Decoding the salt stress mitigation in Vigna radiata by Halotolerant Rhizobacteria Bacillus safensis from Mangrove-Dominant Estuaries of the Sundarban Biosphere Reserve, India: a promising tool for Sustainable Agriculture

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Abstract

The Sundarban delta, which is a hotspot for biodiversity and a UNESCO World Heritage Site, is affected by various biotic and abiotic factors. Due to changes in climate, this area is highly vulnerable to rising cyclonic activity and saline intrusion into agricultural lands. This soil salinity is one of the major limiting factors for crop production worldwide. This study addresses plant growth promoting activities like production of Indole acetic acid (151.6 ± 0.01 µg/ml), Gibberellic acid (114.52 ± 0.05 µg/ml), phosphate and zinc solubilization, siderophore production of halotolerant bacteria isolated from rhizosphere of *Avicennia* sp. of the Sundarban area, India. On the basis of 16S rRNA gene sequencing analysis, SBRK15 was identified as *Bacillus safensis*. Production of IAA was confirmed using HPLC analysis. Resistance to heavy metals such as lead, chromium, arsenic, and cadmium was demonstrated by SBRK15. SBRK15 had significant impact on *Vigna radiata* under a range of NaCl (0.5% – 2.0%) stress conditions. Germination, root and shoot length, chlorophyll content, Relative Water Content were improved in seedlings. Differences were observed in proline and malondialdehyde contents in leaf samples. The seedlings treated with *B. safensis* SBRK15 reported with reduced level of these parameters under various salt stresses compared to the uninoculated plants. Hence, this study had enlightened the salt stress alleviation effect of SBRK15 in mung bean seedlings. This halotolerant rhizobacteria may be applied as a promising tool for sustainable agriculture under stress condition.

Introduction

The Sundarbans, due to its special biological system, has been announced as a World Legacy location in 1989. Indian Sundarban is bound on the west by waterway Muriganga and on the east by waterways Harinbhanga and Raimangal. Other major waterways streaming through this eco-system are Saptomukhi, Thakuran, Matla and Goasaba. Mangroves of Sundarban are found in intertidal and transitional zones that are subject to tidal flooding, saltiness, a need of supplement accessibility, and unforgiving natural conditions that are negative to the development of plants (Spalding and Parrett 2019). More than 50% of the mangroves in the world have been annihilated (Polidoroet al. 2010) primarily since of urban advancement, timber generation, clearing for aquaculture, or for development purposes (Primavera et al. 2004). The rhizosphere of mangroves is a complex microbial living space, counting a consortium of microbes, eukaryotes, organisms. In this manner, the rhizosphere has a coordinate effect on the development of plants (Pallavi et al. 2023; Afridi et al. 2024; Panda et al. 2024). Numerous types of bacteria that promote plant development have been discovered to do so through various mechanisms, which help to regulate plant pests and activate tolerance to various abiotic challenges (Khoso et al. 2024; Mukherjee et al. 2019). The saltiness of the soil is one of the major stresses mindful for restricting crop efficiency (Tchiadje 2007). It impacts the germination of seeds, plant development and advancement (Prittesh et al. 2020). A few locales in the Sundarban are exceedingly anthropogenic due to tourism hones and the fundamental tourism is through boats and dispatch water crafts (Jamal et al. 2022; Paul et al. 2024). Presently these vessels confronted biofouling, which is essentially the aggregation of organic fabric on the surface of submerged structures. The biofouling of traveller vessels causes expanded fuel utilization. Using antifouling (AF) coatings is the

most popular method of preventing biofouling, but this AF cause heavy metal buildup in soil (Hasan et al. 2016; Chakraborty 2024). Members of the genus *Avicennia* are thought to be the most salt-tolerant mangroves and are too frequent to first colonize contemporary dreg stores (Li et al. 2024). Solidity in moving substrates is provided by the spreading root structure. *Avicennia* sp. plant root microbiotas vary by agrotype and plant genotype and significantly affect the health of plants and the stability of biological systems (Bulgarelli et al. 2012; Haichar et al. 2008; Ofek-Lalzar et al. 2014; Pramanik et al. 2019). Plant growth promoting rhizobacteria (PGPR) invigorate development by solubilizing insoluble phosphates, producing essential hormones like Indole acetic acid (IAA) and Gibberellic acid (GA), hence affecting cell division, elongation and fix atmospheric nitrogen (Dey et al. 2024; Kavitha et al. 2023; Huang et al. 2024). Since zinc is a basic mineral needed for plants to grow to their full potential, PGPR can be used in place of chemical zinc fertilizer, which pollutes the environment and depletes soil fertility (Ahmad et al. 2021). As a result, plant development and crop production can be improved and the effects of biotic and abiotic stressors can be precisely and implicitly addressed. Usually halophytes develop their tolerance to stress condition by accumulating proline in enhanced manner. As, proline regulate osmotic balance and help to withstand plants under harsh environmental conditions, it is a vital player in adaptation process (Al-Maskari et al. 2025). Another crucial factor which indicates oxidative stress condition in plants is Malondialdehyde (MDA), indicator of lipid peroxidation (Hnilickova et al. 2021). Rhizobacteria have the ability to control these proline and MDA levels in plants under stress environments. Therefore, the main goal of this study was to examine the capacity of rhizobacterial (*Avicennia* sp.) isolates from five different locations with varying salinities of Sundarban mangrove soil to promote plant development in vitro under both normal and salt stress conditions. The economically and nutritionally significant crop *Vigna radiata* (Mung bean) was used to study plant growth and development (Patel et al. 2016; Pataczek et al. 2018). It can be used as both animal feed and green waste. Therefore, rhizospheric bacteria from mangroves themselves can be employed to save mangroves.

Materials and methods

Sampling Site

The soil samples were collected in triplicate sets from five sites namely Godkhali, Dobanki, Canning, Kumirmari, Fraserganj in Sundarban Biosphere Reserve, West Bengal, India (21°58.706 'N and 088°40.460 'E) during the summers of June-July, 2023 (**Supplementary Fig. 1**). The plant root parts with soil, of 12–28 cm from the rhizospheric region, adjacent to the roots of the true mangrove species *Avicennia* species were collected and kept in sterile zip lock bags at refrigerator till it was further processed.

Isolation of Bacterial Isolates

1 g of each soil sample was suspended in 9 ml of 0.9% saline for the purpose of bacterial isolation. Yeast mannitol extract agar, King's B, Ashby's mannitol salt agar, and Nutrient agar (NA) were among the

selected media on which 100 µl samples from consecutive serial dilutions (10^{-3}, 10^{-4}, and 10^{-5}) were inoculated and incubated at 35 ± 2°C until bacterial growth appeared.

Screening for Indole Acetic Acid (IAA) Production

Assay of IAA was carried out utilizing the procedure of Gordon and Weber (Gordon and Weber 1951). The standard graph of IAA (Sigma-Aldrich, concentration: 10–100 µg/ml) was utilized to calculate the amount of produced hormone. In an incubator shaker, 10 ml of broth supplemented with 0.1% L-tryptophan was inoculated with freshly grown culture and kept at 35 ± 2°C (48 h) at 120 rpm. It was centrifuged (Remi Cooling Centrifuge) at 10,000 rpm for 10 min. Salkowski reagent was mixed with equal volume of supernatant and the mixture was left in the dark. After two hours, the intensity was recorded at 530 nm UV-Vis spectrophotometer (UV-1900, Shimadzu, Japan).

