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**ORIGINAL PAPER**

# Chromium-resistant PGPB: Growth stimulatory impact on *Vigna radiata* L. under chromium stress

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**QAMAR ARSHAD and AMBREEN AHMED***
*Department of Botany, University of the Punjab, Lahore 54590, Pakistan.*
***Author for Correspondence:** Tel: 0092-333-4595101; Email:ambreenahmed1@hotmail.com*

## Abstract

*Five already identified chromium-resistant bacterial strains exhibiting auxin biosynthetic potential (Bacillus sp. AMP2, Halomonas sp. AST, Arthrobacter mysorens AHA, Kushneria avicenniae AHT, Halomonas venusta APA) were used in the present study. Bacterial strains were used to inoculate seeds of Vigna radiata var. NM-2006 under chromium stress and impact of these strains on the growth of Vigna radiata was studied in the absence and presence of various concentrations (0-100µg/ml) of Cr-stress by recording various growth parameters of inoculated and non-inoculated seedlings. Most of the isolates improved plant growth under chromium stress by exerting beneficial impact on shoot length, root length and fresh weight of the inoculated treatments as compared to the growth observed in non-inoculated treatments under chromium stress. Similarly considerable improvement in the biochemical parameters i.e., auxin content and photosynthetic pigments of the inoculated treatments was observed in comparison with the non-inoculated treatments under chromium stress. Among the isolates, Halomonas venusta APA and Arthrobacter mysorens AHA were found to be most effective isolates with phytostimulatory impact on the growth of Vigna radiata L.*

**Keywords:** PGPB, IAA, Chromium, Bioremediation, Auxin

## 1. Introduction

Industrialization is the consequence of increase in population size which has led to many problems including environmental pollution due to the disposal of industrial effluents in the environment. This practice has polluted the soil and water resources with heavy metals and their toxicity is adversely affecting the whole ecosystem [7]. Chromium and its compounds are widely used in industries like steel production, wood preservation, dye manufacturing, paint, textile, fertilizer, electroplating, pulp processing, metal plating, alloy formation and leather tanning and is disposed off in the environment without proper treatment especially in the developing countries like Pakistan [6, 23]. Chromium has different oxidation states i.e., III to VI. Only the trivalent and hexavalent forms of chromium are stable and environmentally significant. Chromium (III) is biologically significant as a trace element required for living organisms. It is usually insoluble and helps to prevent the adverse effects of glucose and lipid metabolism [6]. Hexavalent chromium is toxic and carcinogenic in nature owing to its greater solubility, bioavailability and oxidizing properties [6, 11]. Chromium (VI) has hazardous effects on human health leading to many health problems such as skin problems like rashes, stomach problems and ulcer, weak immune system, respiratory problems, kidney and liver damage, lung cancer and alterations in genetic material which may become carcinogenic [6, 24]. The highly toxic effects of Chromium (VI) are due to its ability to penetrate the cellular membranes where it initiates or takes part in various reactions resulting in the production of toxic intermediate products [6]. Due to its oxidizing activity, it

produces reactive oxygen species which in turn damages DNA. In Pakistan, Kasur district is considered as a house of leather tanning industries where the industrial wastes are spreading diseases in the surrounding localities. Heavy metals in bulk quantity are entering into food chains including air, water, soil and plants. They bind to biomolecules, interfere with biological processes and adversely affect the metabolism of living organisms. Bioremediation processes can be used to decrease deleterious effects of chromium as well as to improve the growth of the plants in the contaminated areas [3]. Metal-resistant bacteria are known to have phytoextraction and phytostabilization activity [19]. Heavy metal tolerance by these bacteria involves variable mechanisms such as conversion of highly toxic form of metals to less toxic form, accumulation of metal ions inside the cell, absorption of metals and pumping of metal ions exterior to the cell [10, 18]. Their application is eco-friendly as compared to the conventional methods used for heavy metal detoxification since these bacteria are useful for plant growth promotion [9].

The present study is concerned with the utilization of beneficial plant growth promoting chromium-resistant bacteria for reducing the adverse effects of chromium on the plants and improving the growth of plants under chromium stress.

