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Chromium-stress alleviation study using plant growth promoting rhizobacteria for maize (*Zea mays* L.)

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Authors' Contribution | **Hanif R.,** conducted the experiment and data analysis, **A. Tariq** performed data analysis and **A. Ahmed** conceptualization and review.

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ABSTRACT

Heavy metal contamination is one of the major environmental threats due to industrialization and urbanization. Among various heavy metals, chromium is of recent concern because it is considered the most hazardous metal pollutant in the environment. Its toxicity alters the process of germination and various other growth parameters ultimately affecting its yield. While maize (*Zea mays* L.) is one of the most widely cultivated cereal crop being used for fodder, feed and food purposes. It is multipurpose cereal crop and is also used as raw material for different industries. Hence, it is mandatory to come up with effective eco-friendly technique to remediate chromium (Cr) to protect the environment from its toxic impacts. During current research, plant microbe interaction was carried out with Cr resistant strains of *Bacillus cereus* (3a), *Pseudomonas aeruginosa* (DS4), *Bacillus nitratireducens* (TP8), *Enterobacter cloacae* (A9G) and *Pseudomonas* sp. (B3) that were selected to observe their beneficial impact on corn (*Zea mays* L.) growth in presence of Cr stress (150 & 300 µg/mL). Results have indicated that growth and biochemical parameters were enhanced due to bacterial inoculation. In comparison to control, maximum increment in shoot length, number of leaves, root length and fresh weight of inoculated plants was noted up to 46, 115, 76, and 42% respectively. Maximum increment in protein and chlorophyll content of inoculated plants was noted up to 304 and 71% respectively as compared to control. The present study suggests the use of above-mentioned highly chromium resistant bacterial strains to remediate chromium contaminated sites and to improve agricultural production.

Keywords: Bioremediation, heavy metal stress, *Zea mays*, PGPR.

INTRODUCTION Environmental pollution is triggered by various heavy metals released from different industries. Chromium (Cr) is considered a hazardous heavy metal with the ability to exist in different oxidation states. Its oxidations states range from -2 to +6, while its most stable forms are trivalent and hexavalent (Hossini *et al.*, 2022). Its toxicity depends on its oxidation states; hexavalent form is more toxic than trivalent form due to its greater solubility (Coetzee *et al.*, 2020). It originates from a variety of industries, including cement plants, electroplating, steel production works, dye, paint, and pigment manufacturing, metal plating, timber processes, pulp and paper production, tobacco smoke, and leaching from unsanitary landfills. Chromium is carcinogenic in nature, can cause skin sores and severe respiratory problems in humans (Sall *et al.*, 2020). In plants, uptake of chromium occurs through a variety of transporters, including phosphate and sulphate transporters (Srivastava *et al.*, 2021). Its toxicity causes alteration in germination process, inhibits shoot and root growth and subsequent biomass accumulation resulting in reduction in chlorophyll production and protein synthesis in plants (Mujahid Farid *et al.*, 2019). Corn (*Zea mays* L.) belongs to grass family *gramineae* and is the most widely cultivated crop in various regions of the world. It is a `multipurpose cereal crop being utilized for fodder, feed and food purposes (Erenstein *et al.*, 2022). It is a staple crop of different countries and provides food to 9.4 million human populations of the world (Sultan *et al.*, 2023). In Pakistan, it ranks as the third most important crop, following wheat and rice, and is cultivated extensively. Maize is cultivated in all the provinces of the country but Punjab and KPK are the primary production regions (Waris *et al.*, 2023). Like all other crops, maize production is also threatened by various abiotic stresses like heavy metals which reduce its quantity and quality (Sultan *et al.*, 2023). Heavy metals are extremely toxic due to their persistence in the environment, as they cannot be easily degraded over time. Therefore, remediation of heavy metals is mandatory to protect the environment from their harmful effects (Li *et al.*, 2019). A number of conventional physicochemical remediation techniques are being utilized to treat heavy metal-contaminated sites, but these techniques are expensive, ineffective, and non-suitable for use on a broad scale (Gong *et al.*, 2018). Among various remediation techniques, the use of chromium resistant rhizobacteria is a promising approach to deal with the problem of chromium contamination because it is environmentally friendly, cost-efficient and least destructive. These rhizobacteria promote plant growth in different ways, such as producing siderophores, fixing nitrogen biologically, solubilizing phosphate, producing ACC deaminase, rhizosphere engineering, displaying antifungal activities, producing phytohormones, inducing systemic resistance, producing volatile organic compounds and disrupting pathogen toxin production (Mushtaq *et al.*, 2022)). They can increase the growth of plant under heavy metal stress (Tirry *et al.*, 2021).

