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Enhancement of Wheat Growth by UV-Mutagenesis of Potential Chromium Tolerant *Bacillus* sp. Strain SR-2-1/1

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Abstract: UV mutagenesis has long been known to improve bacterial strains in their physiological capacity. In the current study, we used the UV mutagenesis approach to increase the PGPR characteristics of a pre-characterized metal-tolerant PGPR strain *Bacillus* sp. strain SR-2-1/1 (KY315919), with the objective of increasing the physiological outcome of its PGPR traits in vitro and post inoculation on wheat plants. After UV irradiation, the results implied a substantial in vitro increase in the phosphate solubilization and ammonia production of two selected mutant strains (M_1 and M_2) as compared to the wild-type strain SR-2-1/1; however, the ACC deaminase enzyme activity was completely lost in the mutant strains, which were originally present in the wild-type strains. However, the UV mutagenesis did not affect the taxonomy of these mutant strains. Moreover, the mutant strains M_1 and M_2 survived in the rhizosphere of wheat plants up to 30 days at an optimum (approximately 7–7.5 Log CFU/mL of rhizosphere soil) population density. The fresh and dry biomass, as well as root and shoot length, of wheat plants inoculated with one of the mutant strains M_2 were significantly higher than in the wheat plants inoculated with wild-type strain SR-2-1/1. The overall results imply that the resulted mutant M_2 was a physiologically competent PGPR strain, which could be tested in field experiments as an inoculum.

Keywords: mutagenesis; PGPR; *Bacillus*; rhizosphere; wheat growth

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1. Introduction

The heavy metal contamination of agricultural soils is one of the hot environmental issues in the current scenario due to the accumulation of metals through municipal, agricultural and industrial activities, such as the discharge of urban and/or industrial sewage in agricultural soils, vehicles exhausts, and waste incineration [1,2]. Hence, the contamination of the biosphere by noxious heavy metals is a worldwide hazard that has quickened, despite the initiation of mechanical rebellion [3]. Mechanical operations such as metal forging, mining, refining, sewage slop application in agronomic practices and the combustion of fossil fuels are the primary source of heavy metal pollution [4]. Additionally, heavy metals antagonistically influence naturally occurring microbial populations and prompt the disturbance of their fundamental natural procedures [5,6]. Moreover, the accumulation of heavy metals in agricultural soils not only decreases crop production due to their persistent nature and toxicity at cellular levels, but also causes health hazards to humans feeding on contaminated grains and tissues [7]. Chromium (Cr) is regarded as the

seventh most abundant element and the second most toxic element on Earth [8]. Cr is used in paints and shades, wood conservation, chemicals synthesis, electroplating, mash, the paper industry and metallurgy [9]. Higher concentrations of Cr are lethal for crop plants due to its persistence in soil, multiple oxidation states, competition with essential elements for uptake and rapid accumulation in plant biomass and grains [10]. The poisonous quality of Cr to plants relies upon its valence state; for example, Cr (VI) is extremely dangerous and movable as compared to Cr (III).

Amongst the multiple other biological approaches to alleviating Cr toxicity in agro-systems, one of the most economical and environmentally friendly ways is the utilization of plant growth-promoting rhizobacteria (PGPR) [11]. After inoculation, metal-tolerant PGPR can reduce the harmful effects of Cr in plants via a variety of mechanisms. For example, metal-tolerant PGPRs are able to immobilize the Cr uptake in plants by adsorption in the extracellular barrier or extracellular sequestration in periplasm. Moreover, metal-tolerant PGPR can reduce the metal ions in cells enzymatically through sequestration by metal-binding proteins [12,13]. Moreover, various plant growth-promoting mechanisms of PGPR support plants' nourishment in harsh environments [14]. For instance, metal-tolerant PGPR can induce physiological vigor at cellular levels in plants by producing ACC deaminase and reducing the concentration of stress ethylene, synthesizing the auxins such as indole-3-acetic acid (IAA), transporting competitively the essential nutrients, reducing radical species production by cells and inducing the production of antioxidant enzymes [15,16].

Amongst the metal-tolerant PGPR, *Bacillus* species have been reported to increase plant growth in harsh environments [17]. The endospore-forming characteristic of the *Bacillus* species provides tolerance to pH extremes, heat, cold, heavy metals, pesticides, and manures [18]. Hence, targeting the *Bacillus* spp. from polluted soils and the plant rhizosphere is a desirable approach to finding useful plant growth-promoting bioresources in harsh conditions [19]. For example, Tirry et al. [20] reported that Cr (VI)-tolerant *Pseudomonas* sp. enhanced the growth of *Medicago sativa* by exhibiting PGPR characteristics. Similarly, Abbas et al. [21] concluded that Cd-tolerant *Acinetobacter* sp. SG-5 could improve the growth of maize cultivars by providing better antioxidant vigor as compared to non-inoculated plants.