## 2. Materials and Methods

**Bacterial growth conditions**

Five already identified bacterial strains i.e., *Bacillus* sp. AMP2, *Halomonas* sp. AST, *Arthrobacter mysorens* AHA, *Kushneria avicenniae* AHT, *Halomonas venusta* APA by Ahmed [2] were used in the present work. The isolates were routinely grown using L-agar and L-broth medium at 37ºC for 24 hrs. To determine the minimum inhibitory concentration of chromium, the bacterial isolates were grown at 37ºC for 24 hours on L-agar medium supplemented with $K_2Cr_2O_7$ in concentrations ranging from 10 to 1000 µg/ml and growth of the isolates was recorded. The strains were able to resist chromium upto 500µg/ml.

**Pot experiment**

Certified seeds of *Vigna radiata* var. NM-2006 were procured from Punjab Seed Corporation, Lahore, Pakistan. Healthy seeds of *Vigna radiata* var. NM-2006 were surface sterilized using 0.1% $HgCl_2$ followed by several washings with sterilized distilled water. Already washed and oven dried petriplates (120mm diameter) were taken. The plates were autoclaved and oven dried after placing two layers of Whatman filter paper no. 1 in each plate. Plates were properly labeled for each strain with different concentrations of working solution of hexavalent chromium salt ($K_2Cr_2O_7$) i.e., 0, 10, 25, 50, 100 µg/ml. Non-inoculated seeds treated with sterilized distilled water in the absence of chromium stress were taken as control treatment. 10 ml of sterilized chromium salt solution with varying concentrations of $K_2Cr_2O_7$ (0, 10, 25, 50, 100 µg/ml) was supplied to each respective plate so that the filter papers were well moistened. Already treated and inoculated seeds of *Vigna radiata* NM-2006 using selected bacterial strains were uniformly spread on the moistened filter paper with the help of sterilized forceps. Petriplates were kept in dark at 30$\pm$2 ºC for three days. After germination, the germinated seedlings were transferred to the labeled pots each containing 140g sieved soil and stress solution containing 0, 10, 25, 50, 100 µg/ml of chromium salt ($K_2Cr_2O_7$) was given to the respective pots. The pots were placed in light (10 Klux, 16 hours duration) at 30 $\pm$2 ºC. The experiment was repeated thrice. After 20-25 days of growth, seedlings were removed from pots and various parameters were studied such as percentage germination, shoot length, root length, number of leaves, fresh weight of plants, auxin content and pigment content. Estimation of auxin content and pigment analysis was carried out following Mahadevan [13] and Lichtenthaler and Wellburn [12], respectively.

# 3. Results

**Pot experiment (*Vigna radiata*)**

Percentage germination of *Vigna radiata* L. var. NM-2006 seeds was recorded at various concentrations of hexavalent chromium salt ($K_2Cr_2O_7$) i.e., 0, 10, 25, 50, 100, 200, 300, 400, 500µg/ml. In non-inoculated seeds, maximum germination percentage was observed at 0µg/ml of chromium stress. Amount of chromium above 100µg/ml exerted extreme toxic effects on seed germination so chromium concentrations up to 100µg/ml were used for further study. Increase in the percentage germination was observed in treatments where seeds were inoculated with bacterial strains *Arthrobacter mysorens* AHA, *Halomonas* sp. AST and *Halomonas venusta* APA under chromium stress as compared to the non-inoculated control treatment (Fig 1a).