Screening for Gibberellic Acid (GA) Production

Bacterial isolate was incubated in nutrient broth medium for 4 days and the culture was centrifuged, the supernatant was adjusted to pH 2.8 by 1N HCl. To 1.5 ml supernatant, 0.2 ml zinc acetic acid was added followed by the addition of 0.2 ml of potassium ferrocyanide solution and centrifuged. Equal volume of supernatant and 30% HCl were mixed and incubated at 27°C for 75 min (Holbrook et al. 1961). Absorbance was measured at 250 nm in UV-Vis spectrophotometer (UV-1900, Shimadzu, Japan) and measured against a standard curve of Gibberellic acid (Sigma-Aldrich).

Additional Plant Growth Promoting (PGP) Traits

Siderophore production The Chrome Azurol S (CAS) assay was used to perform the siderophore production (Pérez-Miranda et al. 2007) ability of PGPR. Bacterial culture was inoculated onto a CAS agar plate and incubated for 48 h (35 ± 2°C) and appearance of orange or bluish zone indicates positive result.

Phosphate solubilization To test the capacity of the strain to solubilize inorganic phosphate, bacterial culture was inoculated on Pikovskaya agar medium (Pikovskaya, 1948) with considering a triplicate. After incubation at 35 ± 2°C for 72 h, observation was recorded for halo zone surrounding the colonies.

Nitrogen fixation To test nitrogen fixation ability, culture was grown on slants of glucose nitrogen-free mineral media. The slant was streaked with the bacterial isolate and was left for incubation at 35 ± 2°C for 7 days. A positive outcome is when the slants change from green to deep green or blue (Döbereiner 1989).

Ammonia and Hydrogen cyanide (HCN) production The following procedure was used to evaluate bacterial isolates for ammonia production: freshly cultured bacterial cultures were injected in 10 ml of nutritional broth and incubated in a rotator shaker (SI600, LAB Companion, Korea) at 35 ± 2ºC for 48

hours. Following incubation 0.5 ml of Nessler's reagent was mixed with 1 ml of culture. A good reaction for ammonia synthesis was suggested by the formation of a yellow to brown colour (Cappuccino and Sherman 2013; Ngoma et al. 2013). For HCN production, petri plates containing King's B medium were streaked with bacterial isolate, and each petri plate cover was fixed with a single filter paper disc and incubated for 72 hours at 35 ± 2°C. For a positive HCN production, the colour observed within the filter paper from profound yellow to light brown (Bakker and Schippers 1987).

Zinc solubilization The examination of in-vitro zinc solubilization was carried out by utilizing plate test. PGPR was tested for its capacity to solubilize insoluble zinc compounds, such as Zinc Oxide (ZnO) and inoculated on zinc medium amended by 1% ZnO (Khande et al. 2017). For seven days, that plate was kept at 35 ± 2°C incubation. Clear zone confirms the potential of zinc solubilization.

Molecular Identification of PGPR

Fresh culture of bacterial isolate was grown in nutrient broth for overnight and genomic DNA was extracted using the phenol-chloroform method (Cheng and Jiang 2006). Universal primers 8F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' TACGGTTACCTTGTTACGACTT 3') had been used to amplify its 16S rRNA gene using Primus25 thermal cycler. 10 ng of template DNA, 2.5 µl of 10× Assay buffer, 1 µl of 5 mM dNTPs, 2 µl of 25 mM $MgCl_2$, 1 µl of 10 mM forward and reverse primers, 0.13 µl of 5 Units/µL Taq DNA polymerase enzyme, and UltraPure water (Himedia) for volume adjustment were all included in the 25 µl PCR reaction mixture. Primary denaturation at 94ºC for 6 min was followed by 35 cycles of denaturation at 95ºC for 55 sec, annealing at 57ºC for 50 sec, extension at 72ºC for 90 sec and final extension at 72ºC for 7 min. This PCR product was purified (PureLinkTM, Invitrogen) and sequenced using Sanger method (Barcode Bioscience, Bangalore, India). The sequences of 16S rRNA was submitted to GenBank, NCBI. Sequence alignment was performed by BioEdit 7.0 software. BLAST searches for closest type strains were done using NCBI. The multiple sequences alignment was done using ClastalW programme and phylogenetic tree was constructed using neighbour-joining method with Bootstrap analysis (1000 repeats) in software MEGA 11.0 (Sengupta et al. 2023). The sequence was submitted to GenBank under the Accession No. OR755836.

Biochemical Characterization and FESEM analysis of selected PGPR

Following Bergey's Manual of Systemic Bacteriology, catalase, oxidase, urease, nitrate reduction activity of the isolate was analysed. The isolate was also investigated for fermentation of different carbohydrates including glucose, fructose, sucrose, maltose, lactose, D-mannitol, carboxymethyl cellulose, L-arabinose, glycerol, xylose, cellobiose, starch along with extracellular enzyme production activity like amylase, cellulase and xylanase at 35 ± 2°C for 24–72 h of incubation. 24 hours fresh PGPR sample was used to observe under Field Emission Scanning Electron Microscope. Sample preparation was performed by washing in phosphate buffer saline (pH 7.5) thrice and kept in 0.25% gluteraldehyde at 37 ºC for 24 hours. On the next day, sample was washed again for three times in sodium phosphate

buffer (7.5). Using ascending ethanol series (30% – 100%, 10 minutes each), sample dehydration had been done. The sample was kept in 100% ethanol for 2 hours. SEM stabs were prepared on adhesive tape (Rasha et al. 2021) and examined (gold coating) under FESEM (Sigma300, CarlZeiss, Germany).

Screening for Heavy metal Tolerance

Lead (Pb), Cadmium (Cd), Arsenic (As) and Chromium (Cr) are the main heavy metals used here to test heavy metal tolerance property of isolated PGPR. Minimum inhibitory concentration (MIC) of heavy metals against the isolated rhizobacteria was derived from agar well diffusion method (Hassen et al. 1998). 100 µl of 24 h fresh culture was spread over the NA plate. Then wells had been cut into the agar and fill with 30 µl of each concentration (range: 0-4000 µg/ml) of metals (Pb, Cd, As, Cr) and incubated for 24 h at 35 ± 2°C. Formation of a zone of inhibition around the well indicated that bacteria cannot grow at that concentration.

High Performance Liquid Chromatography for IAA Production

Selected PGPR was inoculated in 250 ml Erlenmeyer flask containing 100 ml of nutrient broth supplemented with 0.1% L-Tryptophan and incubated at 35 ± 2°C for 48 hours under shaking condition (120 rpm). Culture was centrifuged at 10000 rpm for 12 min. The supernatant was acidified with 1N HCL (pH 2.5–3.5). This was mixed and shaken with ethyl acetate (1:2) and the organic phase containing IAA was extracted using separating funnel and dried in rotary evaporator at 40°C. Finally dissolved in 1 ml methanol (HPLC grade, Merck) and detected in HPLC (C18 column:5 µm 4.6×250 mm, Vanquish, Thermo Fisher, USA) in methanol and water solvent system (40:60) at a flow rate of 1 ml/min, in UV-Vis detector of 280 nm. A standard of IAA (Sigma-Aldrich) of 30 µg/ml was used (Gorai et al. 2021).

