Mitigating NaCl stress in *Vigna radiata* L. cultivars through *Bacillus pseudomycoides* (#95020)

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Mitigating NaCl stress in *Vigna radiata* L. cultivars through *Bacillus pseudomycoides*

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Salt stress is one of the significant abiotic stresses that exert harmful effects on plant growth and production. In this study, different concentrations of NaCl were applied to five cultivars of mung bean (*Vigna radiata* L.), along with the inoculation of a salt-tolerant bacterial strain to assess the growth and yiled. The bacterial strain was isolated from the saline soil of district Sahiwal, Punjab, Pakistan and identified as *Bacillus pseudomycoides*. Plant growth was monitored at 15-days interval and finally harvested after 120 days at seed set. Both sodium and potassium uptake in above- and below-ground parts was assessed using a flame photometer. Fresh and dry mass, the number of nodules, pods, and seeds per plant, as well as the weight of seeds per plant and the weight of 100 seeds, were significantly reduced with an increase in concentration of NaCl from 3 to 15 dSm^{-1}. Plants exposed to NaCl stress without bacterial inoculum exhibited significant reduction in growth and yield, as compared to the plants with bacterial inoculum as these plants showed a significant increase in studied parameters. An inverse relationship was found between growth parameters and salt concentration. It was found that the cultivar Inqelab mung showed the least reduction in growth and yield traits among studied cultivars, while Ramzan mung showed the maximum reduction. Among all the cultivars, maximum Na^+ uptake was found in roots while the least in seeds. The study concludes that NaCl stress significantly reduces the growth and yield among mung bean cultivars, but inoculation of *Bacillus pseudomycoides* can alleviate the salt stress. Such findings will be helpful to cultivate the selected cultivars in the soils with varying concentrations of NaCl.

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Key Words: Biomass, cultivars, growth, mung bean, salt-tolerant bacteria, stress, yield

Abstract

Salt stress is one of the major abiotic stresses that harm plant growth and production. In this study, different concentrations of NaCl were applied to five cultivars of *Vigna radiata* L. (mung bean), along with the inoculation of a salt-tolerant bacterial strain to assess the growth and yield. The bacterial isolation was done from the saline soil of district Sahiwal, Punjab, Pakistan and recognized as *Bacillus pseudomycoides*. Plant growth was monitored at 15-days interval and finally harvested after 120 days at seed set. Both sodium and potassium uptake in above- and below-ground parts was assessed using a flame photometer. There were observed significant reduction in fresh and dry mass, the number of root nodules, pods, and seeds per plant as well as total seeds weight and 100 seeds weight, as the concentration of NaCl increased from 3-15 dSm^{-1}. Plants exposed to NaCl stress without bacterial inoculum exhibited significant reduction in growth and yield, as compared to the plants with bacterial inoculum as these plants showed a significant increase in studied parameters. An inverse relationship was found between growth parameters and salt concentration. It was found that the cultivar Inqelab mung showed the least reduction in growth and yield traits among studied cultivars, while Ramzan mung showed the maximum reduction. Among all the cultivars, maximum Na^+ uptake was found in roots while the least in seeds. The study concludes that NaCl stress significantly reduces the growth and yield among mung bean cultivars, but inoculation of *Bacillus pseudomycoides* can alleviate the salt stress. Such findings will be helpful to cultivate the selected cultivars in the soils with varying concentrations of NaCl.

Introduction

Salinity imposes enormous pressure on agricultural production due to salt deposition in soil (Shahrasbi *et al*., 2020). Salt stress has badly affected about 20% of cultivated land and 50% of cropland worldwide (Naveed *et al*., 2020). The salt-affected area expansion is being accelerated by anthropogenic activities as well as climate change (Arora & Bhatla, 2017). About 50% of agricultural productivity is reduced through salinity in arid and semiarid regions (Kamran *et al*., 2019a). In Pakistan, 6.7 million hectares of land is being degraded annually through salinity, destroying 40,000 hectares of land in Pakistan (Hassan *et al*., 2021). Salt eventually affects some soils as a result of improper salt ~~removal from~~ management the affected site (Saleem *et al*., 2020). Soluble salts are retained in the soil during agricultural practices due to anthropogenic activities such as improper fertilizer use and disposal, which causes excessive nitrification, accelerating soil salinity (Zafar *et al*., 2019). Soil salinization is caused by high evaporation rates, low rainfall, and irrigation with salt-containing water, which results in salinity (Okur & Orcen, 2020).

Salinity causes numerous biochemical and physiological damages in crops, such as imbalanced ionic effect, reactive oxygen species production and lower soil water potential (Rehman *et al*., 2019). Higher concentration of reactive oxygen species disrupts enzymatic processes, cell membranes, as well as the degradation of lipids, proteins, and other biochemicals(Shahid *et al*., 2020). The higher concentration of Na^+ displays a higher Na^+/Ca^+ and Na^+/K^+ ratio, which consequently results in less absorption of K^+ and Ca^+ that disrupts cellular membranes and enzymatic activities, imbalances cellular equilibrium and destroys cellular balance (Kamran *et al*., 2019b). Excess amounts of salt cause oxidative damage by accumulating increased amounts of reactive oxygen species that can cause breakage of nucleic acids, damage plant tissues, and shrinkage of cell and chromatin condensation, which ultimately harms cellular structure (Debnath *et al*., 2021). An increased amount of salt in the soil profile also results in physiological dryness through less absorption of water, ion toxicity, normal cellular metabolic processes and reduced osmotic potential (Safdar *et al*., 2019; Garcia-Caparros *et al*., 2021). Crops show different responses to salt stress at developmental stages (Alsafran *et al*., 2022). The availability of water and nutrient uptake disturbed and ceased the germination of seeds as well as the growth of plants due to ionic and osmotic influences (Nawaz *et al*., 2021).

Bio-inoculation proved highly significant to enhance crop production against different salt stresses (Hyder *et al*., 2023). Plant growth-promoting bacteria can be used to reduce the harmful effects of abiotic stresses (Mumtaz *et al*., 2021). Soil is composed of many living and non-living things, such as fungi, bacteria, plants, insects, and minerals, and these create a sense of interaction with each other (Chen *et al*., 2023). In rhizosphere, soil bacteria help in disease prevention as well as the smooth uptake of nutrients (Kong *et al*., 2021). The induction of bio-formulation with plant growth-promoting bacteria aids in crop long-term fertility and makes modern approaches more appealing than traditional approaches (Backer *et al*., 2018).

The rhizosphere is the area of soil near the roots where soil microbes are abundant and perform microbial activities (Grover *et al*., 2021). Rhizo-microbial activity depends on soil properties, climate changes, and host species (Lyu *et al*., 2020). Bacterial inoculation enhances plant growth through direct and indirect actions (Saleemi *et al*., 2017). Direct mechanisms involve the easy uptake of nutrients from natural resources or phytohormone secretions that improve plant growth (Basu *et al*., 2021). Indirect mechanisms include enhancing stress tolerance as well as suppressing plant pathogens (Fatima & Arora, 2021). The diversity of soil and microbes is the backbone of the agroecosystem and increases plant production at a higher rate even under stress (Fatima *et al*., 2020).

Beneficial microorganisms dwelling in the soil imposed positive influence on plant metabolism, allowing plants to survive under abiotic stresses like salt, drought, heat, and heavy metal (Luna *et al.*, 2007). Various bacterial species such as Arthobacter, Azospirillum, Bacillus, Enterobacter, Klebsiella, Pseudomonas, and Serratia secreted different phytohormones mainly indole acetic acid and cytokinin that increased plant biomass, length, and nutrient uptake efficiency (Rosenburg, Gutnik & Rosenburg, 2006). Cytokinins also transferred signal from roots to shoots to manage environmental stresses (Ansari, Ahmad & Pichtel, 2019). Different bacterial strains, such as *Acinetobacter* sp., *B. massilioanrexius*, *B. pseudomycoides* and *B. thuringiensis* have different defense mechanisms against stress. *Bacillus pseudomycoides* is a mesophilic, spore forming, facultative anaerobic bacterium that forms rhizoid colonies (Paul *et al.*, 2022). It produced different volatile organic compounds that significantly increased plants biomass, resistance against diseases as well as abiotic stresses, regulated ion uptake and endogenous homeostasis of phytohormones (Farag, Zhang & Ryu, 2013).