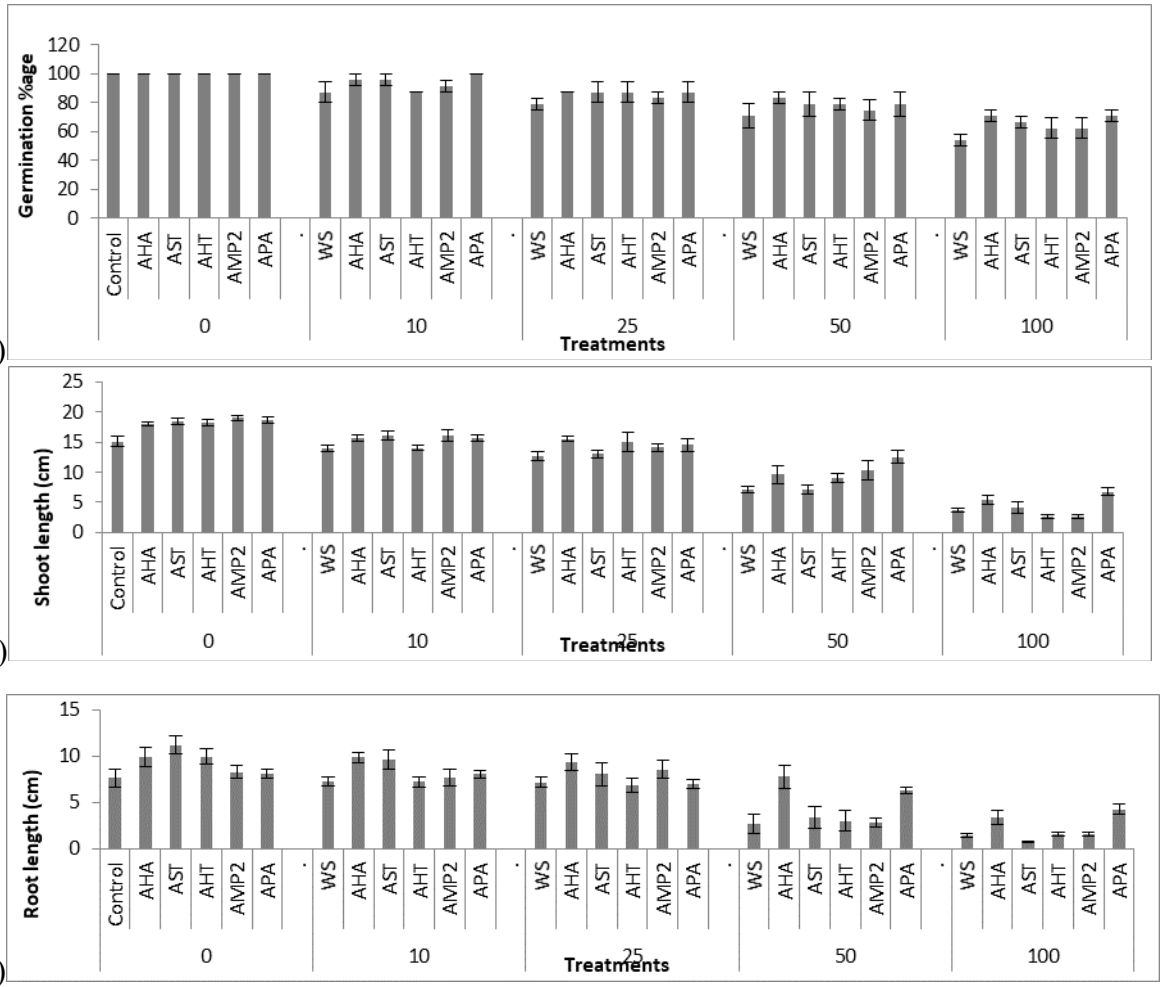


**Fig 1**: Impact of bacterial inoculations (AHA, AST, AHT, AMP2, APA) on **a)** germination percentage **b)** shoot length **c)** root length of *Vigna radiata* L. under chromium stress (0, 10, 25, 50, 100 µgml$^{-1}$ $K_2Cr_2O_7$) [WS = Without bacterial inoculation]

## Growth Parameters

Increase in the concentration of chromium from 0-100 µg/ml caused reduction in the shoot length as compared to the non-inoculated control treatment without chromium stress. Bacterial inoculations enhanced shoot lengths in comparison with the non-inoculated control treatment. At 10µg/ml chromium stress, all the bacterial strains promoted shoot growth with

the exception of *Kushneria avicenniae* AHT compared to non-inoculated respective treatment under chromium stress while at 25µg/ml and 50µg/ml, shoot growth was promoted by all the bacterial strains except AST as compared to the respective control treatment under chromium stress. However, at higher chromium stress i.e., 100µg/ml, *Arthrobacter mysorens* AHA, *Halomonas* sp. AST and *Halomonas venusta* APA caused increment (47, 10 and 83% respectively) in shoot growth while reduction in shoot length was observed in case of treatment with *Kushneria avicenniae* AHT and *Bacillus* sp. AMP2 as compared to the respective non-inoculated control (Fig 1b). In non-inoculated treatments, increase in chromium concentration from 0-25µg/ml did not exert significant impact on the root length of *Vigna radiata* but further increase in the concentration of chromium i.e., 50 and 100µg/ml caused drastic reduction in the root growth. At 10µg/ml chromium stress, bacterial treatment with *Arthrobacter mysorens* AHA and *Halomonas* sp. AST caused increase (35 and 32% respectively) in the root growth as compared to the control. Slight increase in root length was observed with AMP2 and APA (5 and 10%). At 25µg/ml, treatment with *Arthrobacter mysorens* AHA, *Halomonas* sp. AST and *Bacillus* sp. AMP2 promoted root growth in comparison with the control treatment. At 50 and 100µg/ml, prominent increase in root growth was observed with *Arthrobacter mysorens* AHA (187 and 137% respectively) and *Halomonas venusta* APA (132 and 204% respectively) treatments as compared to the respective non-inoculated treatment (Fig 1c).