Such PGPR strains could be further improved genetically and physiologically by various techniques. Ultraviolet mutagenesis is one of the classical and effective techniques that works on the random mutation basis by shaping cross-connects in DNA and pyrimidine dimerization [22]. These mutations bring changes in the synthesis of enzymes and subsequently microbial metabolite synthesis, and thus have practical applications in industry. In current research, an already characterized Cr-tolerant PGPR strain *Bacillus* sp. SR-2-1/1 was used for random mutagenesis through UV radiations. The strain was originally isolated and characterized in our laboratory and has been reported as a potential metal-tolerant PGPR [21,23,24]. We hypothesized that UV mutagenesis could further enhance the Cr-tolerance and PGPR potential of the bacterial strain. Hence, the objective of the current research was to enhance the Cr tolerance and PGPR traits of *Bacillus* sp. SR-2-1/1 through UV mutagenesis, and to evaluate the post-mutagenesis superiority of selected mutant(s).

2. Materials and Methods

2.1. Source of Bacterial Strain

Bacillus sp. strain SR-2-1/1 (KY315919) is a pre-characterized metal-tolerant PGPR that was isolated from wastewater-contaminated soil in our laboratory [25]. The glycerol stock of SR-2-1/1 was collected from −80 °C and streaked on nutrient-agar medium followed by repeated subculturing and morphological examination under a light microscope to confirm the purity.

2.2. Ultraviolet (UV) Mutagenesis

The UV mutagenesis of the selected strain was done by following the technique of [21] with some modifications. The selected bacterial strain was grown overnight at

28 °C in nutrient-agar plates. Petri dishes containing samples were exposed directly to a monochromatic UV lamp (254 nm) for different periods (0, 10, 20, 30, 40, 50, and 60 min). Each irradiated sample was incubated at 28 °C for 24 h. After incubation, morphologically different colonies from plates were picked and re-streaked on new nutrient-agar plates.

2.3. Screening of Mutants for Chromium Tolerance

The morphologically different colonies supposed to be mutants were tested for chromium (Cr) tolerance by adding different Cr concentrations (0, 50, 100, 200, 500, 1000, 1500 mg/L) into the growth media to find the minimum inhibition level. Purified strains were grown in different levels of chromium. The plates were incubated at above-mentioned temperature and tolerance was confirmed by visual examination of colony growth on plates. On the basis of maximum tolerance to Cr, two mutants (M_1 and M_2) were selected for further characterization.

2.4. Phosphate Solubilization

Bacterial mutants and wild-type strains were evaluated for mineral phosphate solubilization by spot-inoculation on Pikovskaya's agar media containing different chromium concentrations (0, 100, 200, 300 mg/L). The Petri plates were incubated at the above-mentioned conditions for the formation of halo zones indicating the phosphate-solubilizing ability of the isolates. The phosphate solubilization index was measured by the following formula: colony diameter + Halo-zone diameter/colony diameter.

2.5. Ammonia Production

Isolates (both mutants and wild-type SR-2-1/1) were inoculated in 4% (*w*/*v*) peptone broth medium with different Cr concentrations (0, 100, 200, 300 mg/L) and incubated at 28 ± 2 °C for 48 h in an orbital shaker at 150 rpm. After incubation, 0.5 mL of Nessler's reagent was added to the bacterial supernatant. The ammonia production was indicated by the production of a yellowish color. The color was quantified by a spectrophotometer at 450 nm. Measurements were carried out based on a standard curve drawn with series ammonium sulphate (Sigma-Aldrich, St. Louis, MO, USA) solutions.

2.6. ACC Deaminase Activity

The isolates (both mutants and wild-type SR-2-1/1) were screened for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity at different chromium concentrations (0, 100, 200, 300 mg/L) using ACC as a sole nitrogen source in DF salt minimal medium [26]. Cultures were grown for 24 h with shaking at 160 g and 28 ± 2 °C to stimulate ACC deaminase activity. Inoculated cultures were observed for their ability to utilize the compound ACC by comparing the culture turbidity with that of the non-inoculated control.

2.7. Molecular Identification of Bacterial Strains

The genomic DNA of the wild-type strain SR-2-1/1 and both the mutants M_1 and M_2 was isolated by using a commercial kit (PuroSPIN^{TM} Genomic DNA purification, Luna Nanotech, Toronto, ON, Canada following the manufacturer's protocol. The primers used to amplify the 16S rDNA genes from the extracted DNA and the complete protocol of mastermix and thermocycler conditions have already been described by Mahmood et al. [25]. The amplicons were purified by gel electrophoresis using a commercial kit (GeneJET Gel Extraction Kit, Thermo Fisher Scientific, Waltham, MA, USA). The purified genes were sequenced commercially by Macrogen, Seoul, Republic of Korea. The trimming of raw sequences, BLASTn analysis, and phylogenetic studies were conducted by the given methods and software packages.

