-like activity in Cyanophyta and microalgae. *J. Appl. Phycol*. **(14):** 215-221 (2002).
42. Takezawa, D., K. Komatsu, and Y. Sakata, ABA in bryophytes: how a universal growth regulator in life became a plant hormone? *Journal of plant research*. **124(4):** 437 (2011).
43. Hameed, A., et al., Induction of non protein thiols by chromium in cyanobacteria isolated from polluted areas. *African Journal of Microbiology Research*. **7(40):** 4806-4811 (2013).
44. Haroun, S. and M. Hussein, The Promotive Effect of Algal Biofertilizers on Growth, Protein Pattern and Some Metabolic Activities of Lupinus termis Plants Grown in Siliceous Soil. *Asian Journal of Plant Sciences*. **2(13):** 944-951 (2003).
45. Montgomery, D.M., et al., Phosphatase production and activity in Citrobacter freundii and a naturally occurring, heavy-metal-accumulating Citrobacter sp. *Microbiology*. **141 (10):** 2433-41 (1995).