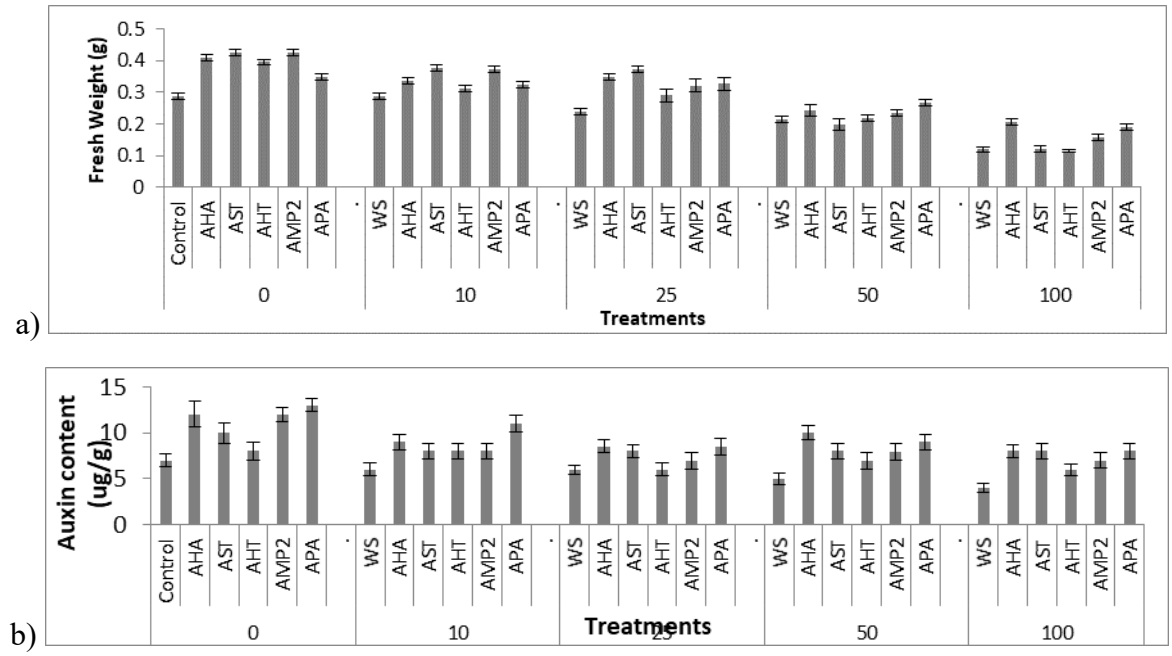


**Fig 2**: Impact of bacterial inoculations (AHA, AST, AHT, AMP2, APA) on **a)** fresh weight **b)** auxin content of *Vigna radiata* L. under chromium stress (0, 10, 25, 50, 100 µgml$^{-1}$ $K_2Cr_2O_7$) [WS = Without bacterial inoculation]

Generally increase in the concentration of chromium from 0-100µg/ml caused reduction in the fresh weight of the plants. Inoculation with the bacterial strains enhanced fresh weight of the treated plants as compared to the non-inoculated treatment. At 10 and 25µg/ml chromium stress, bacterial inoculation with all the isolates resulted in an increase in the fresh weight of the plants as compared to the respective non-inoculated treatment. Similarly, at 50µg/ml chromium stress, inoculation with *Arthrobacter mysorens* AHA (12%), *Bacillus* sp. AMP2 (9%) and *Halomonas venusta* APA (24%) resulted in an increase in the fresh weight.