2.8. Pot Experiment

A pot experiment was conducted using selected mutants (M_1 and M_2) and the wild-type strain SR-2-1/1 as inoculated treatments at different Cr concentrations (0 μM, 50 μM,

100 µM, 200 µM, and 300 µM). An un-inoculated control treatment was also kept for comparison. Selected mutants and wild-type strain SR-2-1/1 were grown in the nutrient broth medium for 24 h at 28 °C. The culture was centrifuged and diluted with sterilized distilled water to maintain the cell density at 1×10^8 CFU/mL. Seeds were surface-sterilized in 5% (*w*/*v*) sodium hypochlorite (NaClO) solution for five minutes followed by washing with sterilized distilled water. Sterile seeds were dipped in beakers with a culture of strain SR-2-1/1 wild-type or mutants for 30 min. The non-inoculated control seeds were dipped in sterile distilled water for the same period of time. Sterilized seeds were sown in pots (8 cm diameter) filled with potting soil (clay loam, NO_3 = 109 mg/L, P = 11 mg/L, organic carbon = 2.3% and pH = 6.4). Five seeds were sown in each pot and the experiment was done in three replicates. Pots were kept in a plant growth chamber (22 °C, light:dark = 16:8, humidity 65%) and different Cr levels (0, 50, 100, 200, 300 µM) were applied to pots in water. Hoagland solution and distilled water were given to plants alternatively to fulfill the nutrition and irrigation requirements of plants. Various growth parameters such as shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot length and shoot length of wheat plants were measured after one month. Bacterial density from the rhizosphere in terms of colony-forming units (CFU) was calculated after 10, 20 and 30 days of sowing.

2.9. Statistical Analysis

The data were analyzed through one-way ANOVA using Statistix software (version 9.0). The significance between the means of different treatments was compared by least significant difference tests at the 95% confidence level.

3. Results

3.1. Bacterial Survival Rate

It was found that the survival rate of the bacterial strain SR-2-1/1 was significantly reduced with an increase in time of irradiation (Figure 1). After one hour of irradiation, only a few colonies were able to survive and grow on the plates. Moreover, our selection criteria for mutants were based on morphological differences in bacterial colonies after irradiation. The colonies picked up as mutants belonged to the plates with 5% survival rate. Based on morphological differences and survival rates, eight mutants (M_1, M_2, M_3, M_4, M_5, M_6, M_7, and M_8) were selected for further characterization. The selected mutants were repeatedly streaked to maintain their purity.

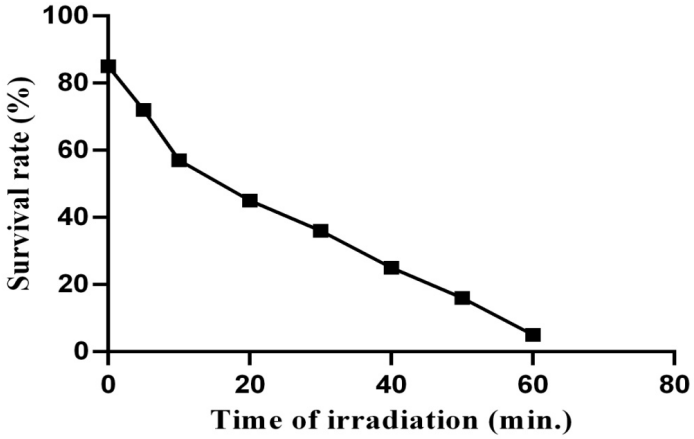

Figure 1. Survival rates of mutants after UV irradiation with the progression of time.

3.2. Minimum Inhibitory Concentration with Chromium (MIC)

In the MIC test to check the Cr tolerance of two mutants (M_1, M_2) including the wild-type SR-2-1/1 strain, the results revealed that the wild-type stain and mutant M_2 tolerated high Cr levels (Up to 500 mg/L), while the M_1 mutant was found resistant against

a 200 mg/L Cr concentration. However, the rest of the selected mutants failed to tolerate a Cr level beyond 20 mg/L (Table 1). Hence, M_1 and M_2 mutants were selected for further experimentation.

Table 1. Minimum inhibitory concentrations of wild-type and selected mutants of SR-2-1/1.

Isolates	Cr mg/L						
	0	**10**	**20**	**50**	**100**	**200**	**500**
W	+	+	+	+	+	+	+
M_1	+	+	+	+	+	+	−
M_2	+	+	+	+	+	+	+
M_3	+	+	−	−	−	−	−
M_4	+	+	−	−	−	−	−
M_5	+	+	−	−	−	−	−
M_6	+	+	−	−	−	−	−
M_7	+	+	+	−	−	−	−
M_8	+	+	−	−	−	−	−

Here, W = wild-type strain *Bacillus* sp. SR-2-1/1, M_1 = selected mutant 1, M_2 = selected mutant 2.

3.3. Comparative Study of Plant-Beneficial Traits

It was found that both mutant strains M_1 and M_2, as well as the wild-type strain, tolerated Cr up to 300 mg/L and rendered phosphate solubilization in Pikovskaya's agar medium. It seems that the UV irradiation increased the phosphate solubilization ability of both the selected mutants; however, a comparatively higher solubilization index was measured in mutant strain M_2, which was 5 at 0 mg/L, 4 at 100 mg/L, 1.8 at 200 mg/L and 1.3 at 300 mg/L (Table 2). A descending trend of the solubilization index is shown with increasing Cr concentrations. Similarly, the ammonia production of the mutants M_1 and M_2 was also higher as compared to the wild-type strain (Figure 2). Comparatively higher ammonia production was recorded in the M_2 mutant as compared with the M_1 and wild-type strain.