Test to Detect Halotolerance and Sodium Uptake by PGPR

Freshly prepared Nutrient broth (NB) supplemented with various concentrations of sodium chloride at 0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M, 1 M, 1.5 M, 2.0 M, 2.5 M, 3.0 M were inoculated with PGPR isolate (OD_{600}=0.5) and incubated at 35 ± 2°C for 24 h. Growth absorbance was measured at 600 nm using UV-VIS Spectrophotometer (UV-1900, Shimadzu, Japan) in triplicates to detect the salt tolerance ability of PGPR. For the estimation of sodium uptake capacity by PGPR, 24 h liquid culture of the selected isolate was inoculated in NB supplemented with different salt concentrations starting from 0.1-3.0 M and incubated for 24 h in orbital shaker (120 rpm) at 35 ± 2°C (Shultana et al. 2020). After incubation cells were centrifuged at 12000 rpm for 15 min. Pellet was washed in sterile distilled water and digested overnight in 0.1 N HCl at room temperature. Supernatant was collected. Sodium uptake had been measured in Inductively Coupled Plasma Optical Emission Spectrophotometer (Perkin Elmer, USA, Optical 2100 DV ICP-OES).

Germination and Growth of Vigna radiata Treated with PGPR

Vigna radiata cultivated in pots, the impact of PGPR inoculation on plant growth was studied (Desai et al. 2023). Seeds were sterilized for 5 min using a 2.5% sodium hypochlorite solution, followed by a 70% ethanol (1 min). They were then thoroughly rinsed with sterile distilled water, dried on Whatman filter paper, and inoculated on the germination medium. Several approaches to seed treatment were used. The ideal moisture level was consistently maintained in each pot. All seeds, with the exception of the control, were submerged in 5 ml of the selected PGPR, which was cultured for 24 h in NB at a concentration of 1×10^8 CFU/ml under aseptic condition. The control seeds were kept in sterile distilled water and dried under the same conditions. 10 seedlings per pot were grown under optimal conditions (32°C/29°C day/night temperature) in a controlled environmental chamber with 10,000 lx of light at a 10 h/14 h day/night cycle. The seeds had been treated under several salt concentrations with or without PGPR like- (a) control (only sterile distilled water), (b) 0.5% sodium chloride (NaCl) salt, (c) 0.5% NaCl + PGPR SBRK15, (d) 1.0% NaCl, (e) 1.0% NaCl + PGPR SBRK15, (f) 1.5% NaCl, (g) 1.5% NaCl + PGPR SBRK15, (h) 2.0% NaCl, (i) 2.0% NaCl + PGPR SBRK15. Plant samples were harvested 45 days to study the impact of salt stress on their growth. Each treatment consisted of triplicates. Root and shoot length along with fresh and dry weight of the mung bean seedlings were measured under various salt concentrations (0.5%-2.0%). The effect of SBRK15 on chlorophyll content under NaCl concentrations had been detected using the Arnone method by applying 80% acetone as leaf chlorophyll dissolving solvent. Absorbance of Chlorophyll a and Chlorophyll b were measured at 663 nm and 645 nm respectively (Kumar et al. 2021).

Relative Water Content of leaf

The relative water content (RWC) of leaves was measured of seedlings. Initially the fresh weight (FW) was detected, and then the leaves were soaked in sterile distilled water for 6 hours. These leaves were air dried and turgid weight was measured. Finally the leaf samples were dried in hot air oven at 60 ºC to take the dry weight (Sobahan et al. 2025).

Measurement of Proline content

The proline content was analysed following the method of Sobahan et al. 2025. About 0.4 gm of fresh leaf tissue was crushed and dissolved in 3% sulfosalicylic acid and centrifuged at 12000 rpm for 15 minutes. 2 ml glacial acetic acid and 2 ml acid ninhydrin reagent were mixed with 2 ml supernatant. The reaction mixture was incubated in water bath (95 ºC) for 1 hour. After this the reaction mixture was cooled using ice and the chromophore was extracted with t5he help of toluene. The absorbance was taken at 520 nm. The prolin content of leaf was detected using the standard curve of L-proline.

Malondialdehyde content detection

Detection of oxidative stress indicator Malondialdehyde (MDA) is an important indicator of lipid peroxidation. This MDA was measured following the method of Sobahan et al. 2025. 0.5 g of leaf sample was crushed in 2 ml of 5% trichloroacetic acid (TCA), centrifuged at 12000 rpm for 15 minutes. 1 ml of supernatant was mixed with 2 ml of 20% TCA containing 0.5% TBA. This mioxture was incubated for 30 minutes in hot water bath and then cooled in room temperature. The OD was measured at 532 nm and

600 nm using UV-Vis Spectrophotometer. By using the extinction coefficient 155 mM-1 cm-1 MDA concentration was determined.

Statistical Analysis

All the tests were done in three sets to obtain standard error. The isolate showed negative result for nitrogen fixation. One way ANOVA had been performed in case of IAA, GA, sodium uptake. For evaluation of plant growth and stress alleviation in *V. radiata*, ANOVA was tested followed by Tukey test to check the significant difference at $p < 0.05$ level (Microsoft word 7.0, R programming).

Results

Isolation of Bacterial Isolates

Fifty-four rhizobacterial strains of differing colony morphologies were isolated by using yeast mannitol extract agar, king's B, Ashby's, and Nutrient agar after 24-72 h of incubation at 35±2°C.

Production of IAA

Among fifty-four tested bacterial isolates nineteen isolates were able to produce IAA (**Supplementary Fig.2**, **Table 1**). Eight isolates out of nineteen produced IAA more than 50 µg/ml. The isolate that produced the highest IAA was SBRK15 (151.6±0.01 µg/ml).

Production of GA

Twelve out of fifty-four isolates produced GA ranged from 10.32-120.09 µg/ml. SBRK08 produced highest amount of gibberellic acid (120.09±0.02 µg/ml) followed by SBRK15 (114.52±0.05 µg/ml, **Supplementary Fig.2, Table 1**). But the amount of IAA produced in case of SBRK08 (2.72 µg/ml) was very low. Further research was carried out with SBRK15.

Additional PGP Traits

The isolate was able to create an orange to yellow halo zone in the CAS agar media. This indicated its ability to produce siderophore. On Pikovskaya agar plate, SBRK15 produced clear halo zone and Phosphate solubilization index 1.67±0.04 (**Table 1**) was reported. SBRK15 was not able to produce ammonia and cannot fix nitrogen also, but showed potential for HCN production. PGPR SBRK15 showed an ability to solubilize zinc (**Table 1**) also.

Morphological, Biochemical, cellular Characterization and Molecular Identification of the Selected PGPR

SBRK15 was Gram-positive (**Fig.1b**), rod shaped, motile, spore-forming bacteria. It produced medium sized white colonies with rough surface. The biochemical features of this strain were summarized in **Table 2**. Selected PGPR was tested positive for the production of extracellular hydrolytic enzymes viz.,

cellulase, protease, lipase and amylase but was negative for xylanase enzyme. On the basis of 16S rRNA gene sequences this isolate was identified as *Bacillus safensis* accession no. OR755836 (**Fig.1a**). The cell morphology of SBRK15 was observed inder FESEM study (**Fig.1c, d**).

Heavy Metal Tolerance of SBRK15

B. safensis SBRK15 demonstrated remarkable heavy metal tolerance. MIC lies between 2500-3000 µg/ml, 100-500 µg/ml, 2500-3000 µg/ml, and 500-1000 µg/ml with lead, cadmium, chromium and arsenic respectively (**Table 3**).