Increasing salt stress in the soil is the factor that limits the production of crops (Xu *et al.*, 2022). The wide range of plant growth-regulating bacteria interacts with the host and helps in the reduction of harmful ramifications of salinity, boosting soil health (Kumawat, Nagpal & Sharma, 2022). The salt-tolerant bacteria indulge in various growth-promoting traits to endure high salt conditions in saline areas (Numan *et al.*, 2018). The salt-tolerant bacteria develop various strategies to overcome the harmful effect of salt such as developing an ion export system to deport extra sodium ions and accumulate chloride and potassium ions to prevent salt stress (Prittesh *et al.*, 2020). Some salt-tolerant bacterial strains substitute glucose with lactoprotein, and anionic phospholipase in the membrane to induce a more negative charge for stability and accumulate solutes like amino acid and sugar to sustain the osmotic pressure of cells (Hanelt & Muller, 2013; Zhang *et al*., 2018; Gupta *et al*., 2021).

Vigna radiata, commonly known as mung bean, is among the beneficent diet, having ecological and economic values, it's a summer crop, mostly cultivated in arid and semi-arid regions (Shafique *et al*., 2023). The main dietary components are protein, carbohydrates, fiber, fatty acids, and amino acids (Aasim *et al*., 2019). Due to nitrogen fixation ability, mung beans can be used for reclamation of soil and enhance soil fertility (Nigar *et al*., 2023). The annual production of this crop is 30 million tons worldwide, which is 5.1% of the total pulses produced (Sehrawat *et al*., 2018). Mung bean major cultivation lands are Africa, Burma, China, India, Pakistan, and Queensland (Pandey *et al*., 2021). Mung bean is considered a salt-sensitive crop and, therefore, susceptible to salt stress (Kumar *et al*., 2023). About 70% mung bean production was reduced at 50 mM NaCl concentration which imposed harmful effects on the crop (Dutta & Bera, 2014). The inoculation of salt-tolerant bacteria in mung beans persuades repulsion against sodium uptake, promotes biomass and length of an above and below-ground plant part, and enhances nodulation with nitrogen fixation and rate of photosynthesis with transpiration (Saha, Chattergi & Biswas, 2010). So far, the impact of salt-tolerant bacteria on these five varieties has not been studied. The objectives of this current study were 1) screening of five cultivars of *Vigna radiata* for NaCl stress 2) utilization of salt tolerant bacteria (*Bacillus pseudomycoides*) to alleviate NaCl stress 3) analyzing the effect of *B. pseudomycoides* inoculation along with salt treatments on growth, yield, and physiological parameters 4) accessing the uptake of Na^+ and K^+ in root, stem, leaves and seeds among the five selected cultivars.

Materials and Methods

Bacterial isolation, screening, and characterization

Three different saline areas in Jahan Khan, district Sahiwal, Punjab, Pakistan, were chosen for soil sampling with geographical location 30° 34' 0" N, 73° 11' 0" E. Salt-tolerant bacteria were isolated from salt-affected soil (Manasi, Rajesh & Rajesh, 2016). Screening of salt-tolerant bacteria was carried out following Zhou *et al*. (2014). Three salt-tolerant bacterial colonies were screened against various concentrations of NaCl ranging from 100 ppm to 30000 ppm salt to determine which concentrations these bacteria can easily survive. Salt-tolerant bacteria samples were prepared and sent to Humanizing Genomics Macrogen in Korea for molecular characterization and identification following Sanjay *et al*. (2018).

Bacterial Inoculum Preparation

Inoculum of bacteria was prepared by adding 8 g of nutrient broth (NB) into one liter of distilled water and bacterial strain loop was inoculated in it. For preparation of slants for inoculum application, it was placed in an incubator at 37 °C for 24 hours.

Seeds collection

Seeds of five different cultivars i.e, NIFA-19, NIFA-17, Ramzan mung, Inqelab mung and Sona mung were collected from the Nuclear Institute for Food and Agriculture Peshawar and the Agriculture Research Institute Dera-Ismael Khan, Pakistan.

Experimental set-up

The pot experiment was conducted to measure plant growth, yield, and physiological parameters for selected cultivars of *V. radiata* as Inqelab mung (V1), NIFA-19 (V2), NIFA-17 (V3), Sona mung (V4) and Ramzan mung (V5). Two set-up were established along the four treatments, one having salt stress without bacterial inoculum (T_0, T_1, T_2, T_3, T_4, T_5) and the other having salt stress with bacterial inoculum (T_0B, T_1B, T_2B, T_3B, T_4B, T_5B) for all cultivars of mung bean. The seeds of cultivars were germinated in the Botanic Garden and five days old seedlings were treated with

NaCl concentration ranging from 3 dSm^{-1} to 15 dSm^{-1} and compared with control lacking any application of NaCl. The bacterial inoculum was applied in the pots having germinated seeds; four pots were used for each treatment, with two replicate plants in each pot (Gamalero & Glick, 2022). Hayyat *et al*. (2021) reported the physico-chemical properties of Botanic Garden soil. Inoculum of bacteria was prepared by adding 8 g of nutrient broth (NB) into one liter of distilled water and bacterial strain loop was inoculated in it. For preparation of slants for inoculum application, it was placed in an incubator at 37 °C for 24 hours. Prepared broth (10 mL) mixed with distilled water and poured into label pots. The experiment was conducted for 120 days (March to June 2023) in ambient conditions at the Botanic Garden of Government College University Lahore Pakistan. During the experiment the mean day-time temperature was 44 °C, relative humidity 26 % and solar radiation 802 watts/m^2. The plants were monitored on weekly basis for their growth and irrigated appropriately to avoid any water stress; equal amount of water was applied to all pots.

Mid and Final Harvesting

During mid-harvest, fresh and dry weight of plant parts (above and below ground) was measured. At final harvest, weight (fresh and dry) of plant parts along with other yield attributes viz. number of pods, total seeds number, total seeds weight per plant and weight of 100 seeds were assessed (Desai *et al*., 2022).

Physiological measurements

The physiological parameters i.e. maximum photosynthetic rate, stomatal conductance, and transpiration rate were measured in the morning from 10:30 am to 11:30 am under ambient conditions before mid-harvest by using IRGA LCA4. The chlorophyll contents were determined using a spectrophotometer (Spectroscan 80D) (Yoshida, Forno & Cock, 1971). The intrinsic water use efficiency of different mung bean cultivars was calculated using the following (Ferreira *et al*., 2018).

Na^+ and K^+ uptake

In order to assess the Na^+ and K^+ uptake, the ash of leaves, stem, seeds, and roots of studied cultivars was prepared by heating the samples at 550 ^{0}C for three hours in muffle furnace (Gallenhamp Size 2). 5 mL of 2N HCl was used to dissolve prepared ash and placed for fifteen minutes followed by the addition of 50 mL distilled water. The solution was filtered by using Whattman No. 42 filter paper to have an extract of particular plant part. The sodium and potassium uptake was assessed by using flame photometer (S20 Spectro lab).

Data Analysis

Statistical analyzes of data was done with Statistix v. 8.1 using a one-way ANOVA (Duncan's multiple range test (DMRT)) involving a comparison of the means across treatments. The descriptive statistics, correlations between parameters and graphics were carried out using Sigma Plot 14.0 version.