OBJECTIVES: The objectives of the current research is to investigate the chromium resistance ability of various bacterial strains and to investigate the potential impact of Cr resistant bacterial strains in increasing growth of corn under chromium stress.

MATERIALS AND METHODS: Screening of Cr-resistant bacterial strains: Twelve previously isolated strains of bacteria were examined for their Cr tolerance ability (figure 1). The minimum concentration of Cr that would inhibit bacterial growth was determined by growing bacterial isolates on various chromium concentrations ranging from 100µg/mL to 400µg/mL. Chromium resistance potential of twelve bacterial strains were evaluated (table 1).

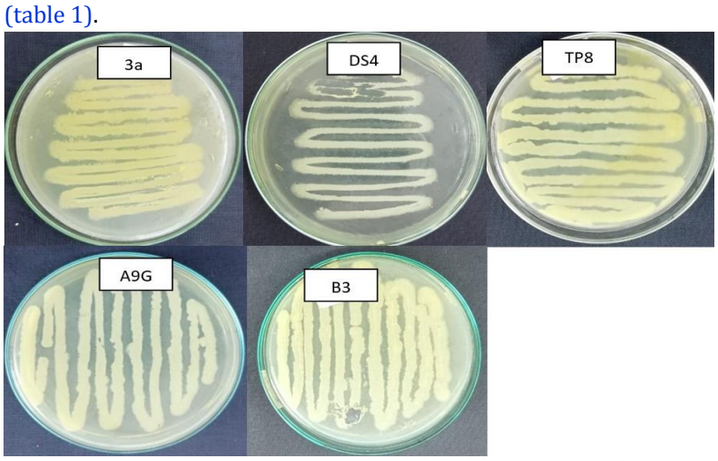

Figure 1: The bacterial strains selected for the current study i.e., *B. cereus* (3a), *P. aeruginosa* (DS4), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and *Pseudomonas sp.* (B3).

Inoculation experiment with *Zea mays* L.: Plant microbe interaction was conducted with selected chromium resistant bacterial isolates i.e., *Bacillus cereus* (3a), *Pseudomonas aeruginosa* (DS4), *Bacillus nitratireducens* (TP8), *Enterobacter cloacae* (A9G) and *Pseuodomonas* sp. (B3) (figure 2 & 3). The Punjab Seed Corporation in Lahore provided certified seeds of corn of variety SG 2002. Three hundred and twenty four healthy seeds were selected, washed with detergent followed by numerous washings of sterile distilled water. The seeds were subjected to surface sterilization by dipping in 0.1% solution of mercuric chloride for 3 minutes and traces of mercuric chloride was eliminated by dipping the seed into sterile distilled water. The seeds were dried on double layer of sterile filter papers and soaked in L-Broth media containing inoculums of bacterial cultures for 1 hour. For control, seeds of SG 2002 were dipped in sterile distilled water. Pots were filled after sieving soil and 155g sterile soil was filled in each pot. Soil obtained from botanical garden of University of the Punjab. Six seeds were sown per pot. Experiment was performed in triplicate. Two different concentrations of chromium were used i.e., 150 & 300 µg/mL. The labelled pots were kept under controlled environment i.e. 10 K lux of light for 16 hours a day, at a temperature of 25+2ºC. After 7 days, germination percentage was recorded. Seedlings were collected from their respective pots after 25-30 days.

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Figure 2: Effect of bacterial inoculations on growth of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). [C – Control, C_{150} – Control with Cr stress of 150 µg/mL, C_{300} – Control with Cr stress of 300 µg/mL, A9G – *E. cloacae*; $A9G_{150}$ – *E. cloacae* with Cr stress of 150 µg/mL, $A9G_{300}$ – *E. cloacae* with Cr stress of 300 µg/mL].

Figure 3: Effect of bacterial inoculations on growth of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). [C – Control, C_{150} – Control with Cr stress of 150 µg/mL, C_{300} – Control with Cr stress of 300 µg/mL, A9G – *E. cloacae*; $A9G_{150}$ – *E. cloacae* with Cr stress of 150 µg/mL, $A9G_{300}$ – *E. cloacae* with Cr stress of 300 µg/mL].