Confirmation of IAA Production by HPLC Analysis

The extracted sample produced by *B. safensis* SBRK15 was characterized by HPLC technique. This gave an identical peak with commercial IAA in **Fig.2a** (Sigma-Aldrich) used here as a standard one. Extract showed a peak with a retention time of 2.268 min in **Fig.2b**.

Halotolerance and Sodium Uptake Capacity

Selected isolate *B. safensis* SBRK15 showed salt tolerance up to 2.5 M salt concentration but failed to grow in media containing 3 M salt concentration. The highest growth was found in 0.5 M salt concentration as evidenced in (**Fig.3a**). In addition to this, salt uptake result was also recorded which showed a gradual increase in sodium uptake capacity starting from 0.1-0.5 M with a maximum uptake capacity at 0.5 M concentration of NaCl which was about 169.12±0.34 mg/l. From 1.0 M of NaCl concentration its uptake potential was gradually decreased as shown in **Fig.3b**.

Germination and Growth of *Vigna radiata* Treated with PGPR SBRK15

B. safensis SBRK15 was selected for plant development in *Vigna radiata* under saline stress conditions after the halotolerant bacterial isolate demonstrated the majority of direct and indirect plant development advancing properties. Compared to control seedlings (treated with sterile distilled water), the seeds treated with SBRK15 exhibited improved growth under stress condition. *V. radiata* treated with 2% NaCl + *B. safensis* SBRK15 showed 96% germination as well as secondary root formation (**Table 4**) in comparison to the control seedlings. Under stress condition *B. safensis* SBRK15 can stimulate secondary root growth as highlighted in **Supplementary Fig**.**3**. Similar positive outcome was noticed in case of root and shoot length of mung bean seedlings in presence of SBRK15 under both normal and saline conditions (**Table 4**). As for example this rhizobacterial strain had increased root length about 2.6, 2.3, 1.9 and 1.4 fold in 2.0%, 1.5%, 1.0% and 0.5% of NaCl respectively. Similarly length of the shoot of seedlings were also enhanced in presence *B. safensis* SBRK15 like 3.9 fold under 1.5% salt stress which was highest shoot length followed by 2.0%, 1.0% and 0.5% recoded in **Table 4**. In addition, this strain had an impact on other metrics such as the fresh and dry weight of seedlings. For instance, applying SBRK15 under saline conditions increased fresh weight was noted by approximately 2.5 fold (1.5% NaCl stress), whereas applying the strain under 1.0% and 2.0% saline conditions fresh weight was increased, but there was no significant difference at $p \leq 0.05$ level in these two treatments. Mung bean seedlings primed with

B. safensis SBRK15 under saline conditions from 0.5% - 2.0% NaCl showed improvements in dry weight. In 2.0% NaCl, there was a significant increase (2.5 fold) in dry weight of seedlings inoculated with *B. safensis* SBRK15. Along with this, dry weight seedlings were also improved by 1.6 (under 0.5% NaCl), 1.8 (under 1.0% NaCl), and 2.4 (under 1.5% NaCl) fold in presence of SBRK15. Apart from these physiological parameters, photosynthetic pigments chlorophyll a and b content was also improved significantly under salt stress in bioprimed seedlings. *B. safensis* SBRK15 had increased the amount of chlorophyll a by 1.7-3.0 folds under saline condition range from 0.5% - 2.0% of NaCl. A similar increased pattern in case of chlorophyll b content was recorded. Under 2.0% NaCl, chlorophyll b was improved by 2.4 fold. Not only this, from 0.5% - 1.5% of NaCl concentrations, chlorophyll b was increased by 1.7- 2.1 folds (**Table 4**). Hence*, B. safensis* SBRK15 aided in the improved growth and development of seedlings under salinity stress.

Relative Water content in leaves

Relative water content (RWC) is a crucial parameter to check the plant health under salt stress. In this study, RWC in case of seedlings under different concentrations of NaCl was quite lower than the control and bioprimed seedlings. As indicated in **Fig.4** lowest RWC (31.2%) was observed in leaf samples of seedlings in presence of 2.0% NaCl. On a contrary, SBRK15 improved RWC (58%) under 2.0% saline stress condition. *B. safensis* SBRK15 had enhanced RWC level from 61% - 72% significantly under 0.5% - 1.5% NaCl treatments.

Proline Content

Proline plays vital role during stress condition in plants as an osmoprotectant. In this study, high proline content was recorded in mung bean seedlings under saline stress condition. Highest proline content 12.34±0.11 nmol/g of fresh weight of leaf was found in seedlings treated with 2.0% NaCl followed by the seedlings treated with1.5%, 1.0% and 0.5% of NaCl (**Fig.5**). In case of Control seedlings proline level was very less in amount as compared to the plants growing under salt stress condition. By applying rhizobacteria *Bacillus safensis* SBRK15 the salt stress condition was ameliorated at significant rate. As for example, seedlings treated with 2.0% NaCl+SBRK15 the amount of proline was decreased by 2.6 fold. Similarly, SBRK15 lowered the proline content by 2.5, 2.0 and 1.9 fold in seedlings treated with 1.5%, 1.0%, 0.5% NaCl respectively as highlighted in **Fig.5**.

Malondialdehyde content

MDA is as an indicator of oxidative stress indicator in plants. Under various salt stress condition, an elevated level of MDA was observed in seedlings. At 2% salt stress condition the MDA level was increased by 3.15 fold compared to the control seedlings. Increasing concentration of NaCl from 0.5% - 1.5%, showed enhanced MDA content in seedlings by 1.74-2.8 fold (**Fig.6**). But inoculation with *B. safensis* SBRK15 mitigated the MDA content in plants under salinity stress. *B. safensis* SBRK15 decreased the MDA level by 2.33 fold under 2.0% NaCl as indicated in the **Fig.6**. This salinity stress

mitigation property by reducing the MDA content by 1.8, 1.9 and 2.12 fold was seen under 0.5%, 1.0% and 1.5% NaCl treatments respectively.