Results

Bacterial screening, identification, and characterization

Three isolated bacterial samples were selected for further screening. After few screenings, only one bacterial strain was chosen for inoculation due to higher tolerance against NaCl. The pure isolate was gram-positive, non-motile, long rod-shaped, and spore-forming under light microscope. Identified as *Bacillus pseudomycoides*, Figure 1 shows the homology of isolated bacterial strain.

Effect of NaCl on *V. radiata* cultivars Biomass

Table 1 represents that salt treatment showed a significant difference in growth and yield among all cultivars while the *Bacillus pseudomycoides* inoculum coped with drastic effect of NaCl. During mid-harvest, plant fresh weight showed a significant increase (63.8% - 93.8%) with

Bacillus pseudomycoides inoculum while a significant decrease (57.5% - 13.6%) was recorded among all the cultivars having salt treatment only. Similarly, plant dry weight with *Bacillus pseudomycoides* inoculum showed a significant increase ranging from (72.3% -84.8%) while decrease (38.6%-16.4%) due to NaCl stress (Figure S1, Supplementary Material).

At final harvest, a significant increase (71.8%-94.3%) in fresh weight of plant with *Bacillus pseudomycoides* inoculum was observed across all the five cultivars while a significant decrease (57.3% - 14.5%) with salt stress (Figure S2, Supplementary Material). The plant dry weight also showed significant increase (67.3%-92.2%) in plants with *Bacillus pseudomycoides* inoculum while decrease from (45.9% - 14.7%) due to salt stress among five selected cultivars (Figure 2). All five studied cultivars of *V. radiata*, Inqelab mung, NIFA-19 mung, NIFA-17 mung, and Sona mung and Ramzan mung showed significant differences in dry weight (above and below ground) (Figure 2 a,b,c,d,e). The measured differences in the fresh and dry weight of cultivars were; $V5<V4<V3<V2<V1$.

Effect of NaCl on Yield

The yield attributes such as number of nodules, number of pods per plant, number of seeds per pod per plant, weight of seeds per pod per plant, and weight of 100 seeds also showed reduction in plants without inoculum of bacteria from T_0-T_5 in all cultivars. The inoculum of *Bacillus pseudomycoides* provided more yield than plants with salt stress only. There were significant differences observed in yield parameters among the *Vigna radiata* cultivars, Inqelab mung (Figure 3 a,f,k,p,u) NIFA-19 mung (Figure 3 b,g,l,q,v), NIFA-17 mung (Figure 3 c,h,m,r,w), Sona mung (Figure 3 d,i,n,s,x), and Ramzan mung (Figure 3 e,j,o,t,y). The percentage increase was observed among all cultivars in number of root nodules (74.3% - 87.5%), pods (65.9% - 92.8%), seeds (72.8% - 94.5%), weight of seeds (64.7% - 85.9%) and weight of 100 seeds (74.9% - 94.8%) in plants with *Bacillus pseudomycoides* inoculum while percentage decrease in number of root nodules (32.8% - 12.7%), pods (27.9% - 9.6%), seeds (33.7% - 15.7%), weight of seeds (45.8% - 15.6%) and weight of 100 seeds (25.7% - 7.9%) due to NaCl stress.

Effect of NaCl on Gaseous exchange and Na^+, K^+ Uptake

The significant differences were found among the studied cultivars regarding their maximum rate of photosynthesis, transpiration, stomatal conductance and water use efficiency (Figure 4 a-t). Plant with inoculum of *Bacillus pseudomycoides* showed percentage increase of photosynthetic rate (75.3%-83.8%), transpiration rate (53.85%-87.1%) and stomatal conductance (67.7%-81.8%) while percentage decrease of photosynthetic rate (25.8%-9.4%), transpiration rate (36.2%-17.3%) and stomatal conductance (47.4%-13.5%) due to salt stress among all cultivars. Water use efficiency also showed increase from 68.3% - 92.1% only with salt stress, the decrease was observed from 28.3% - 13.8% with inoculum of *Bacillus pseudomycoides* in all cultivars. The up-take of Na^+ and K^+ varied in both the above and below ground parts of all varieties. The Na^+ concentration was higher in roots than stems and leaves, while the minimum amount of Na^+ was detected in seeds. The amount of Na^+ was ranging from 0[illegible]-2.77 g kg^{-1} in roots (Figure 5 a-e), 0.40-2.83 g kg^{-1} in stems (Figure 5 f-j), 0.13-2.26 g kg^{-1} in leaves (Figure 5 k-o) and 0.09-1.90 g kg^{-1} in seeds (Figure 5 p-t). Similarly, the concentration of K^+ ranged from 6.73-11.56 g kg^{-1} in roots (Figure 6 a-e), 7.25-10.83 g kg^{-1} in stems (Figure 6 f-j), 7.20-10.40 g kg^{-1} in the leaves (Figure 6 k-o) and 7.03-8.76 g kg^{-1} in the seeds (Figure 6 p-t), across the five cultivars and all treatments with and without *Bacillus pseudomycoides* inoculum.

Significant difference in the Na^+/K^+ ratio was also found in the studied cultivars (Figure S3, Supplementary Material). The increase in ratio was found in order 63.2% - 91.2% in roots, 72.7% - 88.3% in stem, 73.8% - 87.6% in leaves, 69.3% - 91.6% in seeds with *Bacillus pseudomycoides* while decrease as 38.4% - 16.7% in roots, 43.8% - 17.7% in stem, 34.8% - 12.7% in leaves, 28.4% - 11.7% in seeds with NaCl stress among all the cultivars.

Effect of NaCl on Chlorophyll contents

Figure 7 (k-o) shows the total chlorophyll contents across the studied cultivars, chlorophyll a ranged from 0.84 – 2.53 mg g^{-1} (Figure 7 a-e), chlorophyll b 0.39 – 2.72 mg g^{-1} (Figure 7 f-j) and total chlorophyll contents 1.23 – 3.95 mg g^{-1} (Figure 7 k-o). There were observed significant increase in chlorophyll a (68.4% - 87.5%) and chlorophyll b (65.8% - 84.7%) contents with *Bacillus psedomycoides* inoculum while decrease in chlorophyll a (31.8% - 13.7%) and chlorophyll b (27.6% - 9.8%) contents due to salt stress. There were also found significant increase in total chlorophyll contents 73.8% - 92.8% with *Bacillus pseudomycoides* inoculum while decrease 28.4% - 11.8% with NaCl stress among all cultivars. Chlorophyll a contents were higher than chlorophyll b contents under all treatments in all cultivars.

Relationship among the variables

A significantly inverse relationship between fresh (R^2=0.97-0.98) and dry (R^2 = 0.99-0.80) and plant biomass against salt concentration was observed across all the five cultivars. The inverse relationship was also observed among the number of root nodules (R^2= 0.98-0.99), pods per plant (R^2= 0.97-0.96), seeds per plant (R^2= 0.98-0.86) and weight of seeds (R^2= 0.98-0.96) against salt concentration (Figures 8).

