

Physiological and growth responses of two wheat (*Triticum aestivum* L.) varieties inoculated with a new strain of *Bacillus siamensis* under Cadmium (Cd) stress

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Abstract

Bioavailability of cadmium (Cd) metal in the soils due to scarcity of good quality water and industrial waste could be the major limiting factors negatively influencing the growth and yield of crops needs prompt solution to fulfil the requirement of food for increasing world population. In the recent time, variable range of plant growth promoting rhizobacteria (PGPR) are being used on large scale in agriculture to reduce the risk of abiotic stresses on plants and increase crop productivity. Among them, the *Bacillus siamensis* has a huge potential to enhance the plant tolerance against abiotic stress but limited evidences are reported about the putative role of *B.s* in crop plants under heavy metal stress. The current study was aimed to investigate the potential of a new metal tolerant strain of *B.s* on two wheat (*Triticum aestivum* L.) varieties (NARC-2009 and NARC-2011) grown in Cd contaminated soil at different treatments i.e Cd (0, 20, 30 and 50 ppm) and Cd (0, 20, 30 and 50 ppm) + *B.s*. Our results depicted that Cd stress decreased the wheat growth related attributes, biomass, and photosynthetic parameters (Chlorophyll a, b and a + b) which increased in both wheat varieties upon inoculation with *B.s*. Moreover, Cd stress caused significant membrane damage and negatively affected the water content, water potential, and osmotic potential of leaf. However, PGPR considerably increased the soluble sugars to reduce the Cd toxicity. Overall, the plants inoculated with *B.s* enhanced their tolerance index of root and shoot and found better in NARC-2009 than NARC-2011. Therefore, microorganisms efficiently increase the plant growth by reducing the metal toxicity.

1 Introduction

Agriculture is considered as the big source of economy and basic livelihood of people in several countries of the world (Mishra et al. 2014). Agriculture as a pillar in food industry is estimated to provide food for world's increasing population (FAO and <http://faostat.fao.org/>). The global agricultural regions including cereals especially wheat are facing a wide spectrum of challenges, such as biotic and abiotic stresses under normal condition (Rizwan et al. 2016a). Among different type of environmental stresses, the heavy metal stress is getting more focus and becoming a serious environmental issue from last few decades (Hussain et al. 2018). The cadmium (Cd) is thought to be very toxic entity, non-biodegradable, bio-accumulative and a major wheat yield limiting factor (Shoeva and Khlestkina 2018). Cd enters the environment via geogenic and anthropogenic sources such as fertilizer, sewage slough dispersal, industrial waste, electroplating and atmospheric deposition (Rizwan et al. 2018). The Cd firstly accumulated by root directly from the soil and caused reduction in root length, then transferred to aerial parts where it reduced the photosynthesis and resulted in stunted growth and reduced yield (Rizwan et al. 2017). The Cd led to the excessive production of ROS caused oxidative damage and negatively affected the antioxidant defence system of plants (Hussain et al. 2018).

The Cd has high mobility and bioavailability, and enters the food chain via consumption of different vegetables, cereals and cereal grains obtained from cd contaminated soil due to its efficient mobility and bioavailability (Rizwan et al. 2016b). The wheat (*Triticum aestivum* L.) is utilized as staple food by more than 50% population of the world and an important cereal crop worldwide (FAO and

<http://faostat.fao.org/>). The demand for food from wheat is increasing globally day by day and requirement of wheat to feed the increasing population is getting more attention (Curtis and Halford 2014). Wheat has greater potential to accumulate Cd in its various parts as compared to other cereals, resulting the higher Cd compartmentalization in wheat (Naeem et al. 2016). However, the accumulation of Cd varies with wheat cultivars, type of soil and soil contamination level. But the uptake and transfer of Cd from root to shoot depends upon the xylem and phloem loading (Harris and Taylor 2013). Therefore, it is extremely important to reduce the intake and transfer of Cd to aerial parts which is an ultimate risk to humans and other living organisms that consume wheat (Keller et al. 2015).

Plant growth promoting rhizobacteria (PGPR) increase the root development which reflects the accumulation of more water and essential nutrients to a suitable concentration and consequently improve the plant growth by enhancing the photosynthetic apparatus efficiency, linked with chlorophyll concentration and PSII functionality (Mesa-Marín et al. 2018). PGPR are currently used to immobilize and resist the metal toxicity and improve the plant growth by reducing the heavy metal uptake and accumulation within plants (Mallick et al. 2018). PGPR increase the plant growth by restricting the heavy metal accumulation in roots and stopping its transfer toward aerial parts through shoot (Mesa et al. 2015). The higher uptake of heavy metals negatively impacted the photosynthetic carbon consumption during respiration by altering the mitochondrial and electron transport chain configuration however, inoculation with PGPR recovered the plant metabolism by limited translocation of metals in the roots of plants (Mesa-Marín et al. 2018). Previously, it has been reported that *Bacillus megaterium* limited the intake and transfer of Ni and improved the growth of *Sorghum halepense*, *Luffa cylindrica* and *Brassica juncea* (Rajkumar et al. 2013). *Neorhizobium huautlense* considerably increased the growth and biomass production of Chinese cabbage and radish by reducing the uptake of Cd and lead (Pb) (Wang et al. 2016). Enterobacter species has ameliorated the growth of rice seedlings with respect to germination potential, biomass and chlorophyll contents by reducing the Cd stress *in vitro* (Pramanik et al. 2018). Moreover, PGPR provide better resistance to heavy metal infected sites in plants by the synthesis of plant hormones such as indol acetic acid (IAA) and gibberellins. These also facilitate the production of siderophores and solubilising phosphate that increase the plant growth and physiological profile by minimizing the translocation of heavy metals within plants (Gupta et al. 2018).