Shoot length, root length, fresh weight, number of leaves, chlorophyll and protein content were recorded after harvesting. Content of chlorophyll was assessed by following Wellburn (1994). For each treatment, 1g plant material was taken in each test tube after crushing. 80% acetone solution was added in each test tube. These test tubes were then kept in dark for 24 hours and optical density was measured at 663nm and 645nm by using spectrophotometer. Content of protein was checked by following Lowry *et al.* (1951). For each treatment, 1g plant material was crushed with pestle and mortar by using 1N phosphate buffer and centrifuged at 10,000rpm for 10 minutes. Folin's mixture was added to the supernatant and kept at room temperature for 15 minutes. In each test tube, Folin Ciocalteus phenol reagent was added and kept at room temperature for 45 minutes. Optical density was measured at 750nm by using spectrophotometer and protein content was checked by comparing them with standard curve.

Statistical analysis: Statistical analysis of the data was done by applying Duncan's multiple range test. Software SPSS v.16 was used

RESULTS: Screening of Cr-resistant bacterial strains: Based on their chromium resistance ability five bacterial isolates i.e., *B. cereus* (3a), *P. aeruginosa* (DS4), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and *Pseudomonas* sp. (B3) were selected (figure 1). Among five selected bacterial isolates, *P. aeruginosa* (DS4), *B. nitratireducens* (TP8), *E. cloacae* (A9G) have ability to resist chromium stress up to 350µg/mL while *B. cereus* (3a) and *Pseudomonas* sp. (B3) showed resistance against chromium up to chromium stress of 300µg/mL (table 1). Inoculated seeds were exhibiting higher germination percentage as compared to control. Without Cr stress, increment in percentage germination of plants treated with *B. nitratireducens* (TP8), *P.aeruginosa* (DS4), *E. cloacae* (A9G), *Pseudomonas* sp. (B3) and *B. cereus* (3a) was 28.7, 21.5, 14.4, 14 and 7.25% respectively than control. In case of Cr stress of 150µg/mL, increment in percentage germination of plants treated with *P. aeruginosa* (DS4), *Pseudomonas* sp. (B3), *B. nitratireducens* (TP8), *E. cloacae* (A9G), *B. cereus* (3a) was 40, 30, 20, 10.1 and 10% respectively than control. Under chromium stress of 300µg/mL, increment in percentage germination of plants treated with *Pseudomonas* sp. (B3), *B. nitratireducens* (TP8), *P. aeruginosa* (DS4), *B. cereus* (3a) and *E. cloacae* (A9G) was 200, 175, 75, 50 and 25% respectively than control (figure 4).

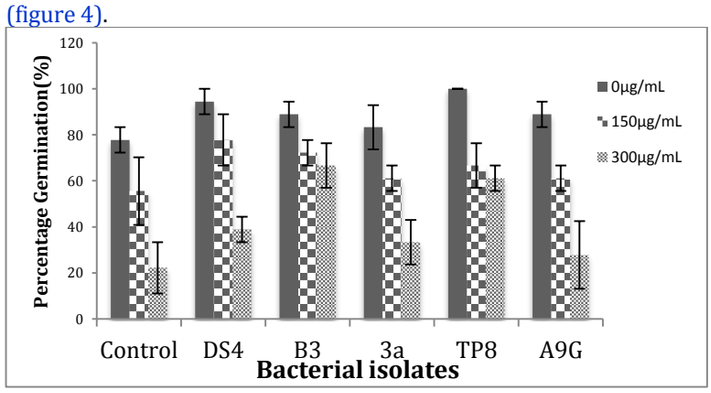

Figure 4: Effect of bacterial inoculations on germination percentage of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). [C - Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and *Pseudomonas* sp. (B3)].

Sr. No.	Bacterial isolates	Cr (µg/mL)						
		100	150	200	250	300	350	400
1.	DS1	+++	++	+	-	-	-	-
2.	DS2	++	+	-	-	-	-	-
3.	DS3	+	-	-	-	-	-	-
4.	DS4	+++	+++	+++	++	+	-	-
5.	E1	+++	++	+	-	-	-	-
6.	ES3	++	+	-	-	-	-	-
7.	B3	+++	++	++	+	+	-	-
8.	3a	+++	+++	++	+	+	-	-
9.	Th1	++	+	-	-	-	-	-
10.	Tp8	+++	+++	++	++	+	+	-
11.	Th6	++	+	-	-	-	-	-
12.	A9G	+++	+++	++	++	++	+	-

Table 1: MIC of bacterial strains

Outstanding growth (+++), Favorable growth (++), moderate growth (+), no growth (-)