Discussion

In this study, five cultivars of *V.radiata* were screened for their NaCl stress tolerance and the salt tolerant bacterial strain *Bacillus pseudomycoides* was used to test whether it can mitigate the applied stress. The selected five cultivars showed significant differences regarding their tolerance to NaCl stress. The application of isolated bacterial strain i.e, *Bacillus pseudomycoides* along the salt stress resulted in enhance~~s~~d growth and yield. This salt stress screening of cultivars and the alleviation through *Bacillus pseudomycoides* provided the novel information to cultivate *V. radiata* cultivars in salt affected areas. Anjana, Rawat & Goswami (2023) identified *Bacillus pseudomycoides* as halophilic bacteria by 16S rRNA sequencing. Studies also showed that gram-positive bacteria belonging to genus *Bacillus* were largely found in saline area (Javed *et al*., 2023). Among the five studied cultivars, Inqelab mung showed more resistance to salt application than other cultivars. This could be due to the more nitrogen fixation ability and more uptakes of minerals, as it had larger number of root nodules and root hairs, balanced phytohormones, and production of reactive oxygen species as well as osmotic adjustment of this cultivar (Mansoor *et al*., 2017). Rehman *et al*. (2019) also reported the interaction of rhizobium strains with mung bean cultivars “Inqelab mung” that showed bacterial strains elevated yield and physiological parameters by increasing nutrient absorption, systematic resistance against pathogens, phosphorus solubilization as well as production of phytohormones. Khattak *et al*. (2020) compared yield of NIFA-17 mung and Ramzan mung in breeding experiment. NIFA-17 was declared as commercial crop from 2017 in Khyber Pakhtunkhawa because it provided more yield in terms of seed production, seed size and number of pods than Ramzan mung. Khattak, Saeed & Ibrar, (2019) also compared NIFA-19 and Ramzan mung. NIFA-19 was recommended as new commercial variety in 2018 because it gave more yields in term of seed size, number and production than Ramzan mung. Ramzan mung was also evaluated as hybridized cultivars with short height, stiff stem, earlier maturity, and moderate grain while no resistance against diseases detected (Khattak, Ashraf & Alam, 2006).

Our findings showed that salt concentrations, *Bacillus pseudomycoides* inoculum and selected cultivars had a significant impact to increase biomass and yield of different mung bean cultivars, indicating that all these three factors could significantly ?? the yield among the mong means. In our study, salt stress decreased biomass and yield of mung bean cultivars while inoculum of *Bacillus pseudomycoides* improved it which was in line with previous study by Malook *et al*. (2020). A substantial imbalance in osmotic and water relation is caused by salinity stress which declined growth. Salinity stress can be alleviated by using plant growth promoting bacteria was also found by Negacz *et al*. (2022). Inoculation of plant growth-promoting bacteria *Acinetobacter bereziniae*, *Enterobacter ludwigii* and *Alcaligenes faecalis* ameliorated the harmful effect of saline stress in *Pisum sativum*. It also enhanced biomass and nutrient uptake efficiency. Our findings support Sapre, Gontia & Tiwari, (2022) showing that biomass and yield of mung bean cultivars decreased with salt stress because Na^+ ion level increased which induced oxidative stress, harmed ionic balance, membrane integrity and photosynthetic efficiency. The inoculation of *B. pseudomycoides* improved biomass and yield significantly reported by Katsenios *et al*. (2022) who treated sweet corn with different types of plant growth promoting bacteria that increased growth and yield in order: *B. mojavensis*> *B. subtilis*> *B. pumilus*> *B. pseudomycoides* compared to control. Li *et al*. (2022) also characterized ability of *Bacillus pseudomycoides* as heavy metal (Cu) resistant bacteria. The salt stress negatively affected root structure; Yousef *et al*. (2023) also found that NaCl stress imposed bad effect on French beans morphological attributes, causing enzymatic degradation, metabolism, physiological rate alteration, and an imbalance in osmotic potential which destroyed root structure. *Bacillus* sp. also worked symbiotically to improve *V.radiata* growth and yield that stabilized metabolism and osmotic balance (Ahmed *et al*., 2020).

In our findings, root nodules, play significant role in nitrogen fixation, also suffered from salt stress as found by Dogra *et al*. (2013) that number of root nodules decreased in chickpeas under salt stress and its affect was ameliorated by *Mesorhizobium cicero* which improved root nodules and nitrogen fixation ability. Hnini, Taha & Aurag (2022) also found rhizobacteria in *Vachellia tortilis* and co-inoculation of *E. hormaechei*, *P. moraviensis* bacteria in *Sulla flexuosa* L. (Hamane *et al*., 2023) developed more root nodules enhancing root colonizing potential and counteracting nutrient imbalance due to salt stress.

Our results are also supported by Medeiros & Bettiol (2021) who investigated *Bacillus* sp. on tomatoes which gave more yield than plants under only stress. It might happened due to increased phytohormones, more ROS scavenging ability as well uptake of minerals and reduced osmotic stress. *Bacillus pseudomycoides* can activate different enzymes such as lipase, pectinase and cellulase that play a major role to minimize the stress and uplifted yield Knezevic *et al*. (2021).

Under salt stress, chlorophyllase break down different photosynthetic pigments, chlorophyll and other enzymes which produced more reactive oxygen species. The chlorophyll contents, rate of photosynthesis, transpiration and stomatal conductance negatively affected by salt stress. Our findings were similar to AbdelMotlb *et al*. (2023) that stress reduced chlorophyll contents and physiological attributes in green gram. The bacterial inoculum enhanced these parameters by increasing electron transport and photosynthetic activity while lowering xylem balancing pressure and stomatal conductance. More positive results were observed in Inqelab mung, and decreasing influence was shown by Ramzan mung. *B. pseudomycoides* also affected water use efficiency of mung bean cultivars against salt stress as reported Obadi *et al*. (2023) in tomato, it might happened due to osmotic stress, imbalanced ion uptake as well as cellular damages, affect the efficiency of cellular process, including water use. *Rhizobium leguminosarum*, a plant growth promoting bacteria showed resistance against drought stress and improved chlorophyll contents and physiological attributes and yield of plants.

The ion uptake results showed that there was comparatively more sodium and less potassium uptake by roots than stems, leaves, and seeds in plant with only salt stress while inoculum of *Bacillus pseudomycoides* improved potassium uptake. Inqelab mung showed less uptake of sodium among all varieties, whereas Ramzan mung presented more uptakes and less resistance against salt stress. Salt stress inflexed more sodium ion in plant cell that initiated osmotic stress, damaged plant tissue, disrupts cellular processes and membrane integrity, Leontidou *et al*. (2020) also found similar result in tomato under salt stress. Higher levels of salt, compartment more Na^+ ions in plant cells that drained plant energy for combating stress rather it could be used for growth. In the presence of excess NaCl salt, the root plasma membrane depolarizes as Na^+ influx which activate guard cell outward rectifying potassium channels. As a result, Na^+ ion diffuses into cell that decrease cytosolic K^+ and increase Na^+ content. Soil-inhabitant bacteria mitigated the uptake of sodium ions by forming an ion export system and prevent cell damage. Inoculation of *Rhizobium* sp. and *Pseudomonas* sp. activated ion exclusion mechanisms that removed extra Na^+ from roots part that prevented seeds and leaf from uptake of salt demonstrated by Ahmed *et al*. (2011). In current study, *Bacillus pseudomycoides* played major role to overcome salt stress and enhanced mung growth and yield also found (ElSharawy, Eid & Ebrahiem, 2023) *B. pseudomycoides* a promising bio-control agent against *Alternaria* early blight in tomato.

Our results showed *Bacillus psedomycoides* inoculum lowered Na^+/K^+ ratio that boosted plant nodulation, yield and physiological attributes against applied stress. Salt stress elevated Na^+/K^+ ratio which disrupts ion homeostasis due to accumulation of Na^+. Similar report presented by Zhang *et al*. (2018) & Jiang *et al*. (2019) that salt stress in rice, cucumber and *Cicer arietinum* increased Na^+/K^+ ratio by inducing oxidative stress, reducing antioxidant system and disrupt cellular functions while inoculation of *Rhizobium* sp. proved significant against salt stress. These results are in agreement with previous findings of Muahtaq *et al*. (2021). It was supposed that bacterial inoculum supported different mechanisms as intra-cellular Na^+ sequestration, cyctolic potassium retention, osmotic adjustment though synthesis of compatible solutes that enhanced crop growth and yield even under stress.