At 100µg/ml, *Arthrobacter mysorens* AHA, *Bacillus* sp. AMP2 and *Halomonas venusta* APA promoted fresh weight (72, 30 and 59%) of the treated plants as compared to the respective non-inoculated treatment. However, AST and AHT did not promote the fresh weight of the treated plants at100 µg/ml in comparison with the respective non-inoculated treatment (Fig 2a). No increase or decrease in the number of leaves of plants in the inoculated treatments was observed as compared to the non-inoculated treatments.

**Auxin Content**

Bacterial inoculation caused significant improvement in the auxin content of the inoculated plants when compared with the non-inoculated control treatment. Increase in the concentration of chromium caused reduction in the auxin content as compared to the control. At 10µg/ml chromium stress, all the bacterial strains caused increment in the auxin content of the treated plants as compared to the respective non-inoculated treatment. However, at 25µg/ml, *Kushneria avicenniae* AHT treatment did not affect the auxin content of the treated plants in comparison with the control. At 50 and 100 µg/ml chromium stress, prominent increases (40-100%) in the auxin content of the treated plants were observed as compared to the respective non-inoculated treatment (Fig 2b).

**Pigment Analysis**

Generally, inoculation with bacterial isolates improved chlorophyll 'a' content of the treated plants as compared to the non-inoculated control treatment. Increasing concentration of chromium stress resulted in a decrease in the amount of chlorophyll 'a'. At 10µg/ml chromium stress, bacterial inoculation has improved the amount of chlorophyll 'a' prominently (28-58%) as compared to the respective control treatment. Similar observations were recorded at 25, 50 and 100 µg/ml chromium stress (Fig 3a). Two of the isolates i.e., *Arthrobacter mysorens* AHA (29%) and *Halomonas* sp. AST (20%) caused increase in the amount of chlorophyll 'b' of the treated plants in comparison to the non-inoculated control treatment in the absence of chromium stress. Increase in the concentration of chromium stress caused reduction in the amount of chlorophyll 'b' in the plants. At 10 µg/ml chromium stress, none of the isolates affected the amount of chlorophyll 'b' as compared to the respective non-inoculated treatment. However, at 25µg/ml, two of the isolates i.e., *Arthrobacter mysorens* AHA and *Halomonas* sp. AST improved chlorophyll 'b' content (12 and 21% respectively) as compared to the respective non-inoculated treatment. The isolate *Halomonas venusta* APA, however, caused slight reduction in the chlorophyll 'b' content of the treated plants in comparison with respective non-inoculated treatment. At 50 µg/ml, *Arthrobacter mysorens* AHA and *Bacillus* sp. AMP2 caused increase in the chlorophyll 'b' content (6 and 18%) whereas *Halomonas* sp. AST and *Kushneria avicenniae* AHT treatments have reduced chlorophyll 'b'. The isolate *Halomonas venusta* APA did not affect chlorophyll 'b' content of the treated plants at 50µg/ml. At 100µg/ml, bacterial inoculations with all the isolates has improved the amount of chlorophyll 'b' in the treated plants except *Halomonas venusta* APA which caused reduction in the amount of chlorophyll 'b' as compared to the respective non-inoculated treatment (Fig 3b).

Bacterial treatment with two of the isolates i.e., *Halomonas* sp. AST and *Halomonas venusta* APA has improved carotenoid content (8 & 4%) in comparison to the non-inoculated treatment while three of the isolates caused reduction in the amount of carotenoids of the treated plants in the absence of chromium stress. At 10µg/ml chromium stress, three of the isolates *Halomonas* sp. AST, *Bacillus* sp. AMP2 and *Halomonas venusta* APA caused increment (7, 12 and 25% respectively) in the carotenoid content of the plants while decrease in the amount of carotenoids was observed with *Arthrobacter mysorens* AHA (10%) and *Kushneria avicenniae* AHT (2%) treatments. At 25 and 50µg/ml chromium stress, slight improvement in the carotenoid content was observed with *Arthrobacter mysorens* AHA,

*Halomonas venusta* APA and *Halomonas* sp. AST treatments. At 100µg/ml, improvement in the carotenoid content were observed with *Arthrobactermysorens* AHA (105%), *Halomonas venusta* APA (80%), *Halomonas* sp. AST (55%) and *Bacillus* sp. AMP2 (60%) treatments whereas *Kushneria avicenniae* AHT treatment has reduced (10%) the amount of carotenoids in the treated plants as compared to the respective non-inoculated treatment (Fig 3c).