Without chromium stress, *Pseudomonas* sp. (B3) showed maximum increment in shoot length up to 31.6%, when compared with control containing no bacterial inoculation. While plants inoculated with other bacterial isolates i.e., *B. nitratireducens* (TP8), *B. cereus* (3a), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) showed increment up to 25.4, 13.1, 11.5 and 9.89% respectively than control. When exposed to chromium stress at a concentration of 150µg/mL, plants treated with the *B. cereus* (3a), *Pseudomonas* sp. (B3), *B. nitratireducens* (TP8), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) exhibited increment in shoot length up to 47.9, 44.1, 40.8, 25.3 and 14.4% respectively in comparison to control. When exposed to chromium stress at a concentration of 300µg/mL, plants treated with *Pseudomonas* sp. (B3), *B. nitratireducens* (TP8), *P. aeruginosa* (DS4), *B. cereus* (3a) and *E. cloacae* (A9G) exhibited remarkable increment in in shoot length up to 39.5, 38.7, 35.9, 24.9 and 24.5% respectively (figure 5). Root length of plants inoculated with bacterial strains was also increased. Without chromium stress, *Pseudomonas* sp. (B3) and *B. cereus* (3a) showed greatest increment in length of root by 38 and 36%, respectively as compared to control plants containing no bacterial inoculation. While plants inoculated with other bacterial isolates i.e., *E. cloacae* (A9G), *B. nitratireducens* (TP8), *P. aeruginosa* (DS4) showed increment in root length up to 24.6, 20.8 and 17.8%,

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respectively. When exposed to chromium stress at a concentration of 150µg/ml, plants inoculated with *Bacillus nitratireducens* (TP8), *P. aeruginosa* (DS4), *Pseudomonas* sp. (B3), *E. cloacae* (A9G) and *B. cereus* (3a) demonstrated significant increment in root length up to 76.3, 57.5, 33.6, 15.9 and 12.5%, respectively. Similarly, under chromium stress of 300µg/ml, plants inoculated with *Bacillus nitratireducens* (TP8), *Pseudomonas aeruginosa* (DS4), *Pseudomonas* sp. (B3), *Bacillus cereus* (3a) and *Enterobacter cloacae* (A9G) showed an increase in root length by 56.2, 48.8, 23.1, 11.7 and 11.4%, respectively as compared to control (figure 6).

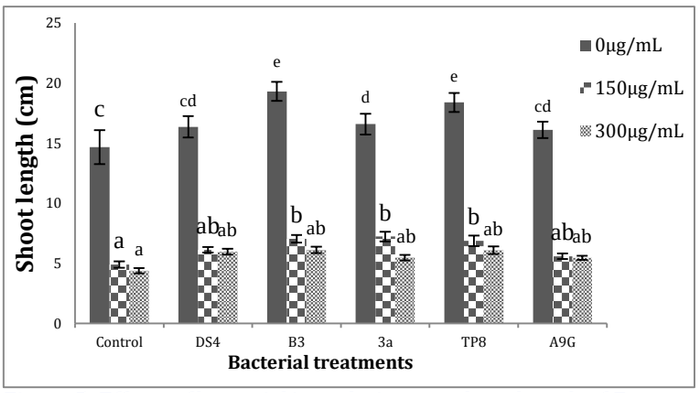

Figure 5: Effects of bacterial inoculations on shoot length of *Zea mays* L. ((SG 2002) under 150 & 300 µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.]

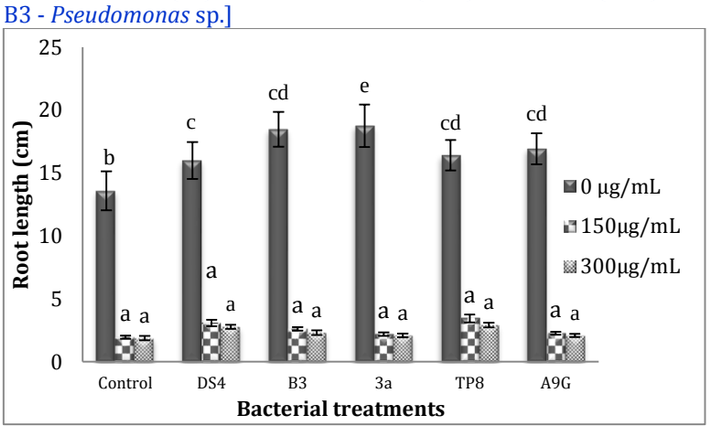

Figure 6: Effects of bacterial inoculations on root length of *Zea mays* L. (SG 2002) under chromium (150 & 300 µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.].