Conclusion

The results of the study showed that the combined effect of NaCl stress and inoculum of *Bacillus pseudomycoides* significantly improve the growth, yield, and physiological attributes of five different cultivars of *Vigna radiata* L. The Na^+ from NaCl applications accumulated in root epidermal cells, impeding plant water uptake and causing oxidative stress. Plants cope with stressed environments through inoculation of *Bacillus pseudomycoides*, which produces phytohormones, ion equilibrium, and easy uptake of nutrients. All parameters decreased as salt stress increased from 3-15 dSm^{-1}. Among all cultivars, Inqelab mung showed the highest resistance to stress, while Ramzan mung demonstrated the lowest resistance.

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Zhou H, Zhou X, Zeng M, Liao BH, Liu L, Yang WT, Qiu QY, Wang YJ. 2014. Effects of combined amendments on heavy metal accumulation in rice (*Oryza sativa* L.) planted on contaminated paddy soil. *Ecotoxicology and Environmental Safety* 101(1): 226–232 DOI:10.1016/j.ecoenv.2014.01.001

Table S1: Physio-chemical analysis of Botanic Garden Soil

Attributes	**Garden soil**
Texture	Loamy Sand
pH	8.23 ± 0.00
EC (dSm^{-1})	0.01 ± 0.00
N (%)	8.2 ± 0.05
P (%)	0.01 ± 0.00
K (ppm)	3.4 ± 0.1
Na (ppm)	6.95 ± 0.15
Ca^{++} (%)	0.11 ± 0.00

Mg^{++} (%)	0.07 ± 0.00

Figure 1 (on next page)

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Figures

Figures

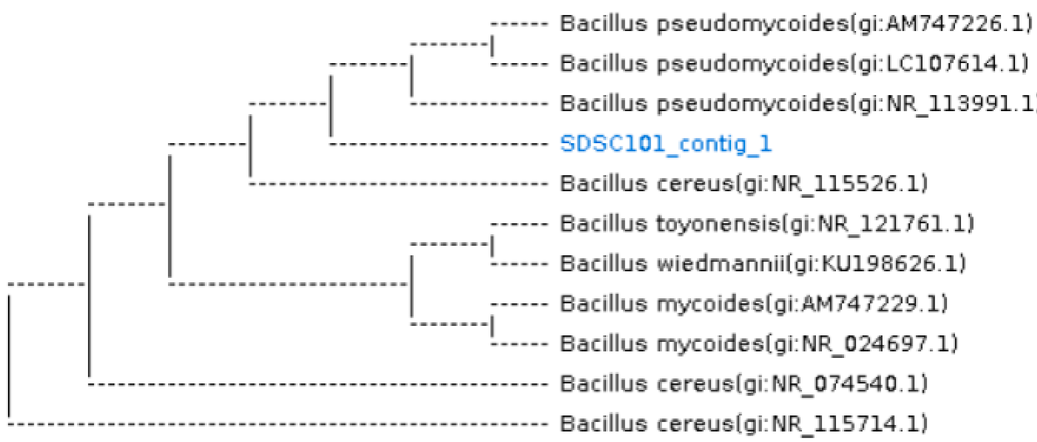

Figure: 1 Dendrogram showing homology of collected bacteria with *Bacillus pseudomycoides* (16S rRNA report). SDSC101_contig_1 showing the present study bacterial sample.

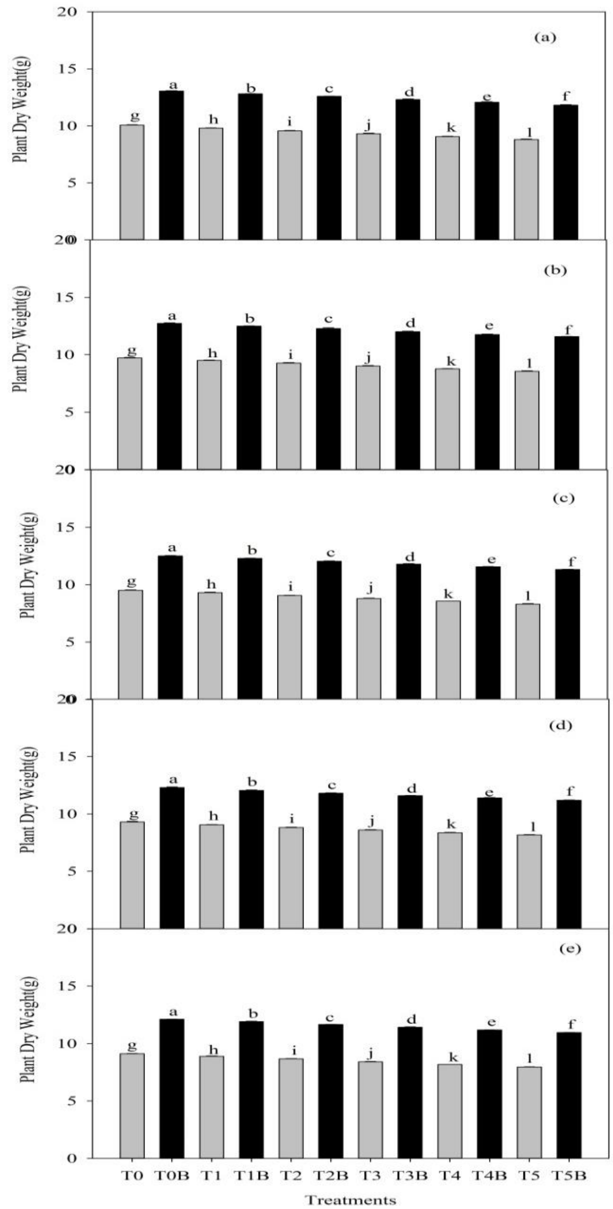

Figure 2: Effect of NaCl on different cultivars of Mung bean (*Vigna radiata* L.), Mean dry weight of plant parts (above and below ground) V1(a), V2(b), V3(c), V4(d) and V5(e), Bars with different letter (s) exhibit significant differences from each other. Grey bars indicate NaCl treatment and black bars indicate NaCl treatment along with *B. pseudomycoides* inoculum.