Discussion

Salt stress is one of the most significant abiotic stress factors limiting growth of the crop globally (Behera et al., 2022). Often the overall fitness of the plant depends upon the microbes present in rhizospheric soil. Currently, application of PGPR is drawing more attention and is seen to be a very promising tool to alleviate abiotic stress in a variety of crops (Kumawat et al., 2022). Plants employed their own mechanisms to withstand salinity stress, but in addition to this soil microbial population also play crucial role for improving the tolerance of the plants. Owing to the several plants growth-promoting (PGP) attributes like production ability of IAA, GA, siderophore, phosphate, and soil microbes have positive impact on the growth and development of plants in adverse environmental conditions. The halotolerant PGPR *Bacillus safensis* SBRK15 exhibited similar PGP traits in this study, including the synthesis of a considerable amount of the phytohormones GA and IAA, which are essential for plant growth. A study showed (Shah et al. 2020) similar outcome of IAA synthesis by *Bacillus* species. IAA and GA strongly promote root growth, phototropism, cell division, seed germination, shoot growth, cell wall synthesis, all of which aid in the growth and development in plants (Bhutani et al. 2018; Kang et al., 2014). Among bacteria the genera Bacilli is one of the most important and common PGPR reported in several studies (Kumar et al. 2012; Mumtaz et al. 2017; Akinrinlola et al. 2018). Species of bacteria, including *Bacillus* spp., have the ability to mineralize and solubilize phosphate in the soil for the plants (Barea and Richardson 2015). *Bacillus* species have the capacity to solubilize insoluble zinc compounds and enhance bioavailability for plants (Mumtaz et al. 2017). The zinc from this current study demonstrated could also be soluble by *B. safensis* SBRK15. Presence of siderophore producing microbes in the rhizospheric soil makes iron available and aids in plant growth while limiting iron availability for phytopathogens (Saha et al. 2016; Sansinenea 2019). In the current investigation, it was discovered that *B. safensis* SBRK15 produced the extra PGP features discussed above. Production of IAA by SBRK15 was also confirmed with the help of HPLC technique and evidence of this was also recorded in other study (Gorai et al. 2021). Despite the ecological and economic importance of mangrove ecosystems, over the past few decades, anthropogenic activities urbanization, deforestation, pollution, and climate change have caused them to decline (Kesavan et al. 2021). Importantly, heavy metal contamination has been the subject of much research worldwide and is one of the main proximate sources of the risk to the mangrove ecosystem (Agoramoorthy et al. 2008; Aljahdali and Alhassan 2020). The ability of *Bacillus safensis* SBRK15 to withstand and thrive in metal-supplemented media indicates that it can be utilized to eliminate heavy metal pollution. In a similar vein, numerous species of *Bacillus* can eliminate heavy metals from soil and lessen pollution (Shameer 2016; Saranya et al. 2019). The survivability of bacteria under salinity condition had been found in many researches from several mangrove areas of all over the world (Mishra et al. 2023; Dey et al. 2024). The rhizospheric bacteria *B. safensis* SBRK15 grew best in a range of concentration 0.2 M to 1.5 M of salt (NaCl), but it could tolerate up to 2.0 M of NaCl. This particular strain exhibited the maximum capacity for sodium uptake 169.12 ±

0.44 mg/l at a salt concentration of 0.5 M. Because mung beans are a highly nutritious crop that humans consume, applying these halotolerant rhizobacteria to them may show to be beneficial for sustainable agriculture (Shahrajabian et al. 2019). Seed priming with *B. safensis* SBRK15 was employed to monitor its effect on the seed germination and other vegetative growth parameters of *V. radiata*, which revealed marked enhancement in the seed germination index under salinity stress condition in comparison to control seedlings (treated with sterile distilled water). Treatment with SBRK15 also enhanced root and shoot length, fresh and dry weight as well as chlorophyll contents in this dicot seedlings. Apart from these parameters, *B. safensis* SBRK15 had significant impact on relative water content in seedlings under various NaCl stress treatments. Due to the loss of turgor pressure and improper hydraulic conductance plant growth declines. One such study also revealed this similar effect of *B. subtilis* and *B. aryabhattai* on RWC of leaves (Siddika et al. 2025). Additionally, proline content was also decreased in *B. safensis* SBRK15 treated mung beans under salt stress. This indicated proper osmoregulation in seedlings under saline conditions supported by several studies (Ashraf et al. 2007; Siddika et al. 2024). Malondealdehyde crucial indicator of oxidative stress was decreased in seedlings primed with *B. safensis* SBRK15 under salt stress. Similar kind of lowering the MDA levels (low lipid peroxidation) by *Bacillus* strains had been recorded in case of other plants like wheat (Ayaz et al. 2022) and tomato (Patani et al. 2023). Under the salinity stress, halotolerance potential of SBRK15 may be the reason to alleviate this stress in the treated seedlings. Therefore this present study on stress mitigation property of plants enhanced by *Bacillus safensis* showed parity with the other researches on the Bacilli genera and that may be used as bioinoculant to promote plant growth (Akinrinlola et al. 2018; Dutta et al. 2023; Kaleh et al. 2022; Ibarra-Villarreal et al. 2021).

Conclusion

The characteristics of halotolerant PGPR that promote plant growth were discovered in the current investigation on *Vigna radiata*. *Bacillus safensis* SBRK15 was chosen depending upon its marked production of various PGP traits especially for IAA and GA production capabilities. Due to this, the isolate also influenced root, shoot length, seed germination index, chlorophyll content under salt stress condition in vitro. Apart from this, this rhizobacteria aided in the adaptation of seedlings under salinity by reducing proline and MDA contents. It also improved RWC of leaves. Therefore halotolerant SBRK15 had the potential to reduce salt stress and increase overall fitness of plant under saline environment. Still, more work needs to be done to enhance the host plant-microbe compatibility in order to boost a wide range of crops; productivity and salt tolerance especially with the PGPR which is one of the most emerging areas of research.

Declarations

The authors declare that the research has no conflict of interest.

Author Contribution

I.C. and S.B. wrote the original manuscript writing, data validation, analysis. R.C. Instrumentation Facility (Inductively Coupled Plasma Optical Emission Spectrophotometer and HPLC, Central Instrument Facility of Birla Institute of technology, Mesra, Ranchi, India). D.P. and A.N. Experimenter and Data Analysis. M.M. Formal analysis, Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Tables

Table 1 to 4 are available in the Supplementary Files section.