Plants treated with bacterial strains showed noticeable increment in leaves number. Without chromium stress, *B. nitratireducens* (TP8) and *B. cereus* (3a) showed greatest increase in number of leaves up to 33.2 and 31.1%, respectively in comparison to control containing no bacterial treatment. While plants treated with other bacterial isolates i.e., *P. aeruginosa* (DS4), *Pseudomonas* sp. (B3) and *E. cloacae* (A9G) showed increment in leaves number up to 27.3, 21.5 and 19.6%, respectively when compared with control. When exposed to chromium stress at a concentration of 150µg/mL, plants inoculated with *B. nitratireducens* (TP8), *B. cereus* (3a), *Pseudomonas* sp. (B3), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) exhibited an increase of 69.7, 35.1, 27.3, 23.4 and 19.5%, respectively when compared with control. Under chromium stress of 300µg/mL, plants treated with the *B. cereus* (3a), *B. nitratireducens* (TP8), *Pseudomonas* sp. (B3), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) exhibited an increase of 115, 71.2, 33.6, 25.3 and 21.1%, respectively when compared with control (figure 7).

An increment in fresh weight was observed on treatment of plants with bacterial isolates. Without chromium stress, *Pseudomonas* sp. (B3) showed maximum enhancement in fresh weight up to 34.8% as compared to control plants containing no bacterial inoculation. Other bacterial isolates *B. nitratireducens* (TP8), *B. cereus* (3a), *E. cloacae* (A9G) and *P. aeruginosa* (DS4) exhibited an increase of 21.5, 16.8, 4.20 and 4.09%, respectively. When exposed to chromium stress at a concentration of 150µg/mL, significant enhancement in fresh weight of plants treated with *Pseudomonas* sp. (B3), *B. nitratireducens* (TP8), *B. cereus* (3a), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) was observed up to 42.5, 39.3, 24.9, 23.8 and 23.6%, respectively in comparison to control. When exposed to chromium stress at a concentration of 300µg/mL, plants inoculated with *Pseudomonas* sp. (B3), *B. nitratireducens* (TP8), *B. cereus* (3a), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) showed an increase of 33.2, 32.1, 22, 15.2 and 14.5%, respectively when compared with control (figure 8).

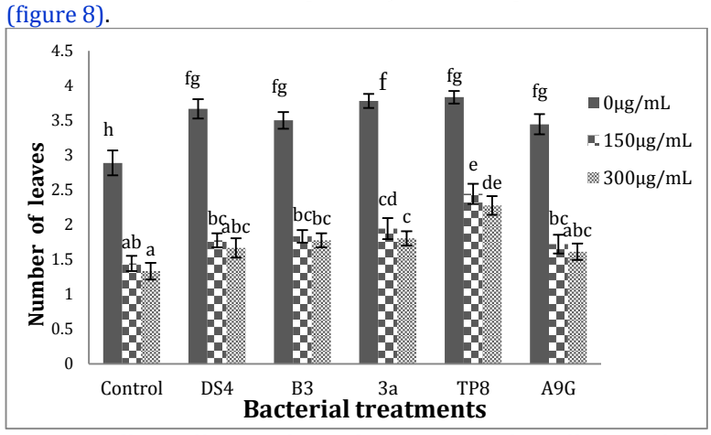

Figure 7: Effects of bacterial inoculations on number of leaves of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL) Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.].

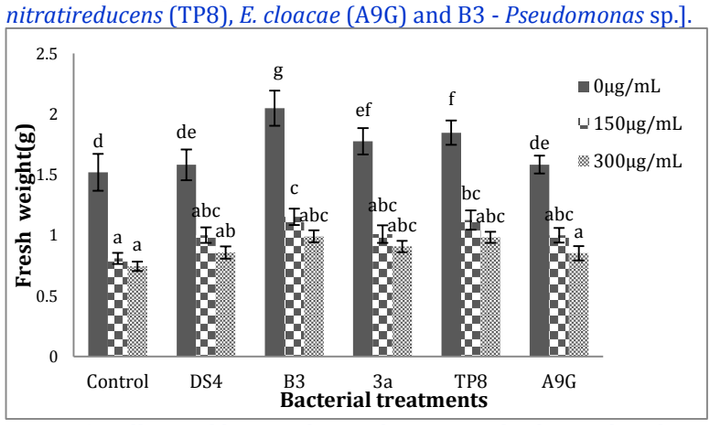

Figure 8: Effects of bacterial inoculations on fresh weight of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P.. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.].