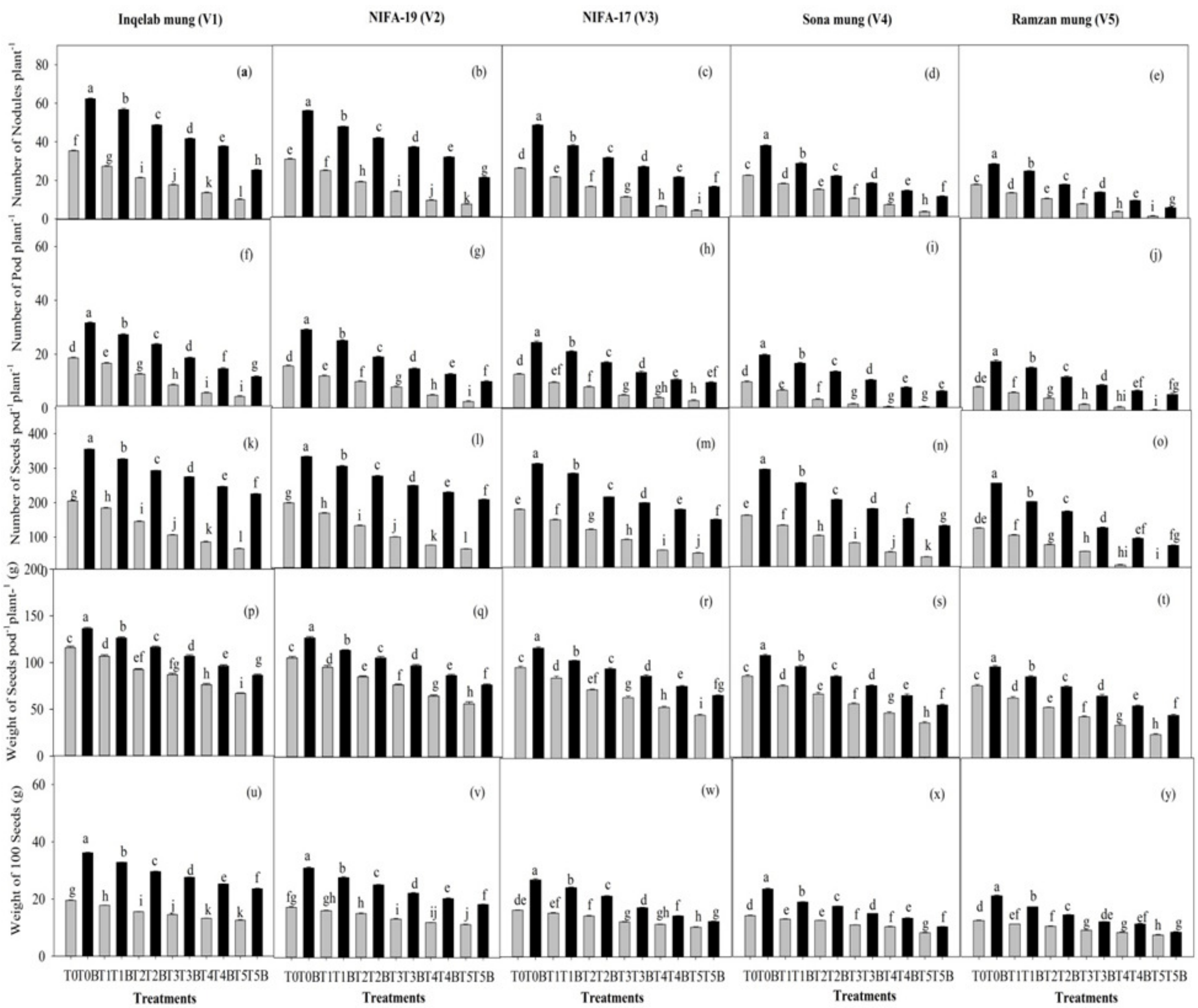

Figure 3: Effect of NaCl on different cultivars of Mung bean (*Vigna radiata* L.), Mean number of root nodules plant^{-1} (a-e), number of pods plant^{-1}(f-j), number of seeds pod^{-1}plant^{-1}(k-o), weight of seeds pod^{-1}plant^{-1}(p-t) and weight of 100 seeds (u-y), Bars with different letter (s) exhibit significant differences from each other. Grey bars indicate NaCl treatment and black bars indicate NaCl treatment along with *B. pseudomycoides* inoculum.

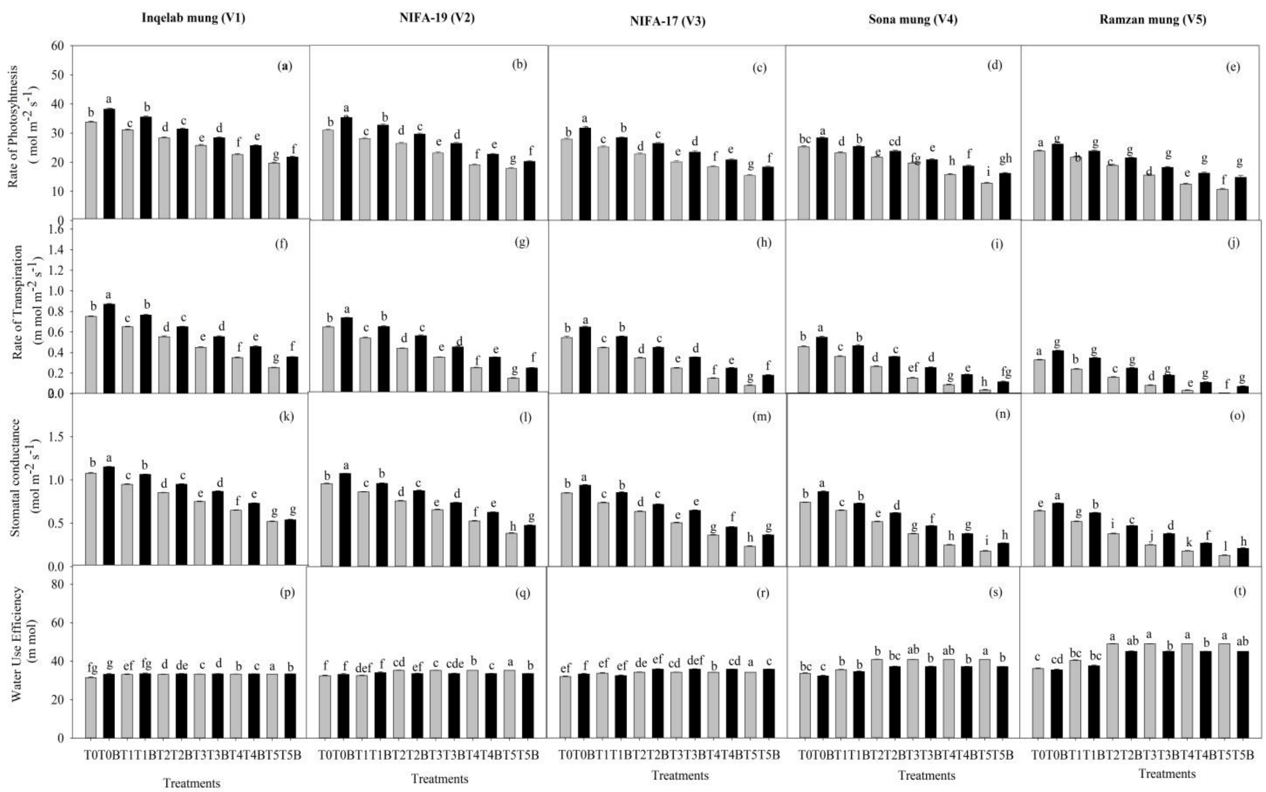

Figure 4: Effect of NaCl on different cultivars of Mung bean (*Vigna radiata* L.), Mean photosynthetic rate (a-e), transpiration rate (f-j), stomatal conductance (k-o) and water use efficiency (p-t), Bars with different letter (s) exhibit significant differences from each other. Grey bars indicate NaCl treatment and black bars indicate NaCl treatment along with *B. pseudomycoides* inoculum.