Figures

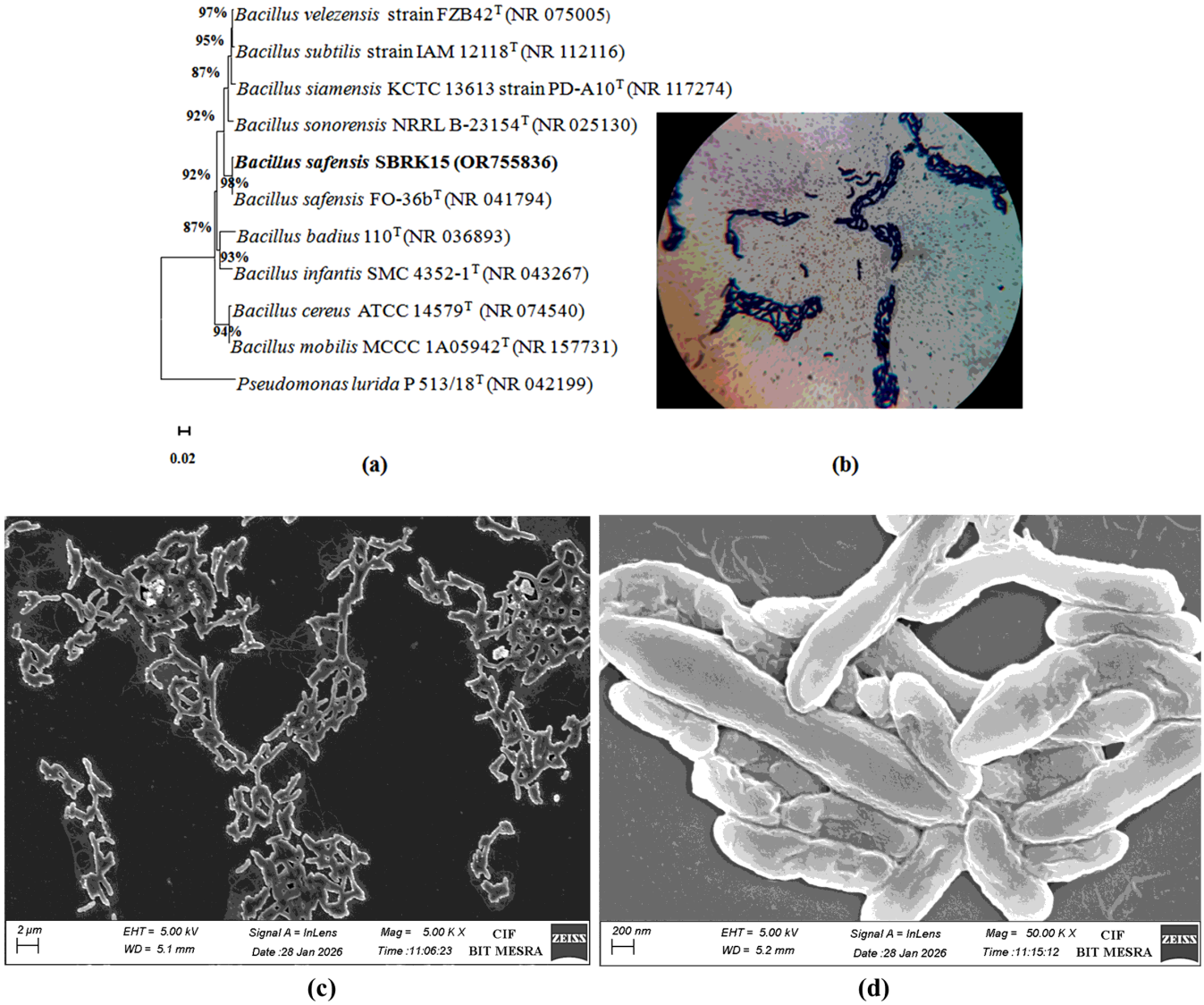


Figure 1

***Bacillus safensis* SBRK15 and its closest type strains positioned in phylogenetic tree (using neighbor-joining method, 1000 bootstrap), in which *Pseudomonas lurida* P513/18^T was presented as an out group (a), Gram staining image of *B. safensis* SBRK15 (b)**

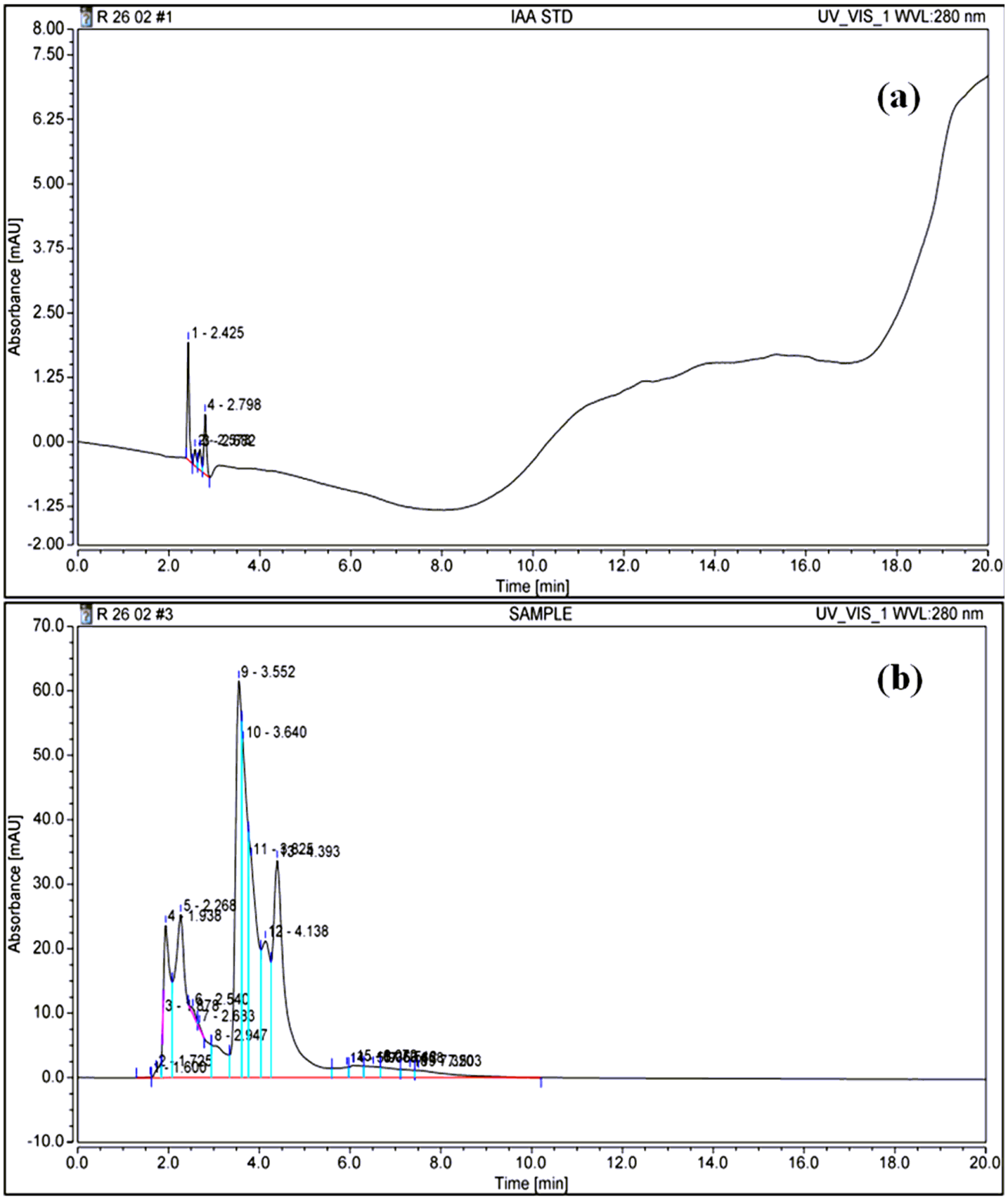
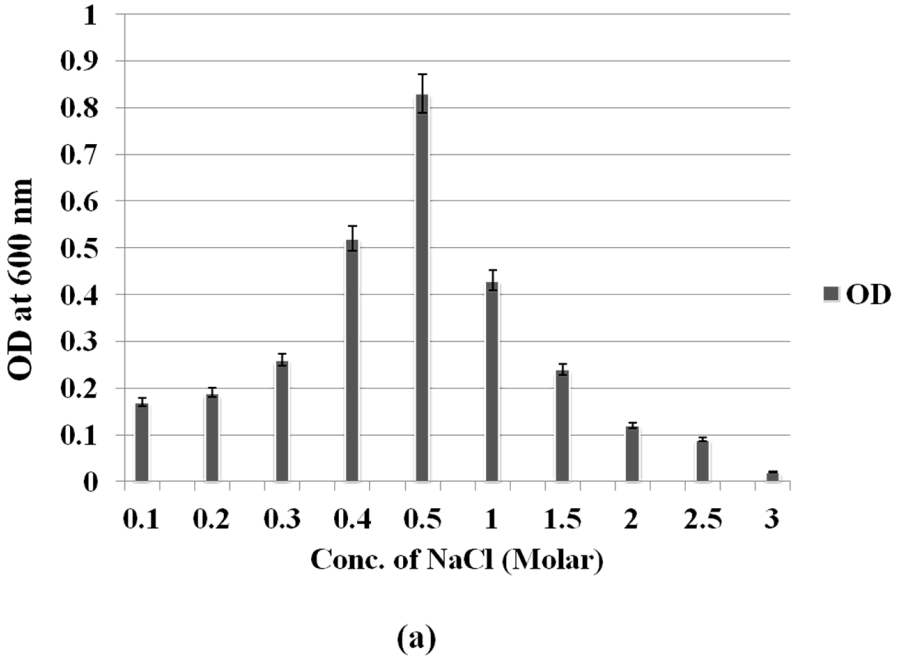
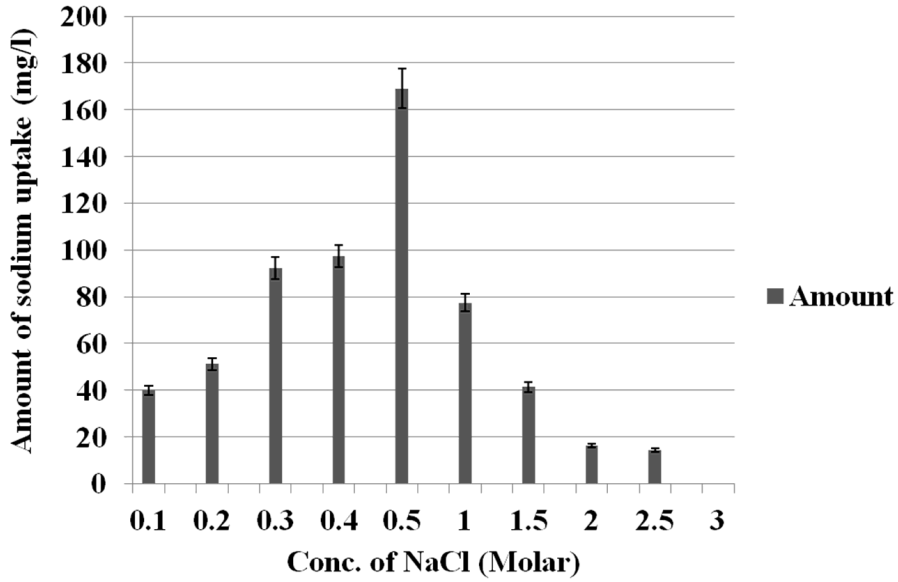