However, it is needed to reduce the harmful impact of heavy metals especially Cd in crop plants while stimulating the plant growth. The present study explored the advantageous role of seed inoculation with *Bacillus siamensis* strain in two wheat varieties with respect to their growth, photosynthetic attributes, biomass and water status of leaf tissues in Cd contaminated soil. To the best of our knowledge, this is the first report which describes the efficient role of the new strain of *Bacillus siamensis* against Cd stress in wheat plants. This study may provide new strategies to increase cereal crop production by ameliorating heavy metal toxicity in plants with application of PGPR.

2 Materials And Methods

2.1 Pot Experiment

A pot experiment was performed in the greenhouse of the department of botany, Arid agriculture university, rawalpindi under natural conditions at 28/20 °C day and night temperature with 65 ± 6% relative humidity. The seeds of two wheat (*Triticum aestivum* L.) varieties (NARC-2009 and NARC-2011) were obtained from National Agriculture Research Centre (NARC) Islamabad. The seeds were surface sterilized with sodium hypochloride (2.6% active chloride) for three mins then properly washed with double distilled water. Afterwards, the half of the seeds of each wheat variety were inoculated with *Bacillus siamensis* (strain no. MH559649 obtained from the department of botany with adjusted concentration of bacteria at 1.2×10^8 cells/ml) for 24 hours at room temperature. Then the PGPR inoculated wheat seeds were air dried. The PGPR inoculated seeds were sown in twenty four (24) pots and rest of twenty (24) pots had un-inoculated seeds (pre-treated with distilled water over night). Eight seeds of each wheat variety were sown in each plastic pot containing 5 kg air-dried loamy soil (1:3) of sand and silt respectively. The soil analysis has given in Table.1. Before sowing the seeds, soil was subjected to Cd stress ($\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$) as (Cd-0, Cd-20, Cd-30 and Cd-50 mg/kg soil) and the remaining four treatments as given as (Cd-0, Cd-20, Cd-30 and Cd-50 mg/kg soil) + PGPR (*Bacillus siamensis*) that gave total of 8 treatments. Field capacity was maintained at 70% throughout the experiment. After 10 days of sowing, wheat plants of each pot were subjected to thinning and five wheat plants were kept in each pot and the experiment was carried out with three replicates of each treatment in a completely randomized design (CRD). Throughout the experimental period, wheat plants were protected under greenhouse to avoid rain. At 30 days after sowing (DAS), the plants were collected for further analysis.

2.2 Growth parameters

At 30 DAS, immediately after plant harvesting, the length of root and shoot, leaf area and fresh weight were measured by using meter rod and electrical balance. These parameters were recorded for each pot and the mean values were determined in triplicate. After that remove the contamination by washing plant roots with distilled water and oven dried at 70 °C to measure constant dry weights and weighed (Rizwan et al. 2019).

2.3 Measurement of Chlorophyll contents

For chlorophyll content, the fresh leaf samples were extracted with 85% v/v acetone at 4 °C for 24 hours under dark conditions. Afterwards, the ready sample's wavelength was measured at 470, 647 and 664 nm by using a spectrophotometer. The chlorophyll contents were calculated according to method described by (Lichtenthaler 1987).

2.4 Determination of total soluble sugars content

The leaf tissues were taken into 10 ml centrifuge tube with 80% ethanol (5 ml). The reaction mixture was incubated in water bath with shaking for 30 min at 80 °C and centrifuged for 5 min at 4000 rpm to get the supernatants. Pellets were treated with 80% ethanol for two more extractions. Supernatants were collected and diluted with 80% ethanol and mixed to form whole volume upto 25 ml, and kept at -20 °C for further analysis. The total soluble sugars were measured by following the method of (Seifter et al. 1950).

2.5 Determination of Membrane Stability Index (MSI)

The leaf from each sample was cut into small pieces (100 mg) and washed with double distilled water. Afterwards, leaf pieces were inserted in test tubes and placed in a water bath at 40°C for 30 min. Then, (C_1) electric conductivity was measured by using EC meter. Again the samples were placed in a water bath at 100°C for ten mins and electric conductivity (C_2) was measured. the MSI was calculated according to formula given by (Sairam et al. 2005).

Membrane stability index = $[1 - C_1 / C_2] \times 100$

2.6 Determination of Osmotic and Water Potential

To calculate the osmotic potential and water potential under water deficit conditions, the pressure chamber was utilized with pressure measurement value of 6.0 MPa (Turner and Begg 1981). A fully expanded leaf was taken to determine the osmotic potential with vapour pressure osmometer. Osmotic potential was obtained by measuring the difference at 100% relative water content with water scarcity relative water content. According to the (Turner 1986), the equation was used to calculate osmotic potential;

$OP_{100} = OP \text{ (RWC-Assumption of apoplastic water)}/100 - \text{Assumption of apoplastic Water}$

OP stands for osmotic potential and RWC stands for the relative water content of the leaf.