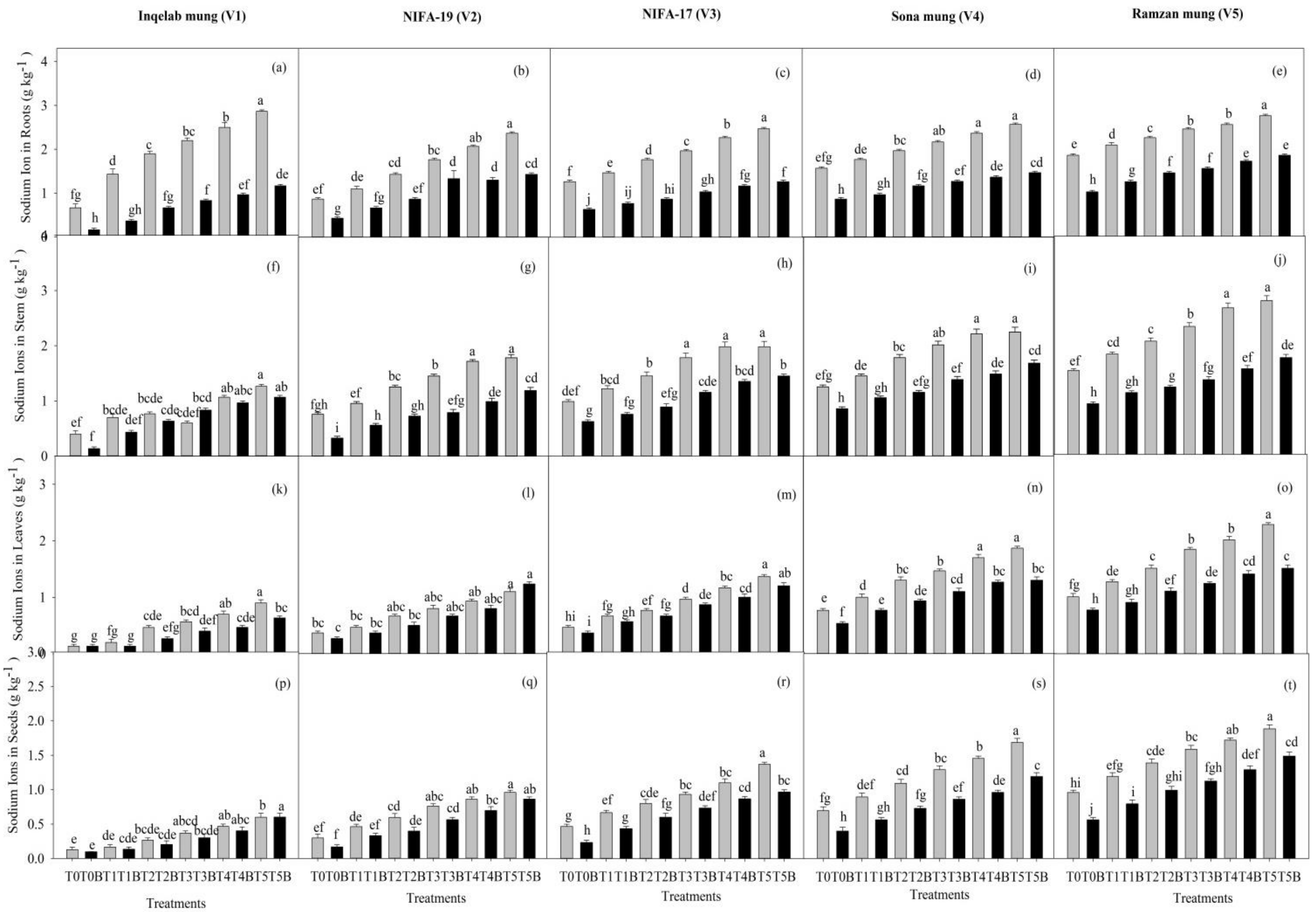

Figure 5: Effect of NaCl on different cultivars of Mung bean (*Vigna radiata* L.), Mean sodium ion uptake in root (a-e), sodium ion uptake in stem (f-j), sodium uptake in leaves (k-o) and sodium ion uptake in seeds (p-t), Bars with different letter (s) exhibit significant differences from each other. Grey bars indicate NaCl treatment and black bars indicate NaCl treatment along with *B. pseudomycoides* inoculum.

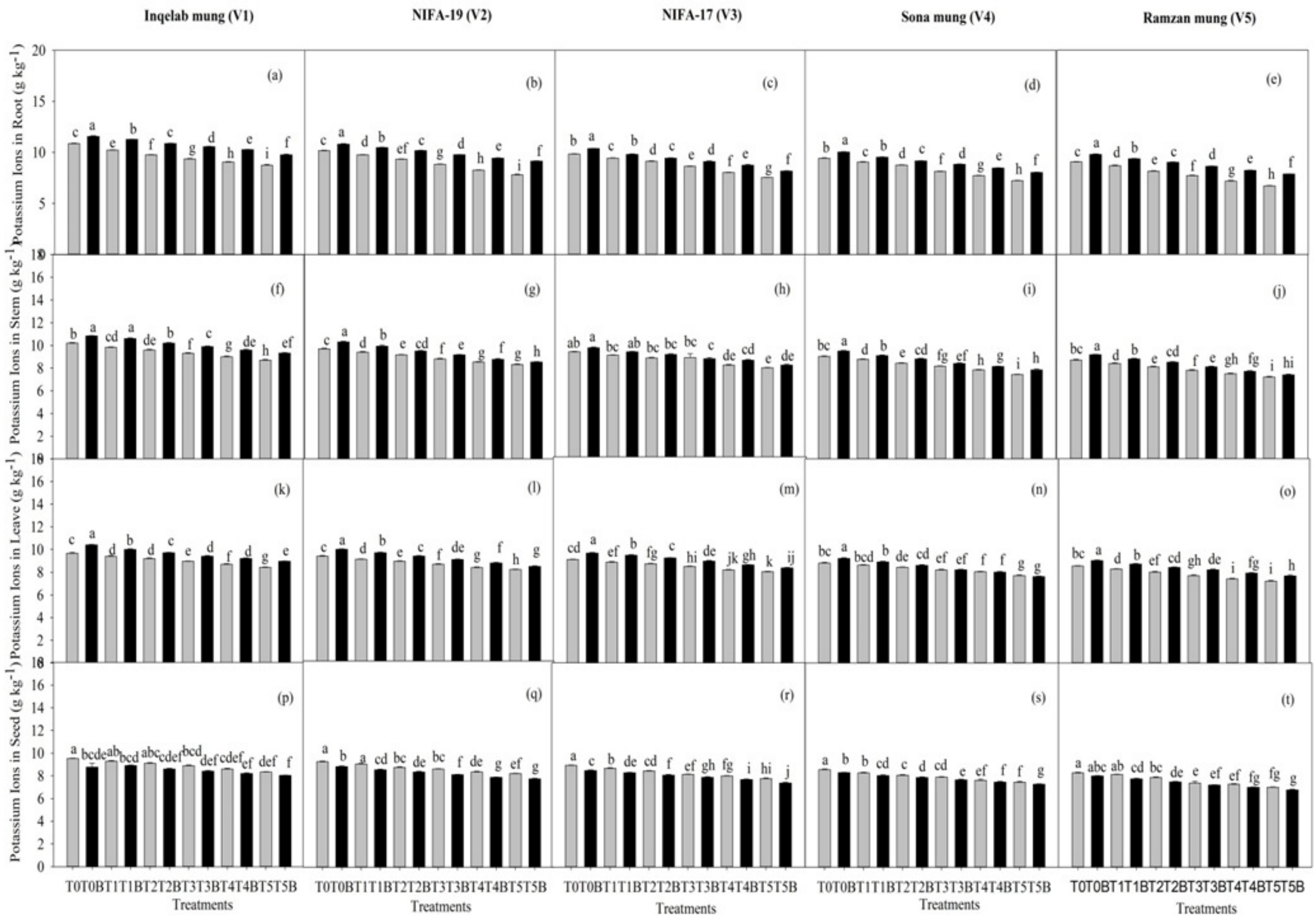

Figure 6: Effect of NaCl on different cultivars of Mung bean (*Vigna radiata* L.), Mean potassium ion uptake in root (a-e), potassium ion uptake in stem (f-j), potassium uptake in leaves (k-o) and potassium ion uptake in seeds (p-t), Bars with different letter (s) exhibit significant differences from each other. Grey bars indicate NaCl treatment and black bars indicate NaCl treatment along with *B. pseudomycoides* inoculum.