Table 2. Phosphate solubilization index of wild-type strain and its mutants.

Isolates	Colony Diameter (cm)	Zone Diameter (cm)	Solubilization Index
Cr 0 mg/L			
W	1 ± 0.008	1.4 ± 0.03	2.4 ± 0.2
M_1	0.6 ± 0.005	1.2 ± 0.01	3 ± 0.3
M_2	0.5 ± 0.004	2 ± 0.06	5 ± 0.4
Cr 100 mg/L			
W	1.2 ± 0.01	1 ± 0.008	1.8 ± 0.05
M_1	0.7 ± 0.006	1.1 ± 0.009	2.5 ± 0.2
M_2	0.4 ± 0.003	1.2 ± 0.01	4 ± 0.4
Cr 200 mg/L			
W	1.5 ± 0.03	0.2 ± 0.002	1.1 ± 0.009
M_1	1.3 ± 0.01	0.4 ± 0.003	1.3 ± 0.01
M_2	0.7 ± 0.006	0.6 ± 0.005	1.8 ± 0.05
Cr 300 mg/L			
W	1.7 ± 0.02	0.1 ± 0.001	0.9 ± 0.012
M_1	1.2 ± 0.02	0.3 ± 0.002	1.1 ± 0.003
M_2	0.9 ± 0.02	0.4 ± 0.004	1.3 ± 0.001

W = wild-type strain *Bacillus* sp. SR-2-1/1, M_1 = selected mutant 1, M_2 = selected mutant 2.

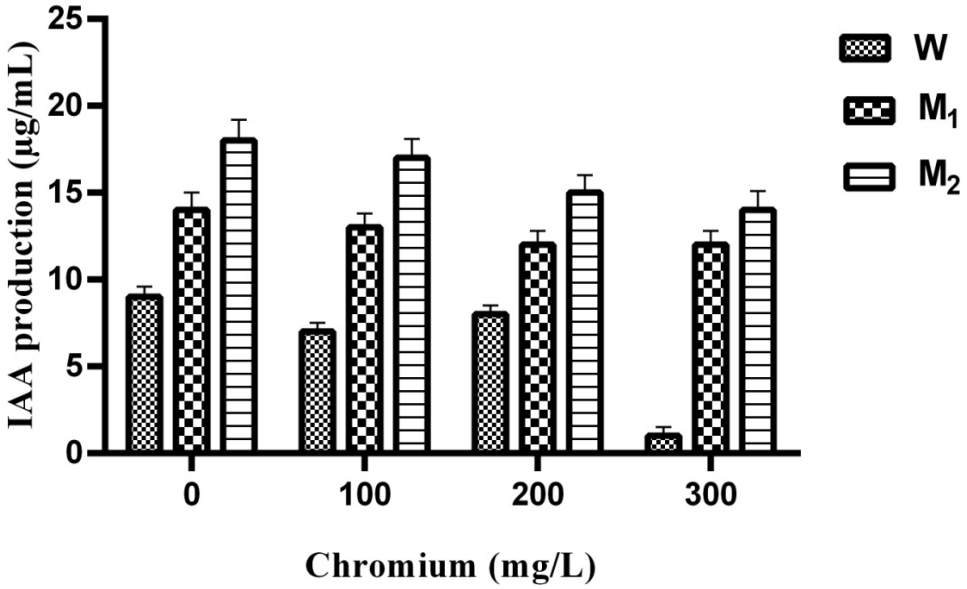

Figure 2. Ammonia production by wild type SR-2-1/1 and its mutants at different levels of Cr. W = wild-type strain *Bacillus* sp. SR-2-1/1, M_1 = selected mutant 1, M_2 = selected mutant 2.

Moreover, similar to the phosphate solubilization, a decreasing trend of ammonia production was measured with increasing concentrations of Cr. Interestingly, at the 300 mg/L Cr concentration, the ammonia production in the wild-type strain was almost negligible; however, both the mutant strains produced a considerable amount of ammonia at this Cr level. Qualitatively, the wild-type strain *Bacillus* sp. SR-2-1/1 was found to be positive for ACC deaminase activity up to a 300 mg/L Cr concentration; however, the ability to use ACC in the culture medium was completely lost in the mutant strains M_1 and M_2. The ACC deaminase activity was not recorded in the mutant strains even in medium without the addition of Cr.

3.4. Molecular Identification of Bacterial Strains

In the phylogenetic analysis of the 16S rRNA gene sequence, the wild-type strain SR-2-1/1 and both the mutants M_1 and M_2 shared the same clade, and this clade clustered itself with the *Bacillus cereus* strain NCDO 1769^T (X60646). Thus, we confirm that the taxonomic identity of the wild-type and mutant strains was not changed after UV irradiation (Figure 3).