2.7 Relative water content and tolerance index of root and Shoot

To measure the leaf relative water content (LRWC), the procedure given by (Turk and Erdal 2015) was used. Immediate after harvesting the plants, fresh weight (FW) of seventh leaf of wheat plant was calculated. Then leaf was cut into segments and dipped in distilled water over night to get the turgid weight (TW). Afterwards, the samples were subjected to an oven at 70C and measured the dry weight (DW). The RWC was measured according to given formula as;

$RWC = [(FW - DW) / (TW - DW)] \times 100.$

To find out the tolerance index of root and shoot, the formula of (Turner and Marshall 1972) was used as given below;

$$\textit{Tolerance Index} = \frac{\textit{Mean length in Cd solution}}{\textit{Mean length in control}}$$

2.8 Statistical analysis

The analysis of data was accomplished by using SPSS. The significance of data was analysed with one-way analysis of variance ANOVA. All values are given as mean of three replicates. The 5% level of probability was used to compare the mean with least significance difference (LSD) test.

3 Results

3.1 Plant morphological traits and leaf area

The results of current study depicted that inoculation with *B.s* positively improved the growth of both wheat varieties grown in Cd contaminated soil (**Figure.1 and 2**). At 30 DAS, the plants inoculation with *B.s* significantly increased the morphological traits such as length of root, shoot and leaf area as compared to non-treated plants. In NARC-2009, the root length and shoot length were increased by 15% and 13% at *B.s* alone, over the control, respectively. While on the other hand, Cd treatment decreased the root length by 54% and shoot length by 35%, at the highest level (50 ppm) over the control (Figure.1A and B). In contrast to NARC-2009, the NARC-2011 decreased the root- and shoot length by 55% and 43% at 50 ppm Cd over the control. While *B.s* improved the root length by 11% and shoot length by 7% over the control, in NARC-2011. Pre-treatment with *B.s* increased the leaf area by 12% and 14% in NARC-2009 and NARC-2011 over the control of both varieties, respectively. Conversely, the Cd application reduced the leaf area by 10% and 9% at 50 ppm Cd as compared to control of NARC-2009 and NARC-2011 respectively (Figure.2A). In both wheat varieties all tested levels of cadmium considerably decreased the morphological traits however, the application of *B.s* mitigated the effect of Cd and improved the wheat growth. Similar trend was notice with respect to membrane stability index in both wheat varieties treated with Cd stress. The maximum decrease in MSI was 34% and 37% in NARC-2009 and NARC-2011 at the highest level Cd-50 ppm whereas, *B.s* ameliorated the injury caused by Cd toxicity and improved the MSI by 11% and 7% respectively, over the control (Figure.2B).

3.2 Total biomass accumulation

Different levels of Cd negatively impacted the total biomass accumulation however, the application of *B.s* lessened the harmful effects of Cd and enhanced the biomass production when compared with non-treated plants in both wheat varieties (Figure.3). Cd treatment at 50 ppm, decreased the total biomass (fresh and dry) accumulation by 35% and 40% in the plants of NARC-2009, and 33% and 45% in NARC-2011 over the control of both varieties, respectively at 30 DAS. In contrast, the highest biomass (fresh and dry) accumulation was 15% and 49% in the plants of NARC-2009 treated with *B.s* alone over the control (Figure.3A and B).

However, in NARC-2011, the maximum accumulation of biomass (fresh and dry) was 17% and 37% in the plants inoculated with the *B.s* over the control. On contrary, at highest level of Cd-50 ppm, the maximum reduction in fresh and dry biomass was 33% and 45% over the control, respectively. In addition, the NARC-2009 significantly enhanced the total biomass accumulation as compared to NARC-2011, inoculated with *B.s* and grown in Cd contaminated soil.

3.3 Chlorophyll content

The plants inoculation with *B.s* enhanced the chlorophyll contents and improved the MSI of both wheat varieties grown in Cd contaminated soil (Figure.4). In NARC-2009, the Cd treatment at 20, 30 and 50 ppm decreased the chlorophyll a (Chl a) by 25%, 27%, and 50%, chlorophyll b (Chl b) by 18%, 39% and 56%, and chlorophyll a + b (Chl a + b) concentrations by 22%, 32% and 53% over the control, respectively. However, the plants pre-treated with *B.s* increased the chl a, chl b and Chl a + b concentrations by 2%, 14% and 7% over the control, respectively (Figure.4A, B and C). Whereas, the Chl a: b ration was decreased in *B.s* treatment as compared with all Cd treated levels (Figure.4D). However, more decrease in Chl contents were observed in NARC-2011 due to Cd toxicity as compared to NARC-2009. Results showed that 28%, 45%, 48% decrease in Chl a, 28%, 47%, 65% in Chl b and 27%, 46%, 55% in Chl a + b was noticed in plants exposed to Cd at 20, 30 and 50 ppm over the control. In addition, *B.s* application improved the Chl a, b and a + b contents by 8%, 17%, 12% over the control.

3.4 Total soluble sugars

The Cd treatment negatively affected the total soluble sugars in both wheat varieties at all levels however, the application of *B.s* enhanced the soluble sugars and improved the plant growth significantly (Figure.5). In the wheat plants, the maximum reduction in soluble sugars was 35% and 32% at Cd-50 ppm while the maximum production was 13% and 14% when plants were inoculated with *B.s* for NARC-2009 and NARC-2011, respectively.