Figure 2

Detection of IAA produced by *B. safensis* SBRK15. (a) Standard IAA (Retention time 2.425 min), (b) Partially purified IAA from SBRK15 (Retention time 2.268 min)



(a)



(b)

Figure 3

Growth (tolerance) of *B. safensis* SBRK15 in presence of various concentration of NaCl (a), Uptake of sodium by *B. safensis* SBRK15 from NaCl supplemented media (b). Each bar represents Mean±Standard Deviation from triplicate tests

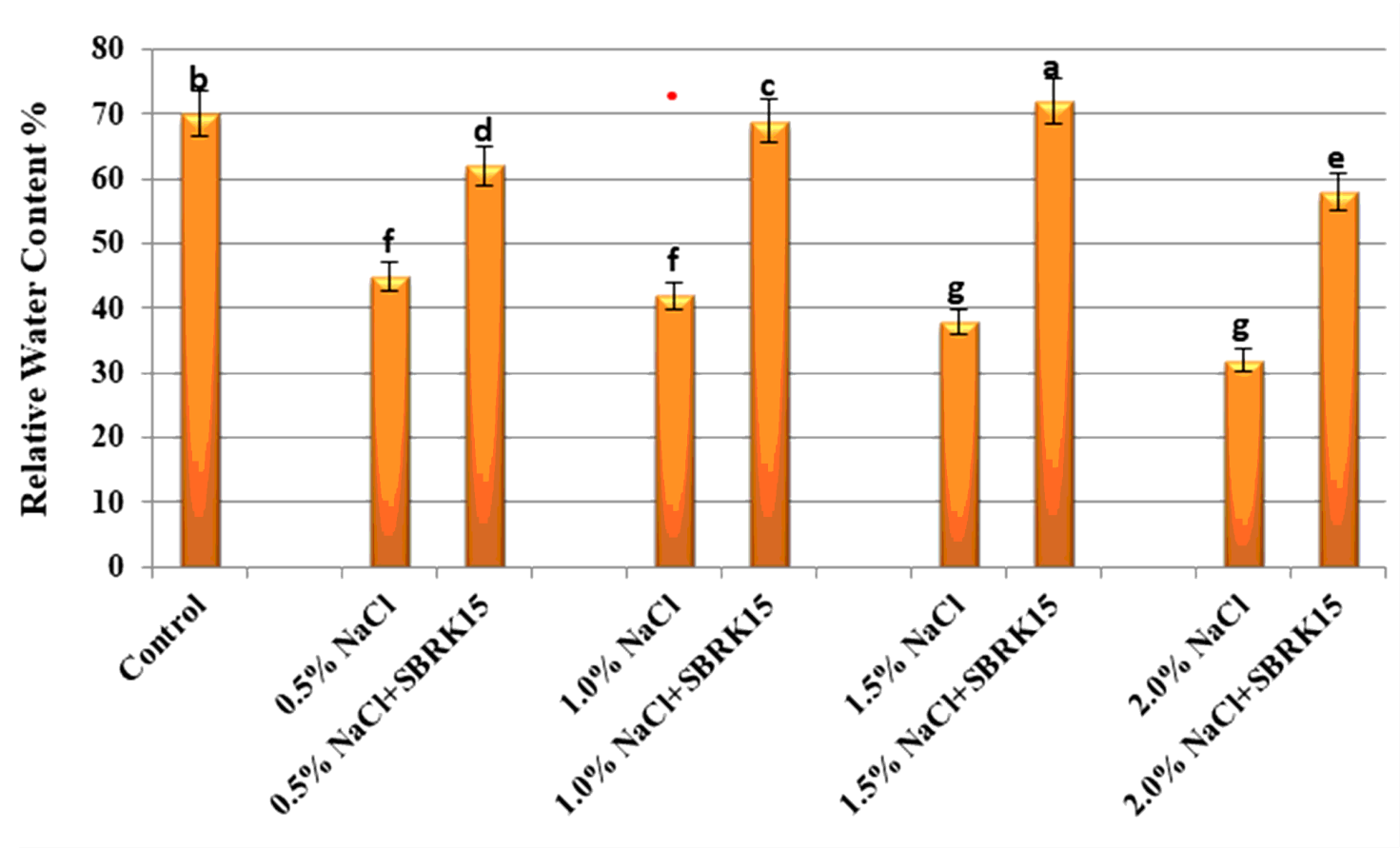


Figure 4

Effect of *B. safensis* SBRK15 on Relative Water Content in Mung bean leaves under salt (NaCl) stress conditions. Each bar represents Mean±Standard Deviation from triplicate tests. Same alphabet on bar represents no significance at p≤0.05