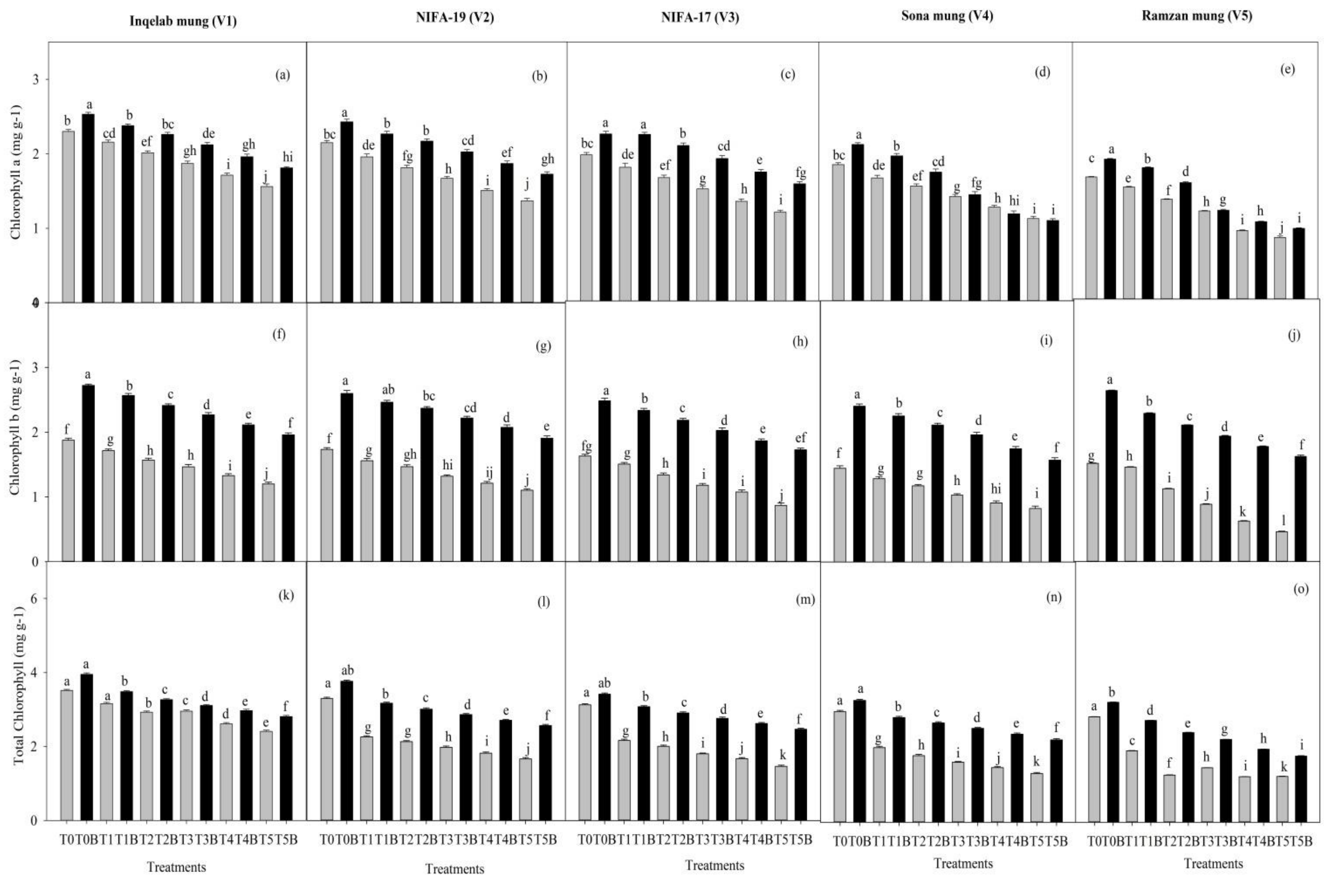

Figure-7: Effect of NaCl on different cultivars of Mung bean (*Vigna radiata* L.), Mean chlorophyll a (a-e), chlorophyll b (f-j) and total chlorophyll (k-o) contents, Bars with different letter (s) exhibit significant differences from each other. Grey bars indicate NaCl treatment and black bars indicate NaCl treatment along with *B. pseudomycoides* inoculum.

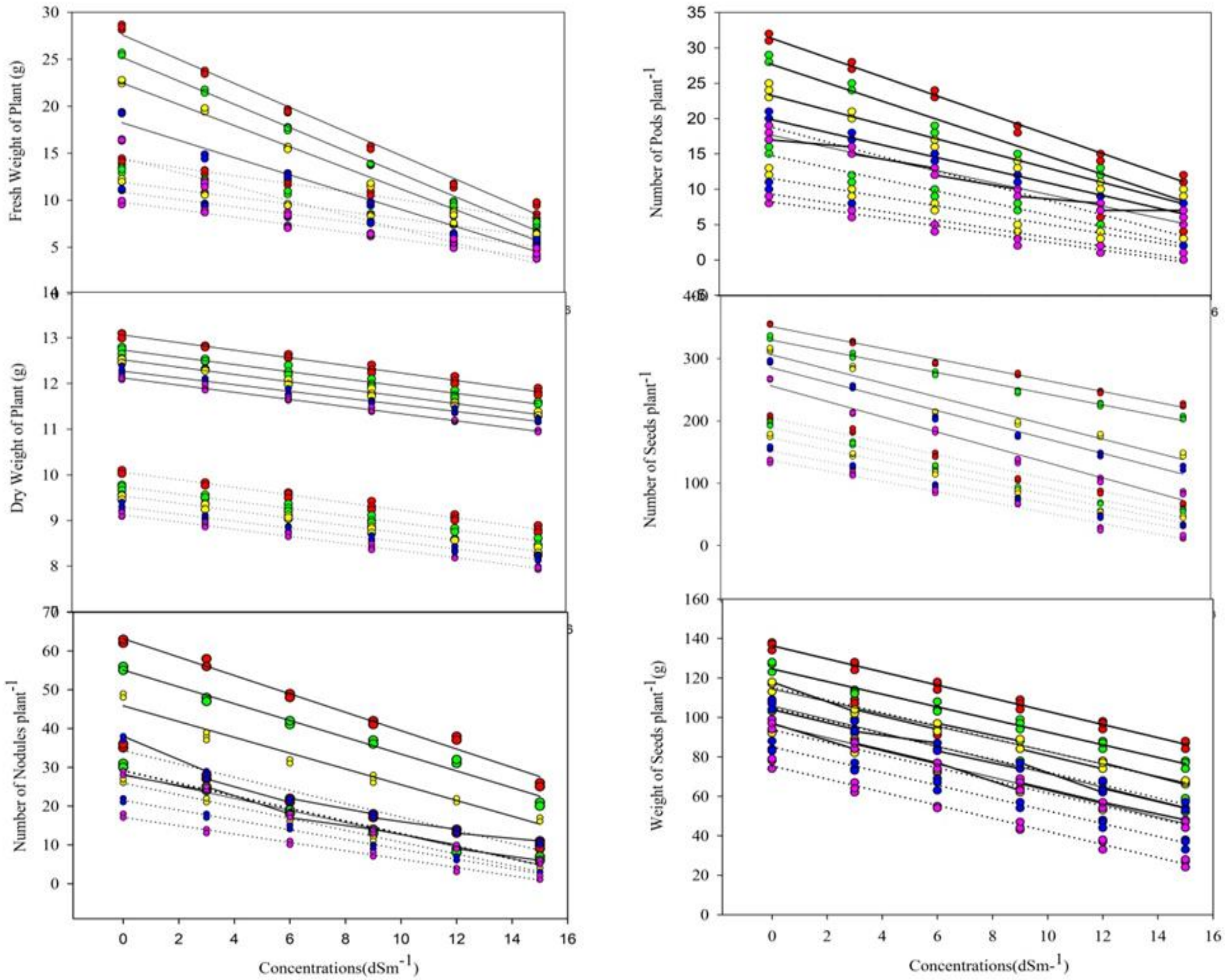

Figure 8: Effect of NaCl on different cultivars of Mung bean (*Vigna radiata* L.), Representation with dotted line = plant without inoculum of bacteria, solid line = plants with inoculum of bacteria, Red dots=V1, Green dots= V2, Yellow dots= V3, Blue dots= V4, Purple dots= V5.

Table 1(on next page)

Table

Tables

Table 1: Two-ANOVA model for interaction among measured parameters, salt stress and *Bacillus pseudomycoides* that represent significance ($p \leq 0.05$) level

Parameters	DF	SS	MS	F	P value
Plant biomass	44	0.065	0.0015	1.22	0.0000
Weight of 100 seeds plant^{-1}	44	596.10	13.548	123.03	0.0000
Number of seed plant^{-1}	44	35096	798	113.00	0.0000
Number of pods plant^{-1}	44	246.79	5.609	16.59	0.0000