3.5 Determination of Water Potential and Osmotic potential

The Cd at different levels of drastically effected the water potential and osmotic potential however, the seed inoculation with *B.s* positively impacted the water and osmotic potential in wheat as compared to non-treated plants in both varieties (Figure.6). The Cd at highest level 50 ppm the values for potential and osmotic potential were 3.03 and 4.94 -MPa whereas, the application of *B.s* improved the water potential and osmotic potential as 0.95 and 2.7 -MPa, respectively in NARC-2009. Similar results were observed in NARC-2011, the Cd at 50 ppm negatively affected the water and osmotic potential as 3.15 and 5.09 - MPa, improved with *B.s* application as 1.64 and 3.23 -MPa respectively (Figure.6A and B).

3.6 Leaf Relative water content (LRWC) and tolerance index

The application of *B.s* positively impacted the LRWC and tolerance index of both wheat varieties under Cd stress (Figure.7 and 8). The maximum increase in LEWC was noted 94% in NARC-2009 and 90% in NARC-2011 after inoculation with *B.s* alone. In contrast, the maximum reduction in LRWC was recorded at the highest level of Cd-50 ppm. At highest level of Cd-50 ppm, the LRWC was noted as 70% and 65% in NARC-2009 and NARC-2011, respectively. Moreover, the tolerance index was found more in NARC-2009 as compared to NARC-2011 which is shown in the (Figure.8A and B) as root and shoot tolerance index separately.

4 Discussion

The current study depicted that Cd treatment impaired the growth of wheat plants with respect to all morphological traits and Cd had severe impact at highest level of Cd-50 ppm (**Figure.1 and 2**). In contrast, the seed inoculation with *B.s* positively affected the plant growth profile exposed to Cd stress. The detailed molecular mechanism of Cd toxicity is poorly understood yet however, few researchers explained the damaging effects that Cd may destroy the soil microbial communities, reduce the water and nutrients uptake, and impair the cell division and elongation process ultimately decrease the crop growth (Khanna et al. 2019). Our results reaffirm the findings of (Ahmad et al. 2015), who described a significant reduction in root and shoot length of *B.juncea* exposed to Cd stress. In addition, the limited growth of root and shoot and leaf chlorosis on exposure to heavy metals has been suggested in previous studies (Hussain et al. 2019). The decline in root and shoot elongation is directly associated with the inhibition of root and shoot metabolism and ultimately affected the overall plant growth (Khanna et al. 2019). Seed inoculation with microbial strain (PGPR) considerably improved the wheat growth in the current study (**Figure.1**). Microorganisms facilitate the plant growth and development, and increase the supply of phosphate through siderophores formation, root hairs growth, and hormonal stimulation that reduce the heavy metal translocation (Gupta et al. 2018). PGPR induces changes in metabolic activities involve in solubilization and mineralization of organic phosphorous. These metabolic activities helps in the efflux of proton and other various anions, and then phosphatase enzymes release that enables the hydrolysis and mineralization of phosphorus (Ahemad and Kibret 2014). (Liu et al. 2018) reported the microbial treatment enhanced the growth of maize plant under Cd stress.

Moreover, the Cd stress declined the biomass (fresh and dry) accumulation in both wheat varieties as shown in (**Figure.3**). Similar findings were achieved by (Verma et al. 2008) in *B. juncea* under Cd stress. A significant decrease in the plant biomass under Cd stress could be due to its harmful impact on root and root hair development, essential nutrient uptake via roots, chlorophyll biosynthesis in leaf, photosynthesis, less water and more Cd accumulation in different organs of plants (Qadir et al. 2014). The total biomass accumulation and distribution were observed to be decreased in Russian knapweed (Rasouli-Sadaghiani et al. 2019) and *M lupulina* (Jian et al. 2019) due to severe oxidative stress and root damage caused by Cd stress. On contrary, our results showed that *B.s* enhanced the total biomass in both wheat varieties. Therefore, the increased plant growth and biomass production, and distribution is directly correlated with PGPR applications in *Eruca sativa* under Cd stress (Kamran et al. 2015). (Treesubstorn et al. 2018) reaffirms our study, who described that *B. subtilis* and *B. cereus* increased root and shoot biomass when inoculated to *O. sativa* exposed to Cd toxicity. The possible explanation for increasing biomass accumulation and distribution could be the solubilization of organic minerals from soil towards plants organs, phytoremediation of heavy metals, metal resistance ability of PGPR and the regulation of hormonal production require for plant resistant to heavy metals (Jian et al. 2019; Khanna et al. 2019). Therefore, the PGPR have widely being used to improve the plant growth under various types of environmental stresses.

Chlorophyll as a major component of chloroplast is efficiently associated with plant photosynthetic ability whereas, Cd and rest of the heavy metals negatively affected the chlorophylls and caused chlorosis in leaves (Rizwan et al. 2016a). Several types of heavy metals such as Cd, Zn, Cu, Hg and Pb

induce toxicity to cell wall and thylakoid membrane integrity which lead to the inhibition of enzymes i.e Rubisco, chlorophyll synthase, involved in the synthesis of chlorophyll and resulting in the degradation of chlorophylls (Hashem 2014). (Rascio et al. 2008) stayed with our results who reported that Cd reduced the Chl a, b and a + b content in rice. The photosynthates produced by plants with the help of chlorophyll directly linked with increase in plant biomass production whereas, decline in chlorophylls leads to the lower biomass production influenced by Cd stress (Khanna et al. 2019). The increase in nitrogen content an important molecule of chlorophyll structure, was observed in PGPR inoculated *M.lupulina* which is associated with more production of plant biomass under heavy metal stress (Jian et al. 2019). Moreover, the Cd stress severely affected the membrane permeability and enhanced the protein degradation in *B.juncea* (Ahmad et al. 2015). However, our results are in line with (Pramanik et al. 2018) who revealed that Enterobacter species has stimulated the growth and improved the chlorophyll content in *Oryza sativa* seedlings by reducing the toxicity of Cd stress.