3.5. Bacterial Survival in the Rhizosphere

It was found that the wild-type strain SR-2-1/1 and both the mutants were able to survive in the rhizosphere of wheat plants at an optimum population density (approximately 7–7.5 Log CFU/mL of rhizosphere soil) even up to the 300 µM Cr concentration. More interestingly, this level of population density was retained by the strain SR-2-1/1 and the mutant strains up to 30 days after sowing (Figure 4). Compared to the population density of wild-type SR-2-1/1 and mutant strains, the indigenous soil bacteria of control pots were not able to survive, and their population density was significantly dropped from approximately 7 to 4 Log CFU/mL of rhizosphere soil at a 300 µM Cr concentration at all intervals of measurement (10, 20 and 30 days after sowing).

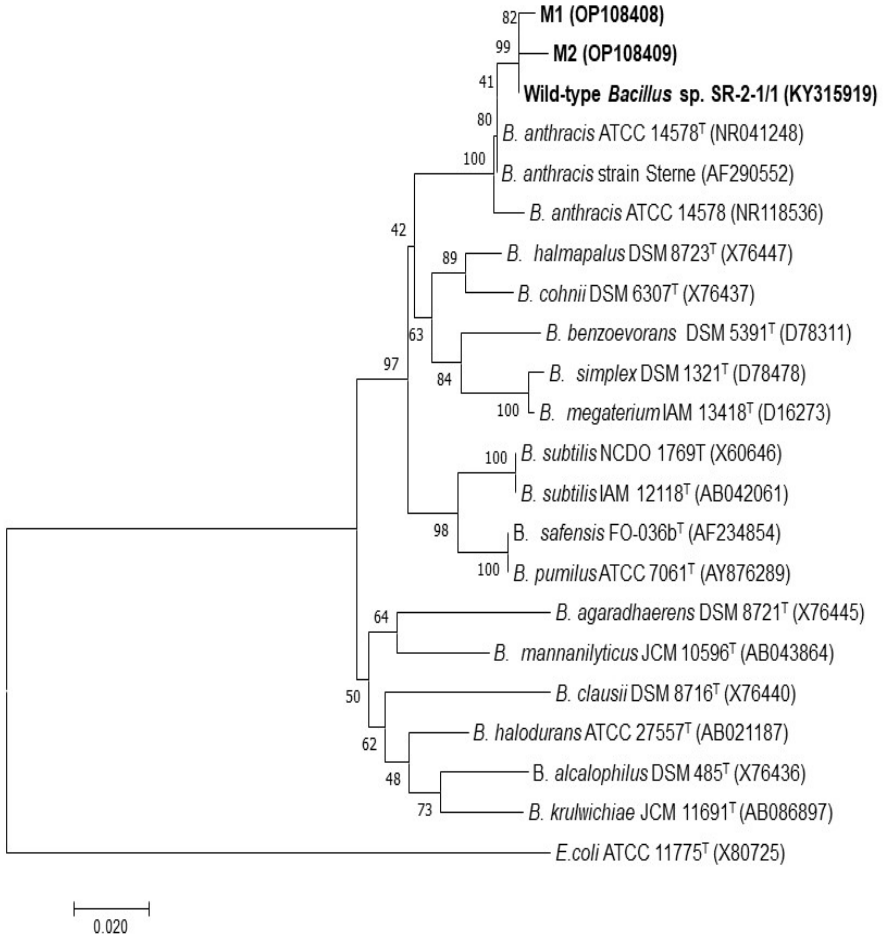

Figure 3. Phylogenetic tree of wild-type *Bacillus* sp. SR-2-1/1 and mutants M_1 and M_2 with the type strains of genus *Bacillus*. The evolutionary history was inferred using the Neighbor-Joining method. The percentages (50%) of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown. The evolutionary distances were computed using the Maximum Composite Likelihood method, and are in units of the number of base substitutions per site.

3.6. Growth Measurement

The results of growth parameters measured after 30 days show that the wild-type strain SR-2-1/1 and both the mutant strains M_1 and M_2 had significantly ($p \leq 0.05$) higher root and shoot lengths and root and shoot fresh and dry weights as compared to non-inoculated control wheat plants at all the Cr concentrations. However, when we compared the wild-type strain with the mutants, it was computed that the M_2 strain had significantly higher root and shoot lengths, shoot fresh and dry weights and root fresh weights as compared to the strain M_1 and the wild-type strain SR-2-1/1 at all Cr concentrations. However, the root dry weight of strain M_2 was found statistically on a par with the wild-type strain SR-2-1/1 and the strain M_1 at 0, 50 and 100 µM Cr concentrations, while at a Cr concentration of 200 and 300 µM, the root dry weight of strain M_2 was found to be significantly higher than the wild-type strain SR-2-1/1 and the strain M_1 (Figure 5).