Biochemical parameters: Significant increment in biochemical parameters of treated plants was noted. Inoculation of bacterial strains led to increment in content of chlorophyll **'a'** content. Under no chromium stress, plants inoculated with the bacterial strains *E. cloacae* (A9G), *Pseudomonas* sp. (B3), *B. cereus* (3a), *B. nitratireducens* (TP8) and *P. aeruginosa* (DS4) showed increment in chlorophyll 'a' up to 69.2, 67.7, 59.1, 49.2 and 44.8%, respectively when compared with control. Under chromium stress of 150µg/mL, plants inoculated with the bacterial strains *B. cereus* (3a), *B. nitratireducens* (TP8), *Pseudomonas* sp. (B3), *E. cloacae* (A9G) and *P. aeruginosa* (DS4) showed increment in chlorophyll 'a' up to 66.4, 64.3, 33.1, 6.11, and 2.46 %, respectively when compared with control. When exposed to chromium stress at a concentration of 300µg/mL, plants inoculated with *Pseudomonas* sp. (B3), *E. cloacae* (A9G), *P. aeruginosa* (DS4), *B. nitratireducens* (TP8) and *B. cereus* (3a) showed increment in chlorophyll 'a' up to 41.8, 12.6, 3.14, 2.30 and 2.26%, respectively in comparison to control (figure 9).

The chlorophyll 'b' content also increased significantly due to inoculation of bacterial strains. Plants treated with the bacterial strains *E. cloacae* (A9G), *Pseudomonas* sp. (B3), *B. cereus* (3a), *B. nitratireducens* (TP8), *P. aeruginosa* (DS4) showed increment in chlorophyll 'b' up to 71.2, 70.1, 61.7, 49.7 and 45.4%, respectively as compared to control. When exposed to chromium stress at a concentration of 150µg/mL, plants treated with *B. cereus* (3a), *B. nitratireducens* (TP8), *Pseudomonas* sp. (B3), *E. cloacae* (A9G) and *P. aeruginosa* (DS4) showed increment in chlorophyll 'b' up to 68.2, 65.1, 33.9, 5.31 and 2.52%, respectively when compared with control. Similarly, under chromium stress of 300µg/mL, plants inoculated with the bacterial strains *E. cloacae* (A9G), *Pseudomonas*

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sp. (B3), *P. aeruginosa* (DS4), *B. cereus* (3a) and *B. nitratireducens* (TP8) showed increment in chlorophyll 'b' up to 44.7, 14.7, 3.54, 3.14 and 2.03%, respectively when compared with control (figure 10).

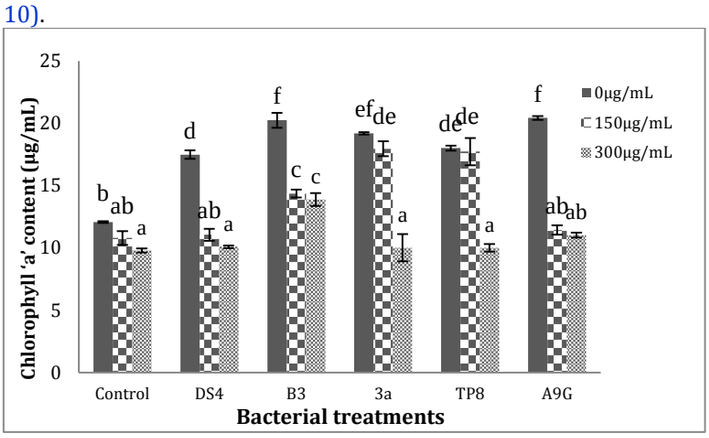

Figure 9: Effects of bacterial inoculations on content of chlorophyll 'a' of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.].

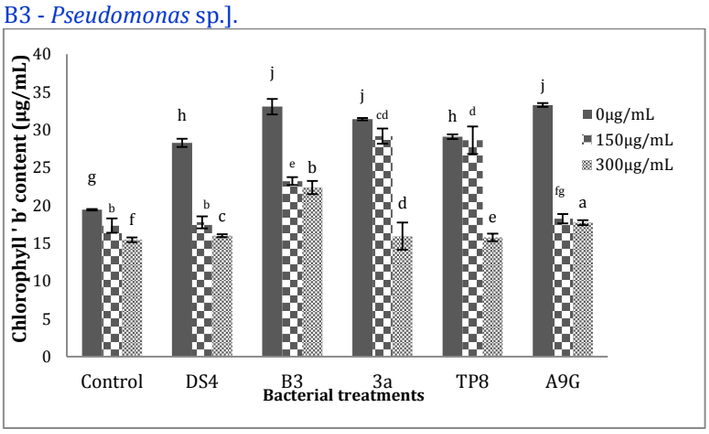

Figure 10: Effects of bacterial inoculations on content of chlorophyll 'b' of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.].