In our study, the Cd treatment significantly decreased the water potential, osmotic potential and LRWC in both wheat varieties while the highest values for these parameters was observed under PGPR application. Cd stress in the soil decreased the microbial community and damaged the root tips to reduce the uptake of water and disturb the water balance of cells in leaf resulting in the reduction of stomatal conductance and transpiration rate (Qadir et al. 2014). Consequently, this is directly linked with decline in chloroplast amount as well as cell enlargement and ultimately reduced the plant growth and biomass formation (Rucińska-Sobkowiak 2016). In addition, Cd reduced the surface area of cells that absorb water indicating the disturbance of water balance (Sun et al. 2016). However, the PGPR improves the LRWC and water potential in different plant species exposed to different types of environmental stresses (Naveed et al. 2014). It is reported that PGPR improves the stomatal aperture to uptake more water via roots and enhances the stomatal conductance as compared to non-PGPR inoculated plants (Vejan et al. 2016). (Ahmad et al. 2016) described that PGPR enhanced the water uptake, RWC and membrane stability in the leaf of maize plant under Cd stress which support our study. Moreover, PGPR efficiently improved the tolerance ability of plants exposed to various environmental stresses including heavy metals and increased the yield of plant (Enebe and Babalola 2018).

It is depicted that the effect of Cd is dose dependent that varies with its concentration, duration of exposure and the nature of plant species at different growth stages (Hussain et al. 2019) thus, the response of NARC-2009 was found better than NARC-2011 on exposure to different levels of Cd. Besides, the PGPR enhanced the plant efficiently alone or with Cd treatment as shown in (Figure.8). Overall, it is estimated that Cd stress at all levels severely impacted the growth of both wheat varieties while the inoculation with PGPR reduced the Cd toxicity and improved plant growth attributes. However, further studies are needed to find out the actual mechanism of Cd toxicity in plants at molecular level with the application of PGPR.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human and animal participants. Consent to participate: Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All the analysed data for this study are included in this article. Conclusions of the current study is included in this article.

Competing interests

Authors declare that there is no conflict of interest.

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Authors' contributions

Conceptualization, I.K., S.A.A. and A.R.; methodology, M.A.R., I.K., R.T. and M.A.; formal analysis, S.A.A., G.A.S. and N.A.; investigation, L.H., M.B. and A.K.; writing—original draft preparation, I.K., S.A.A.; writing—review and editing, L.H., M.R., M.S. and S.A.A.; funding acquisition, L.H. All authors have read and agreed to the published version of the manuscript.

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Unsectioned Tables

Table.1. Analysis of the soil.

pH	7.45
EC dSm ⁻¹	1.28
Organic matter (%)	1.92
Phosphorus (mg/kg)	6.2
Potassium (mg/kg)	100
Zn (mg/kg)	1.04
Cu (mg/kg)	0.71
Mn (mg/kg)	2.64
Fe (mg/kg)	2.95
Cd (mg/kg)	4.36
Saturation (%)	33

Figures

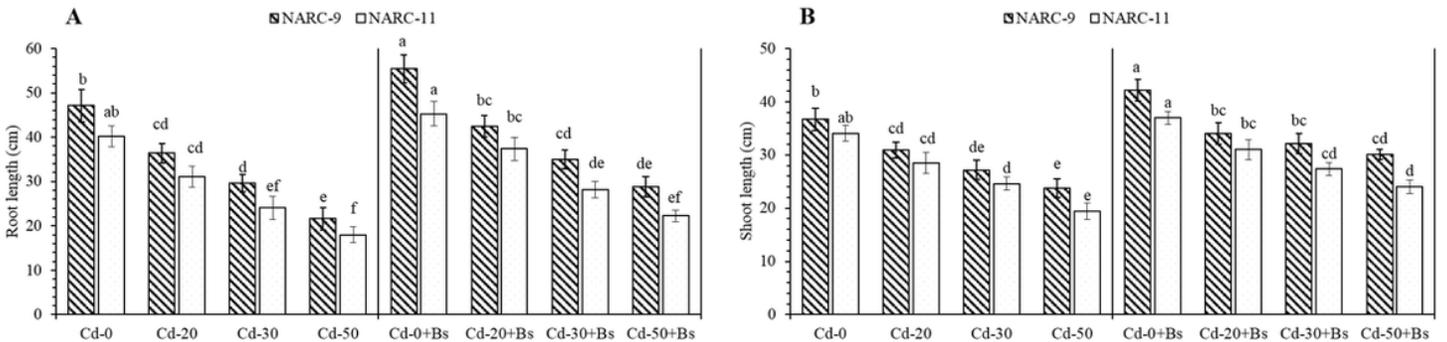


Figure 1. Morphological attributes of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments. (A) Root length (B) Shoot length at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 1