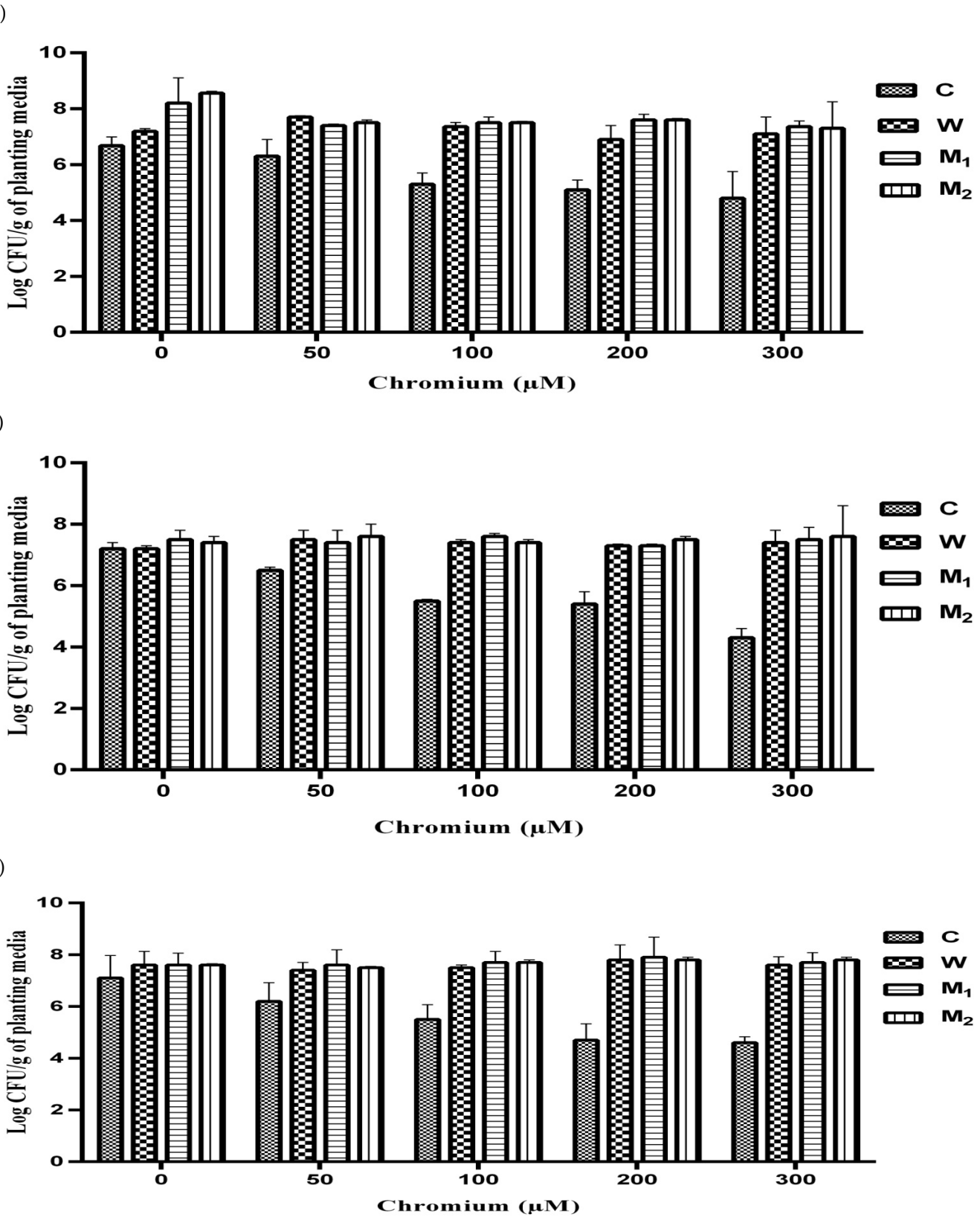

Figure 4. Bacterial population density count in the rhizosphere of wheat plants inoculated with wild-type and selected mutants under different Cr concentrations (**A**) 10 days after sowing, (**B**) 20 days after sowing, (**C**) 30 days after sowing. W = wild-type strain *Bacillus* sp. SR-2-1/1, M_1 = selected mutant 1, M_2 = selected mutant 2, C = control.