The inoculation of bacterial strains enhanced the total chlorophyll content of the inoculated plants. Under no chromium stress, plants treated with the *E. cloacae* (A9G), *Pseudomonas* sp. (B3), *B. cereus* (3a), *B. nitratireducens* (TP8) and *P. aeruginosa* (DS4) showed increment in total chlorophyll content up to 70.5, 69.2, 60.6, 49.5 and 45.2 %, respectively when compared with control. Under chromium stress of 150µg/mL, plants inoculated with the bacterial strains *B. cereus (*3a), *B. nitratireducens* (TP8), *Pseudomonas* sp. (B3), *E. cloacae* (A9G) and *P. aeruginosa* (DS4) showed increment in total chlorophyll content up to 67.5, 64.8, 33.6, 5.62 and 2.50%, respectively when compared with control. Under chromium stress of 300µg/mL, plants inoculated with *Pseudomonas* sp. (B3), *E. cloacae* (A9G), *P. aeruginosa* (DS4), *B. cereus* (3a) and *B. nitratireducens* (TP8) showed increment in total chlorophyll content up to 43.6, 13.8, 3.34, 2.76 and 2.10 %, respectively when compared with control (figure 11). Total soluble protein content was also improved significantly in comparison to control due to inoculation of bacterial strains. In the absence of chromium stress, plants treated with the *E. cloacae* (A9G), *P. aeruginosa* (DS4), *B. nitratireducens* (TP8), *Pseudomonas* sp. (B3) and *B. cereus* (3a) showed increment in content of protein by 16, 14.3, 13.7, 12.1 and 5.14%, respectively when compared with control. When exposed to chromium stress at a concentration of 150µg/mL, plants inoculated with *B. nitratireducens* (TP8), *P. aeruginosa* (DS4), *Pseudomonas* sp. (B3), *B. cereus* (3a) and *E. cloacae* (A9G) showed increment in protein content by 304, 225, 160, 54.2 and 1.24%, respectively when compared with control. When exposed to chromium stress at a concentration of 300µg/mL, plants inoculated with *B. nitratireducens* (TP8), *B. cereus* (3a), *Pseudomonas* sp. (B3), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) showed an increase in protein content by 273, 156, 151, 106 and 26.1%, respectively when compared with control (figure 12).

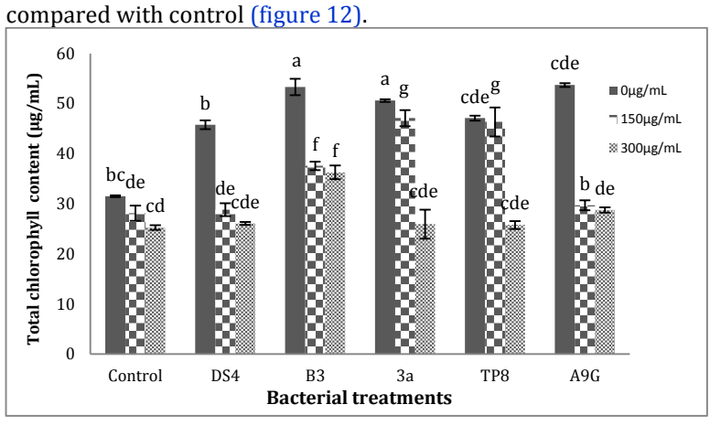

Figure 11: Effects of bacterial inoculations on content of total chlorophyll of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C - Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.].

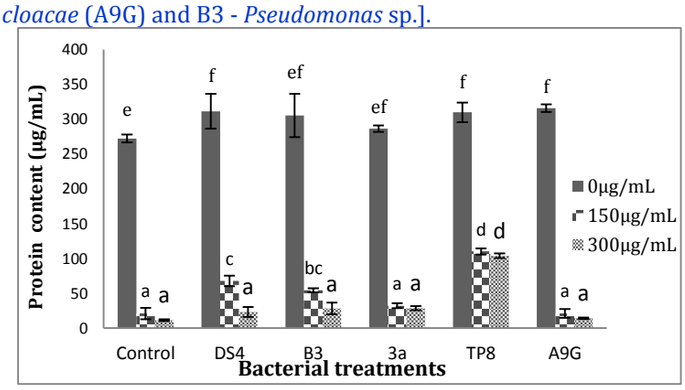

Figure 12: Effects of bacterial inoculations on protein content of *Zea mays* L. (SG 2002) under two different concentrations of chromium (150 & 300 µg/mL). Alphabetical letters show significant differences between the treatments as determined by Duncan's multiple range test (P=0.05). [C-Control; *P. aeruginosa* (DS4), *B. cereus* (3a), *B . nitratireducens* (TP8), *E. cloacae* (A9G) and B3 - *Pseudomonas* sp.].