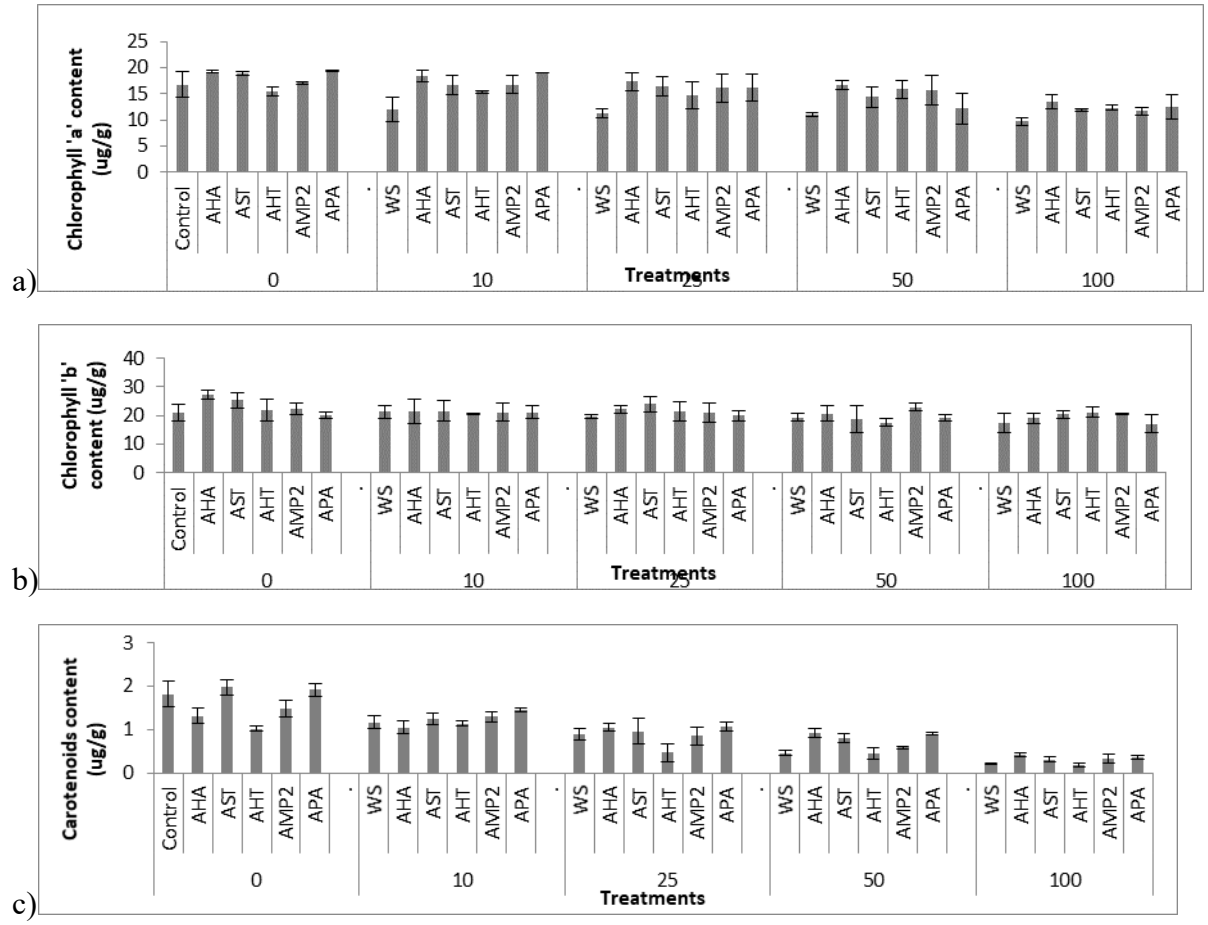


**Fig 3**: Impact of bacterial inoculations (AHA, AST, AHT, AMP2, APA) on **a)** chlorophyll 'a' **b)** chlorophyll 'b' **c)** carotenoid content of *Vigna radiata* L. under chromium stress (0, 10, 25, 50, 100 µgml$^{-1}$ $K_2Cr_2O_7$) [WS = Without bacterial inoculation]