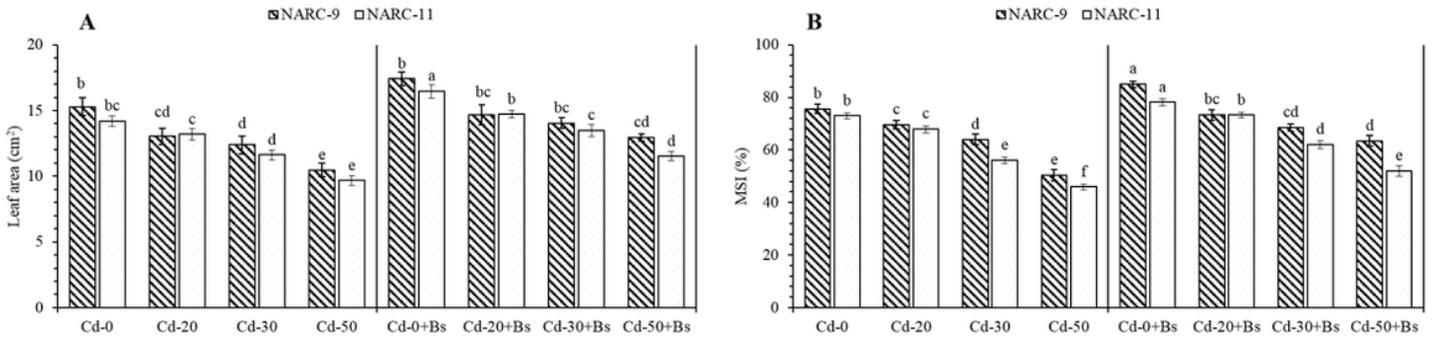


Figure 2. Leaf traits of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments. (A) Leaf area (B) Membrane stability index (MSI) at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 2

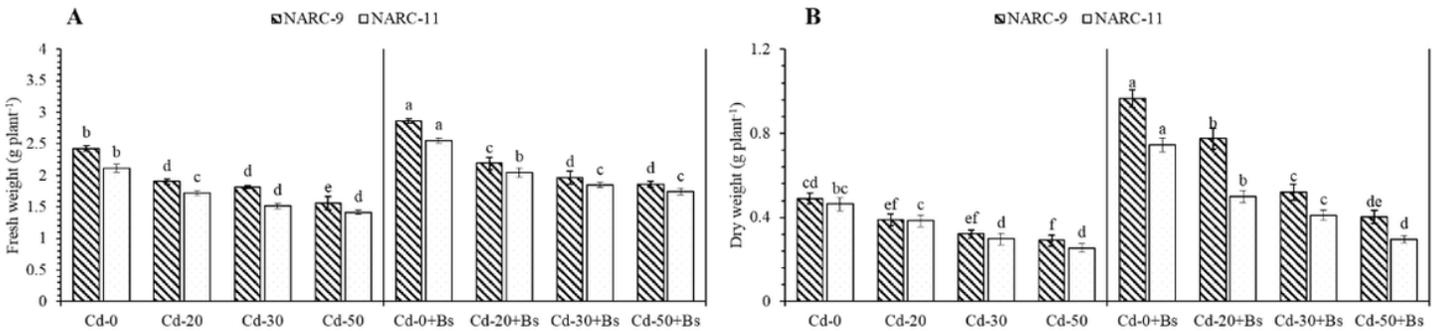


Figure 3. Biomass of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments. (A) Fresh weight (B) Dry weight at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 3