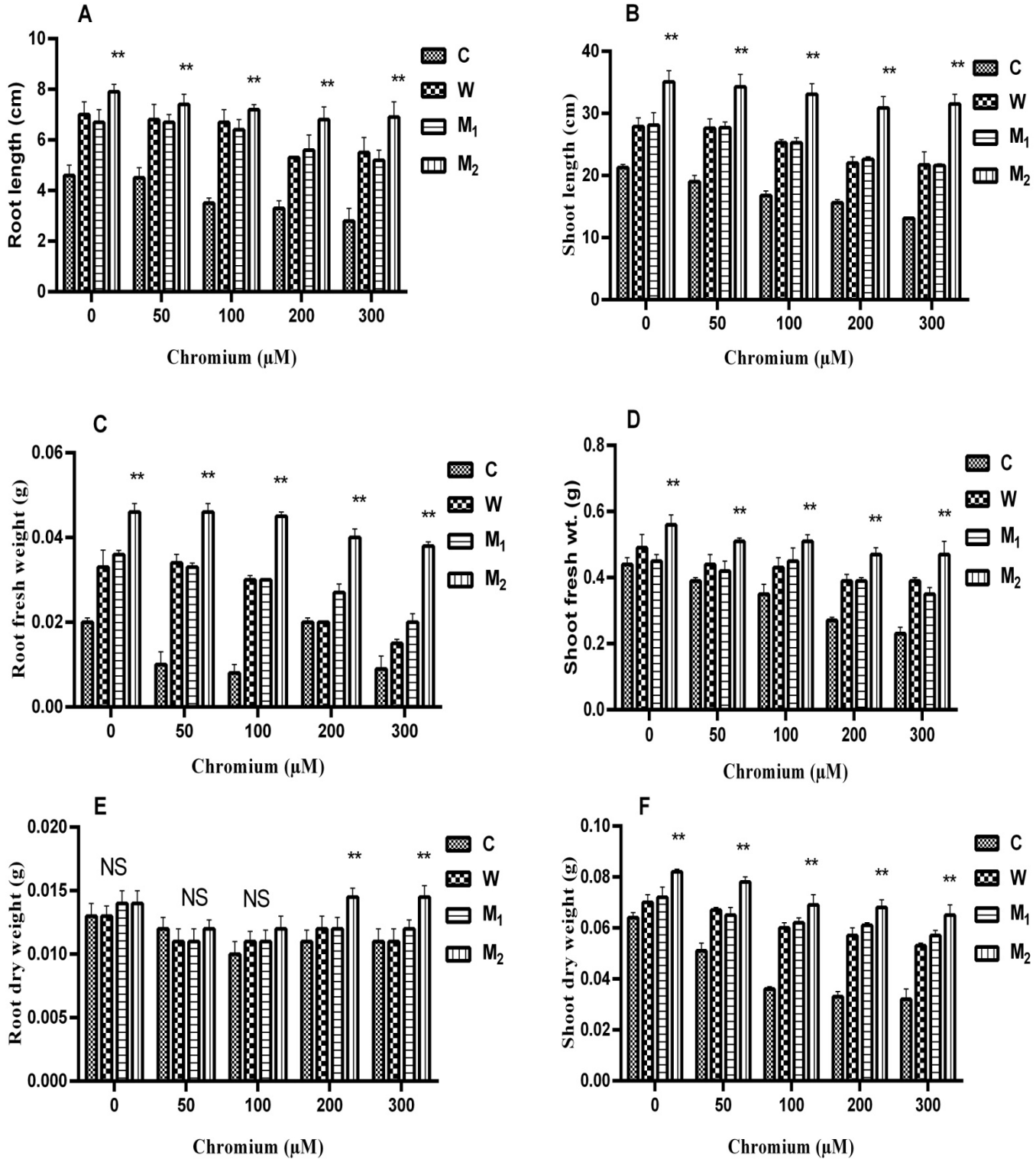

Figure 5. Measurement of the growth parameters of wheat plants: (**A**) root length, (**B**) shoot length, (**C**) root fresh weight, (**D**) shoot fresh weight, (**E**) root dry weight, (**F**) shoot dry weight. Each value is the mean of three replicates and the values differing significantly are represented by **, while those having no significant difference are denoted as NS. Here, W = wild-type strain *Bacillus* sp. SR-2-1/1, M_1 = selected mutant 1, M_2 = selected mutant 2, C = control.

4. Discussion

The current research investigated ultraviolet mutagenesis as a tool to create mutations in a pre-characterized metal-tolerant PGPR strain (*Bacillus* sp. SR-2-1/1) isolated, reported and preserved in our laboratory. The strain was chosen for its bioremediation potential together with PGPR characteristics reported in our previous study [25–27]. The current study hypothesized increased bioremediation and PGPR benefits of the strain SR-2-1/1 after UV mutagenesis, and sought to translate these benefits into the physiological competence and plant growth of wheat under harsh conditions. We observed that the survival rate of the bacterial strain after UV irradiation was inversely proportional to the time of irradiation, and the mutant colonies selected for further characterization belonged to the plates with only 5% survival rates to ensure the possibility of maximum mutagenic alternations in the genome of strain SR-2-1/1 (Figure 1). UV radiation causing mutagenic effects and minimizing bacterial survival is a well-established fact [28]. Additionally, random mutagenesis based on UV irradiation has been reported to increase bioremediation potential in bacterial species [29]. Out of the eight mutants that were selected, only two (M_1 and M_2) were able to tolerate the Cr level of 200 mg/L, while one, namely, M_2, survived at a Cr concentration of 500 mg/L (Table 1).