DISCUSSIONS: Environmental pollution due to heavy metals has become major concern due to detrimental impacts it is causing around the globe (Ajibade *et al.*, 2021). Moreover, exposure of heavy metals to plants lead to crop yield reduction (Gupta and Seth, 2021). Hence it is necessary to come up with effective eco-friendly technique to reduce heavy metals toxicity to protect the environment from their negative effects (Singh *et al.*, 2023). For this purpose, certain plant growth promoting rhizospheric bacteria (PGPR) have been proven useful in minimizing toxicity of heavy metals. For the recent work, five chromium resistant bacterial strains i.e., *Pseudomonas* sp. (B3), *B. cereus* (3a), *B. nitratireducens* (TP8), *P. aeruginosa* (DS4) and *E. cloacae* (A9G) were used to remove toxicity of chromium (table 1). Without bacterial inoculation all growth parameters were significantly reduced in chromium contaminated medium. Significant increment in parameters of growth was noted as a result of treating corn seeds with chromium resistant bacterial isolates. Germination percentage of treated seeds with bacterial isolates was greater than control in chromium contaminated medium. When exposed to chromium stress at a concentration of 0, 150 and 300µg/mL, maximum percentage germination of treated seeds was up to 28, 40 and 200%, respectively. This significant increment could be attributed to synthesis of gibberellins which enhance the functioning of certain enzymes i.e., *a*-ß-amylase, nuclease, and protease that enhance seeds germination. Zaib *et al.* (2023) also reported the similar results of enhanced seed germinations in barley due to inoculation of *Pseudomonas* species. When exposed to chromium stress at a concentration of 0, 150 and 300µg/mL, treated plants showed increment in length of shoot up to 31, 47 and 39%, respectively. This increment in shoot length may be due to solubilization of phosphate by these rhizobacteria. Abd El-Mageed *et al.* (2022) also observed similar results of increased shoot length due to bacterial inoculation. When exposed to chromium stress at a concentration of 0, 150 and 300µg/mL, greatest increment in root length of inoculated plants was noted up to 38, 76 and 56% respectively. Phytohormone production (e.g. IAA) may increase the

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network of the lateral roots and roots hairs. Due to large surface area of roots, uptake of nutrients was increased which lead to shoot growth increment of corn seedlings. Chamkhi *et al.* (2023) also observed the increment in number of fresh leaves due to inoculation of different bacterial strains indicating that these strains are effective in enhancing leaf turgor and chlorophyll content due to which plants generate more fresh leaves. When exposed to chromium stress at a concentration of 0, 150 and 300µg/mL, maximum increment in fresh weight of inoculated plants was recorded up to 34, 42 and 33% respectively. Da-Silva *et al.* (2023) also noted an enhancement in fresh weight when plants were treated with *Azospirillum brasilense.* Biochemical analysis indicated that the chlorophyll content of inoculated plants was significantly higher than untreated plants when exposed to stress of chromium stress. When exposed to chromium stress at a concentration of 0, 150 and 300µg/mL, treated plants showed maximum increment in content of chlorophyll 'a' noted no up to 69, 66 and 41%, chlorophyll 'b' was up to 71, 68 and 44% and total chlorophyll was up to 70, 67 and 43% respectively as compared to control. This increase in chlorophyll content may be due to nitrogen fixation. As nitrogen is important component of chlorophyll molecule so when nitrogen fixation process increases due to rhizobacteria, then ultimately chlorophyll synthesis also increases leading to darker color of plants. These bacteria take atmospheric nitrogen and convert it into its bioavailable form i.e., ammonia. Content of plant soluble protein was also greater in inoculated plants than control. Results indicated that in presence of chromium stress of 0, 150 and 300µg/mL, maximum increase in protein content of treated plants was up to 16, 304 and 273% respectively. Due to phytohormones production, chlorophyll and plant soluble protein content may increase. Sultana *et al.* (2024) also observed the similar findings of considerable increment in photosynthetic pigments (chlorophyll and protein) upon inoculation of PGPR.

CONCLUSIONS This study suggests the use of these highly efficient chromium resistant bacterial strains i.e., *B. cereus* (3a), *P. aeruginosa* (DS4), *B. nitratireducens* (TP8), *E. cloacae* (A9G) and *Pseudomonas* sp. (B3) as bio fertilizers for promoting growth (*Zea mays* L.) and reducing chromium toxicity. Further research can be done to know science behind the plant growth increment under chromium toxicity.

CONFLICT OF INTEREST: All the authors mentioned in this paper declared that they have no conflict of interest regarding this paper.

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