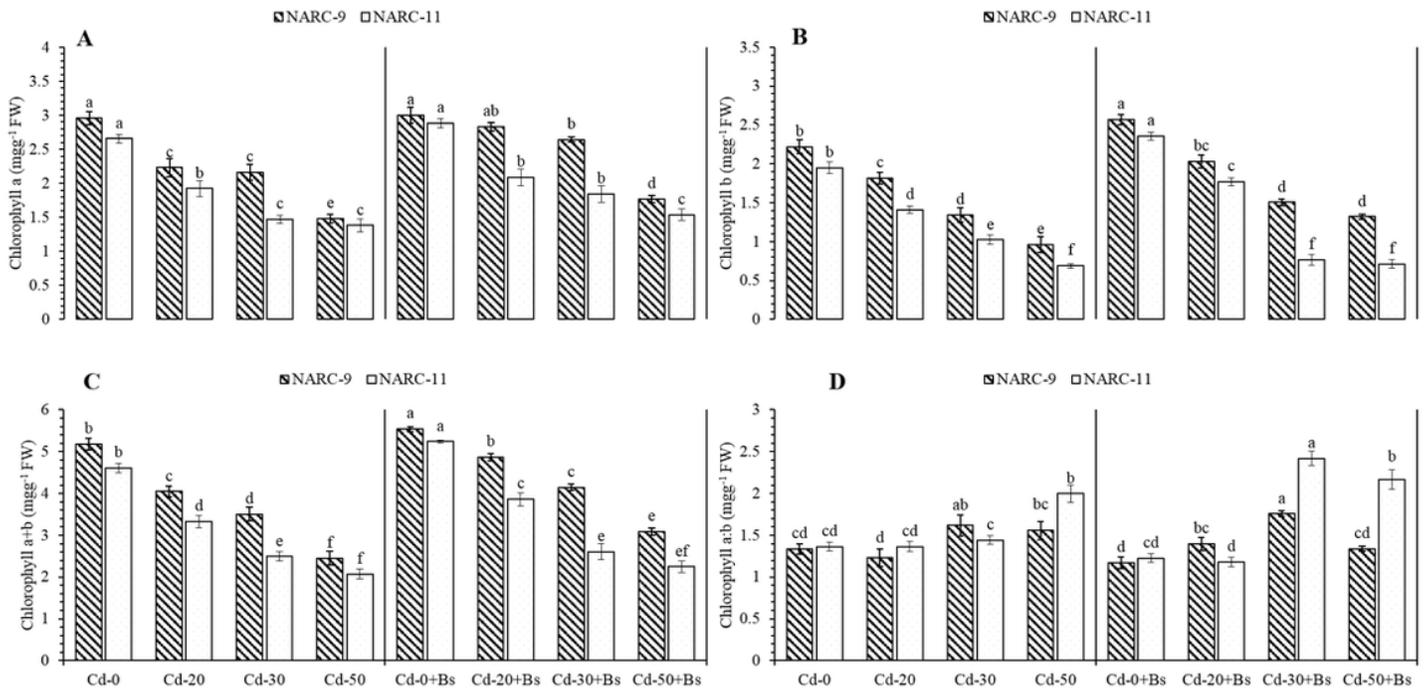


Figure 4. Photosynthetic pigments of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments. (A) Chlorophyll a (B) Chlorophyll b (C) Chlorophyll (a + b) (D) Chlorophyll (a : b), at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 4

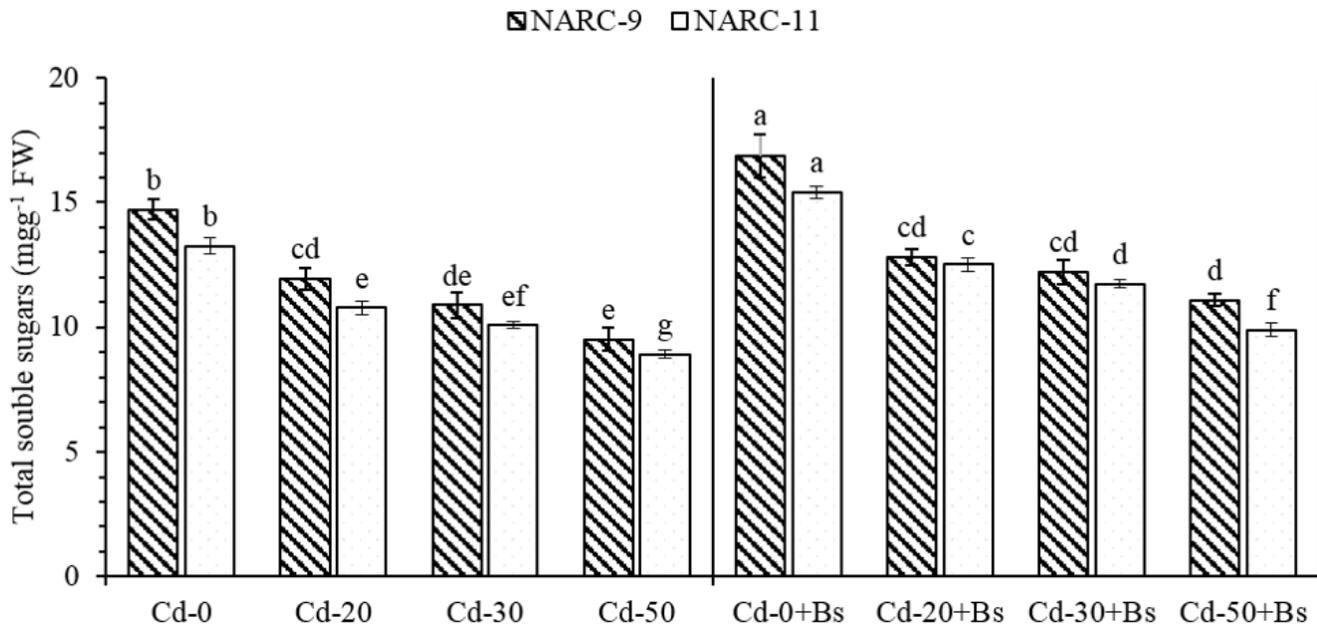


Figure. 5. Total soluble sugars content of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 5

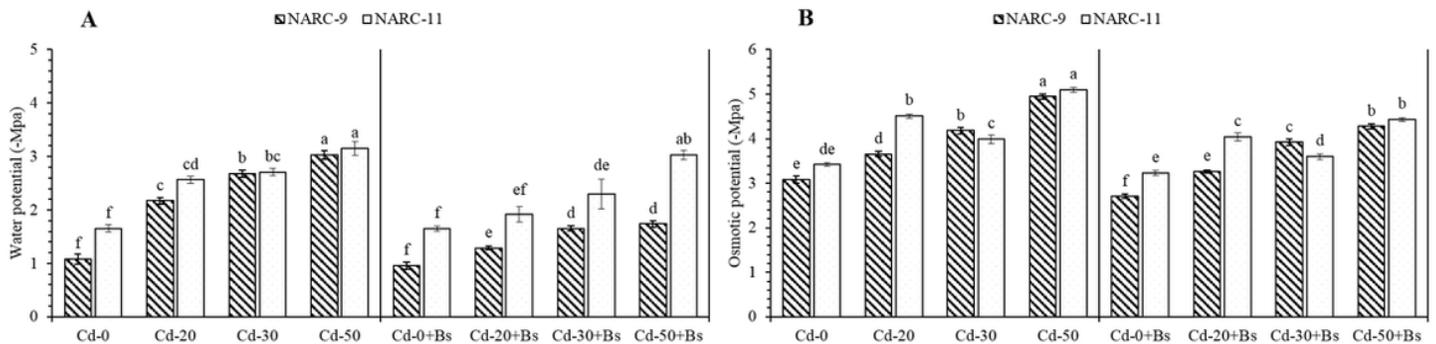


Figure. 6. Water status of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments (A) Water potential (B) Osmotic potential, at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 6