Hence, these two mutants were selected for further experimentation. Compared to the wild-type strain, both the mutants were able enhance the solubilization of inorganic phosphates in the agar media, which implies that the mutagenesis resulted in an increase in the physiological competence of mutants M_1 and M_2 in terms of their phosphate solubilization potential. On the other hand, the descending trend of phosphate solubilization with ascending Cr concentrations implies that elevated Cr stress proved lethal for bacterial cells, and restrained them from using calcium bicarbonate in the culture medium. Our results are corroborated by an earlier study conducted by Achal et al. [30], in which it was stated that the UV mutation of *Aspergillus tubingensis* increased its phosphate solubilization. Many PGPR strains with a phosphate solubilization ability are also reported to cause auxin production [31,32]; however, in the previous study [25], the wild-type strain SR-2-1/1 did not produce IAA in the culture media, and the same findings were reproduced in the current study both in the case of wild-type and mutant strains. The UV mutagenesis also enhanced the ammonia production of both the mutants M_1 and M_2 as compared to the wild-type strain up to 300 mg/L. The maximum increase was calculated in the mutant strain M_2, which was 77%, 58%, 87% and 704% greater than the wild-type strain *Bacillus* sp. SR-2-1/1 at 0, 100, 200 and 300 mg/L Cr concentration, respectively. In an earlier study [33], Ai-xiang et al. (2017) reported a 12% increase in the ammonia production of *Providencia* sp. JAT-1 after UV-mutagenesis. Our results on ammonia production are comparable to those reported by Lindquest et al. [34] when they employed a yeast strain *Yarrowia lipolytica* for UV mutagenesis. Our assumption is that the mutant strains were used to utilize the proteins in the culture medium more effectively than the wild-type strain, hence triggering the metabolic pathways for ammonia production.

Plant growth-promoting rhizobacteria with the ACC-deaminase enzyme could improve plant development under stress conditions [28]. Surprisingly, the wild-type strain SR-2-1/1 was found positive for ACC deaminase production up to 300 mg/L Cr stress in the culture medium; however, the ability to utilize ACC as the sole nitrogen source in both the selected mutant strains was absent. The reason for the loss of ACC deaminase activity in mutant strains might be the mutation of core genes responsible for coding ACC deaminase. The core gene responsible for ACC deaminase production is AcdS; however, some other regulatory genes are responsible for the synthesis of ACC deaminase, and mutations in any of the core and regulatory elements could result in the loss of ACC deaminase activity [35,36]. Researchers reported polymorphism in the DNA fingerprints between wild-type and mutant strains after being subjected to mutagenic techniques [37]. Hence, re-validating the taxonomic positions of bacterial mutants becomes important. Therefore, we re-sequenced the 16S rRNA genes of wild-type and mutant strains to verify their taxonomic classification after UV-mutation. After BLASTn and phylogenetic analyses

of wild-type and mutant strains, it was concluded that the taxonomic genera of wild-type and mutant strains were *Bacillus*, and they grouped together in the same clade (Figure 3).

In the pot experiment, the wild-type strain SR-2-1/1 and both the mutant strains M_1 and M_2 survived in the rhizosphere of wheat plants at a population density of approximately 7-7.5 Log CFU/mL of rhizosphere soil, even up to the 300 µM Cr concentration. It has been reported in earlier studies that the PGPR strains colonize plant roots and sometimes enter into the root epidermal cells, and this colonization is essential for plant growth and survival [32]. Our findings related to the rhizosphere colonization competence of mutant strains was further validated by the drop in population density from approximately 7 to 4 log CFU/mL of the rhizosphere soil at a 300 µM Cr concentration at all intervals of measurement in the non-inoculated control treatment. This implies that the indigenous microflora present in control pots failed to survive at high Cr stress levels; however, our wild-type strain as well as the mutants not only survived in the rhizosphere of wheat plants, but also showed growth enhancement. Researchers have already transformed the rhizosphere competence of the PGPR strain into the better growth of underground and aerial parts of the plants [37]. This was further validated when higher root and shoot lengths, as well as fresh and dry weights, were recorded in the plants inoculated with wild-type strain SR-2-1/1 and its resulting mutants. The highest biomass in wheat plants treated with the M_2 mutant was linked to the greater phosphate solubilization, Cr tolerance and ammonia production of this mutant strain as compared to mutant M_1 and the wild-type strain. There is literature available on the enhancement of the metabolic potential of bacterial strains to synthesize valued industrial products using mutagenic tools [34]; however, only a few reports have documented the use of mutated bacterial strains as PGPR in greenhouse experiments for plant growth enhancement or to combat plant pathogens [38]. Hence, the current research adds value to using conventional UV mutagenesis for improving the PGPR characteristics of bacterial strains.

5. Conclusions

A study that attempted to further improve the PGPR potential of a pre-characterized metal-tolerant *Bacillus* sp. SR-2-1/1 resulted in promising results in terms of improving its phosphate solubilization and ammonia production ability; however, the ACC deaminase activity was completely lost in mutants strains. The resulting mutant strains M_1 and M_2 were proven effective as wheat rhizosphere colonizers and were able to improve the wheat's above- and below-ground growth more effectively than the wild-type strain.

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