## 4. Discussion

Heavy metal pollution is one of the most important environmental concerns today since it is introducing toxicity in the biological systems as well as in the environment exerting adverse effects on microbiota, fauna, flora and human beings [22]. Presently, biological solutions are gaining attention to reduce the heavy metal contamination from environment. These are considered as safe and environment friendly [22]. Metal-resistant bacteria have the ability to tolerate higher concentrations of heavy metals. Certain enzymes such as chromium reductase are found to be involved in reducing the toxic effects of chromium by chromium-resistant bacteria [23]. PGPR improve plant growth through various mechanisms [14, 1]. Among these mechanisms, nitrogen fixation and auxin production by the bacteria are considered as the major factors responsible for plant growth stimulation by bacteria [21]. Five identified chromium-resistant bacterial strains (*Bacillus* sp. AMP2, *Halomonas* sp. AST, *Arthrobacter mysorens* AHA, *Kushneria avicenniae* AHT, *Halomonas venusta* APA) by

Ahmed [2] were used for this study. With increasing concentration of chromium stress from 0-100 µg/ml, reduction in the germination percentage and shoot length was observed in non-inoculated treatments. However, bacterial inoculations were observed to enhance germination percentage and shoot growth under chromium stress in comparison to the respective non-inoculated treatment (Fig 1a, b). Among the isolates, *Halomonas venusta* APA and *Arthrobacter mysorens* AHA were found to stimulate plant growth more effectively especially at higher concentrations i.e., 50 and 100 µg/ml. Reduction in the germination percentage may result from the unavailability of water as water uptake is extremely reduced in the presence of higher concentrations of chromium in the soil. Bacterial inoculations reduce the adverse effects of chromium stress thus increasing the germination percentage especially at higher concentration [3]. Chromium affects all the metabolic processes of the plants such as photosynthesis, respiration, etc., thereby causing an overall damage to the plant growth and development. Retardation of root growth was generally observed with increase in chromium stress. However, at higher concentrations i.e., 100µg/ml of Cr, only two isolates *Arthrobacter mysorens* AHA (137%) and *Halomonas venusta* APA (204%) stimulated root growth (Fig 1c). An increase or decrease in root length in response to bacterial treatment has been speculated to be the consequence of IAA production by the isolates. Any change in the level of IAA in plant environment immediately affects the growth and development of plant roots [15]. Other workers have also reported that bacterial inoculation affects plant roots [5]. Chromium-resistant strains have the ability to absorb chromium through various mechanisms which involve the removal of chromate ions from the cells and help to change toxic form of Cr i.e., hexavalent chromium to less toxic trivalent form [20]. Heavy metal toxicity exerts adverse effects on the cell division of the plants reducing water uptake by the plants which results in the decrease of plant fresh weight. Increase in the biomass production is known to be the consequence of improvement in the mutual plant-microbe interactions through enhanced metal tolerance [14]. Prominent increases in the auxin content of the inoculated plants as compared to the non-inoculated treatments is a clear manifestation of the impact of auxin production potential of these isolates leading to growth improvement of the plants under chromium stress (Fig 2b). Any change in the auxin content of the treated plants affects the overall biology of the plants. *Arthrobacter mysorens* AHA was found to be most effective isolate in improving chlorophyll content of the treated plants under chromium stress while both *Arthrobacter mysorens* AHA and *Halomonas venusta* APA were effective in improving carotenoid content under chromium stress (Fig 3). Among the different pigments found in plants, chlorophylls are very important as these play significant role in absorbing light and energy and thus helping the plants in their development [17]. Bacterial inoculations were found to improve the amount of chlorophyll, thereby improving plant health and supporting plant for better growth under chromium stress as compared to the non-inoculated control treatments. Mishra *et al*. [16] have also reported improvement in chlorophyll content following bacterial inoculation. The phytostimulatory effect of the isolated chromium-resistant bacteria on *Vigna radiata* L. is the consequence of their ability to release growth promoting substances such as IAA [23]. Malekzadeh *et al.*[14] have also reported phytostimulation of Maize through bacterial inoculation under Cd stress. Similarly improvement in plant growth by bacterial inoculation under chromium stress has also been reported by Hemambika and Kannan [8].

## 5. Conclusion

The above study indicates the effectiveness of chromium-resistant bacterial isolates for their plant growth stimulatory potential under chromium stress by reducing the toxic effects

of chromium on plant growth. These biological agents can be efficiently utilized for bioremediation purpose in chromium-contaminated areas making these contaminated sites available for plantation and minimizing the hazardous impact of chromium on environment especially on the soil.

## 6. Acknowledgements

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