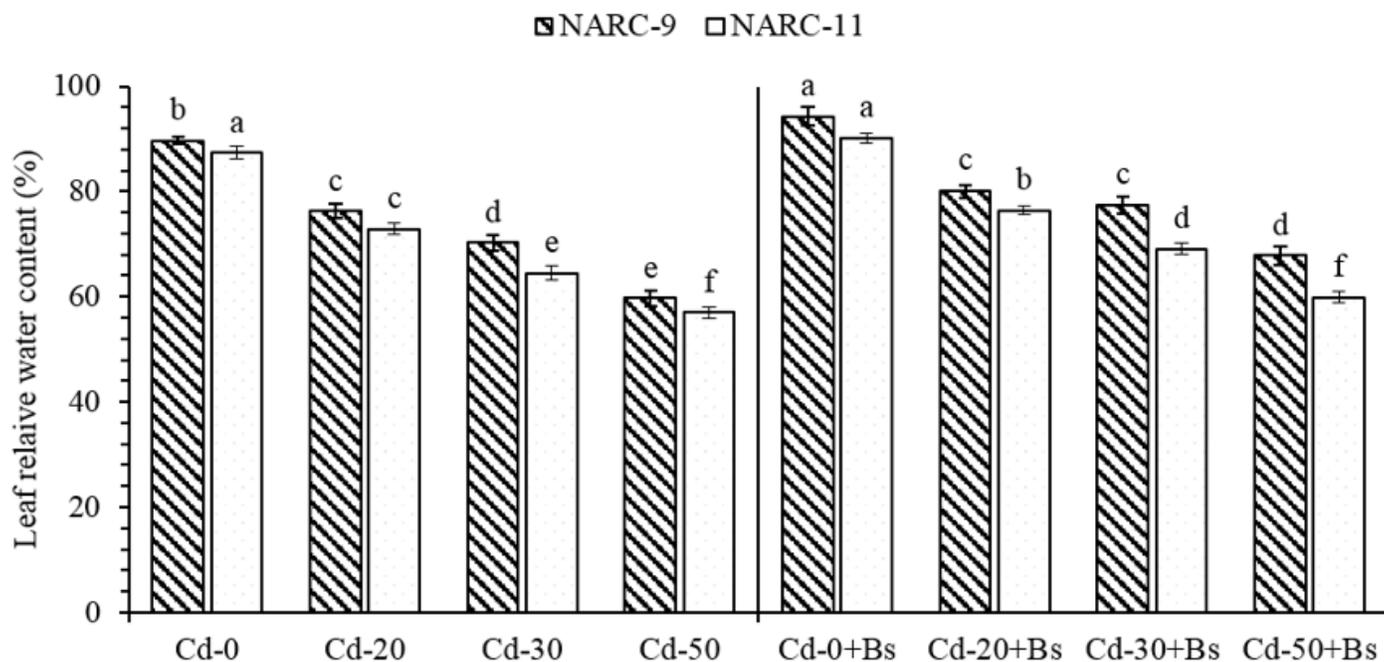


Figure. 7. Leaf relative water content of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 7

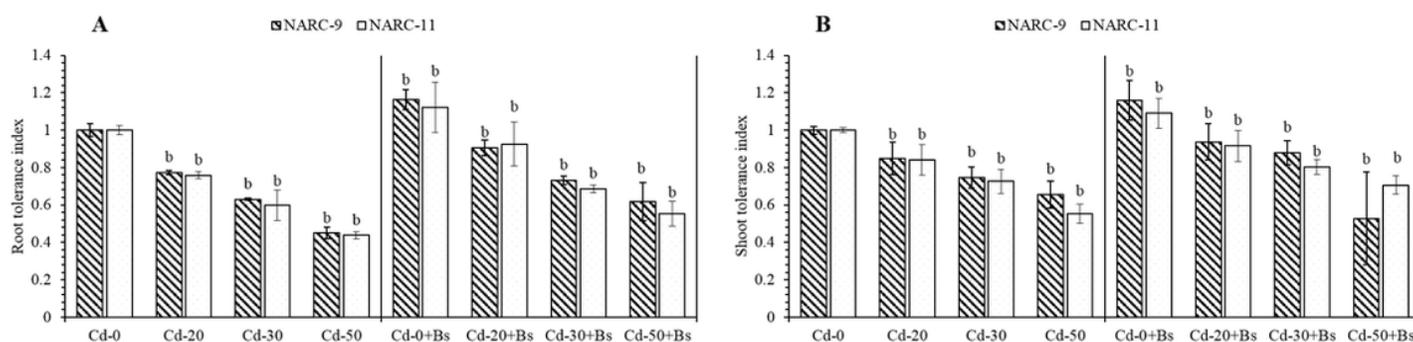


Figure. 8. Tolerance index of two wheat varieties i.e NARC-9 and NARC-11 inoculated with *Bacillus siamensis* under different cadmium treatments (A) Root tolerance index (B) Shoot tolerance index, at 30 days after sowing (DAS). Data are expressed as the average of three replicates. Bars show \pm SE within a bar and lowercase letters show a significant difference at 0.05 level.

Figure 8