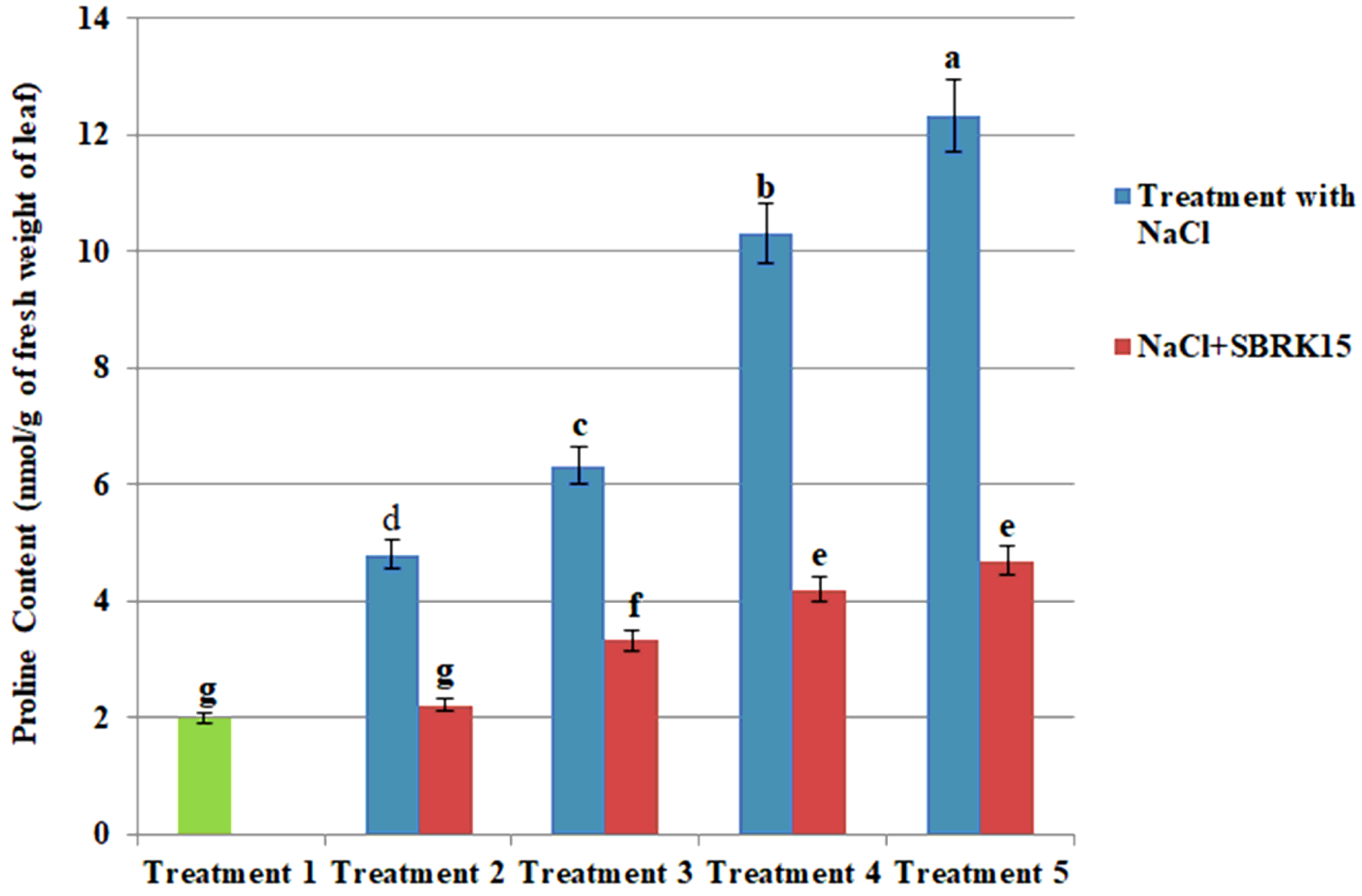


Figure 5

Proline content detected in seedlings in control (only distilled water; Treatment 1), 0.5% NaCl (Treatment 2), 1.0% NaCl (Treatment 3), 1.5% NaCl (Treatment 4), 2.0% NaCl (Treatment 5).

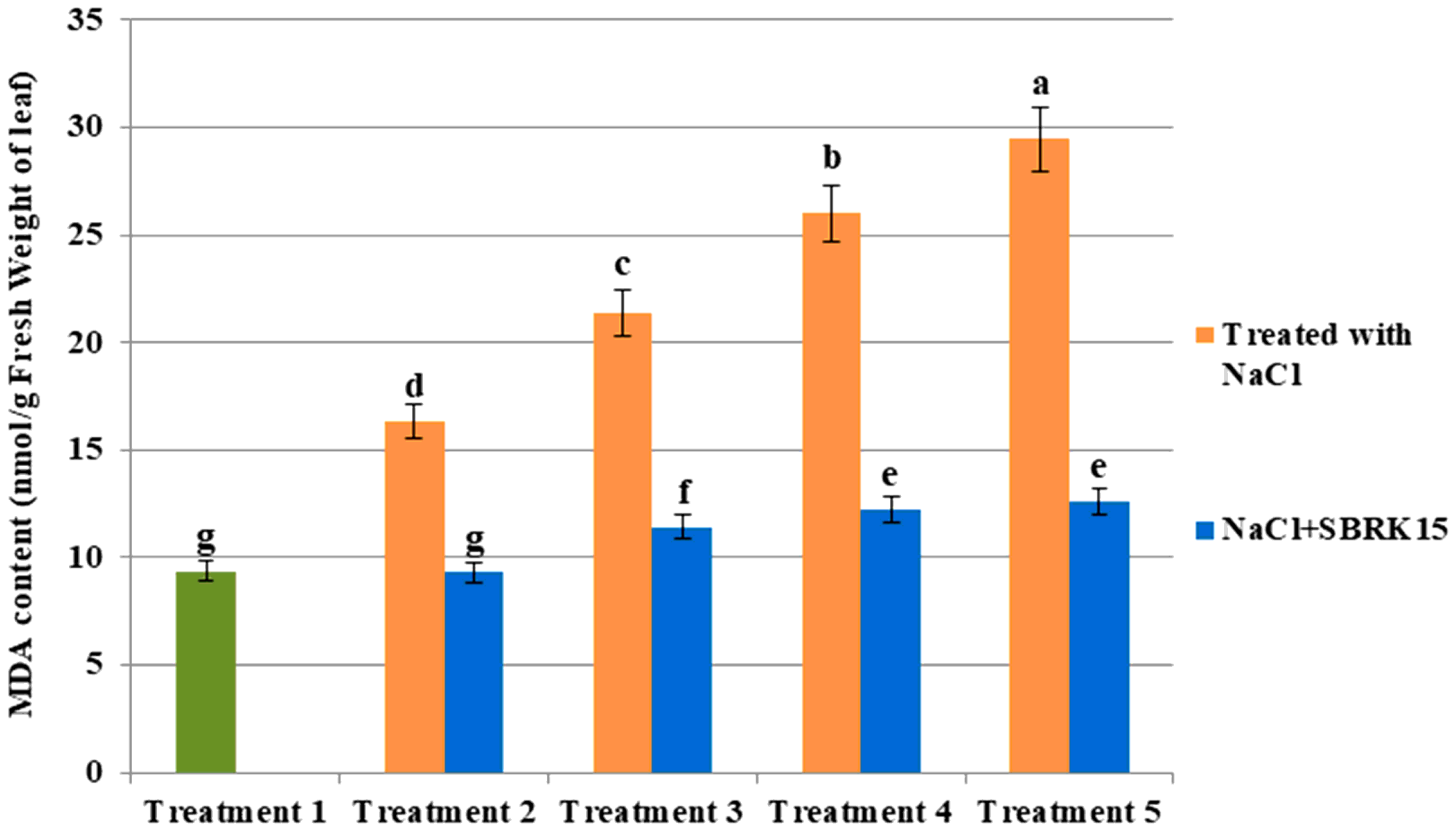


Figure 6

Malondialdehyde level in seedlings in control (only distilled water; Treatment 1), 0.5% NaCl (Treatment 2), 1.0% NaCl (Treatment 3), 1.5% NaCl (Treatment 4), 2.0% NaCl (Treatment 5).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile.docx
- TABLES.docx