PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.7793

eISSN 2348-1900

RESEARCH ARTICLE

Inoculation of indole acetic acid-producing bacteria modulates growth and biochemical response of aromatic rice

Nasrin Parvin[1*], Biyas Mukherjee[1,2], Sanchali Roy[1] & Sikha Dutta[1]

[1]Applied and Molecular Mycology and Plant Pathology Laboratory, Department of Botany, The University of Burdwan, Purba Bardhaman 713 104, West Bengal, India

[2]East Calcutta Girls' College, P 237, Lake Town Road, Sreebhumi, Kolkata 700 089, West Bengal, India

*Correspondence email - parvinnasrin3@gmail.com

Received: 18 February 2025; Accepted: 07 June 2025; Available online: Version 1.0: 23 July 2025

Cite this article: Parvin N, Mukherjee B, Roy S, Dutta S. Inoculation of indole acetic acid-producing bacteria modulates growth and biochemical response of aromatic rice. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.7793

Abstract

Indole-3-acetic acid (IAA) represents a pivotal phytohormone amongst the essential compounds involved in fostering plant growth and development, exerting its influence by provoking cell elongation, cellular enlargement and cellular division. Here, we aimed to optimize the IAA production by this selected strain and investigated its effect on the growth and biochemical status of two local aromatic rice cultivars Gobinda Bhog (GB) and Badshah Bhog (BB) as well as the soil nutrient status and their bioavailability were analyzed. The experiment was conducted in Molecular Plant Pathology and Fungal Biotechnology Laboratory, University of Burdwan, from June to December 2024. Our results indicated that fortification of rice plants with the microbial inoculant *Bacillus cereus* enhanced growth and biochemical characteristics as well as improved soil nutrient status. Culture conditions like incubation time, tryptophan concentration, carbon and nitrogen sources and their concentrations were optimized to get auxin-enriched postbiotics using the selected bacteria. Results showed maximum IAA (8.23 µM/mL) synthesis at 18 hrs of growth in 0.5 % tryptophan supplementation. Cellulose and sodium nitrate were the carbon and nitrogen sources respectively chosen by the isolate as suitable ones for the highest IAA production. Bio-priming as seed coating, seedling root inoculation and soil application, showed enhanced germination percentage, seedling height, dry biomass and tiller numbers. Further, the inoculated plants showed higher pigments, primary metabolites like soluble sugars and proteins, secondary metabolites like flavonoids and polyphenols and proline content compared to uninoculated plants. In conclusion, using this strain (*Bacillus cereus* MCC4850) has a high potency of implementing environmentally benign, economically viable and management techniques for sustainable crop production.

Keywords: aromatic rice; *Bacillus cereus*; bio-priming; fortification; indole-3-acetic acid; postbiotics

Introduction

Rice (*Oryza sativa* L.) holds significant prominence as a staple food crop, serving as sustenance for approximately 60 % of the global population (1). It is one of the highly consumed staple crops which is chiefly cultivated in developing and equatorial countries (2). Nevertheless, the significance of aromatic rice on a global scale primarily stems from its distinctive aroma properties, which have a profound impact on quality evaluation, consumer acceptance and substantial market demand. There are two special types of rice; one of them is the basmati rice cultivated in India and Pakistan, while other rice is Jasmine rice originating from Thailand fetching a higher price in the international market (3). The aroma of basmati rice is determined by intricate interplays involving the recessive gene (badh2), climatic factors, soil characteristics and geographical positioning (4). The aroma characteristics of basmati and other aromatic rice cultivars are undergoing a progressive decline (1, 4). The gradual decline in the aroma characteristics of basmati rice could potentially be attributed to the disruption of rhizospheric microflora resulting from the implementation of contemporary agricultural techniques and the excessive application of inorganic fertilizers (5).

The rhizospheric microflora encompass a group of microorganisms known as Plant Growth-Promoting Rhizobacteria (PGPR). These PGPR have the ability to enhance plant growth, increase crop yields and improve soil characteristics. They achieve this by suppressing the growth of harmful plant pathogens, mitigating the impact of both non-living and living environmental stressors and augmenting the diversity of microorganisms present in the soil (6). PGPR have many beneficial traits that directly or indirectly stimulate plants. Some of the mechanism include the auxins (IAA), cytokinins and gibberellins production, aminocyclopropane-1-carboxylic acid deaminase (ACC) production, atmospheric nitrogen fixation, phosphorus solubilization, lytic enzymes production (chitinase, cellulase, protease, glucanase), siderophore production, Induced Systemic Resistance (ISR) and antibiotic lipopeptides production (7). Various PGPR have been isolated from paddy

soil such as *Acinetobacter, Aeromonas, Agrobacterium, Allorhizobium, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Caulobacter, Chromobacterium, Delftia, Enterobacter, Flavobacterium, Frankia, Gluconacetobacter, Klebsiella, Mesorhizobium, Micrococcus, Methylobacterium, Paenibacillus, Pantoea, Pseudomonas, Rhizobium, Serratia, Streptomyces, Thiobacillus* and *Variovorax* (5, 8-10). One such PGPR is *Bacillus cereus* occur in rice rhizosphere and is capable of direct stimulation of plant growth and secondary metabolite production (11). Growth regulator, like IAA acid production, is one of the major mechanisms exhibited by soil *Bacillus* which directly play role in plant growth promotion (12). As, IAA is the primary constituent of the auxin group and serves as a pivotal factor in various plant processes spanning from seed germination to plant maturation (13). IAA is known to stimulate both rapid responses (e.g. increases in cell elongation) and long-term responses (e.g. cell division and differentiation) in plants (14). L-tryptophan, an essential amino acid, plays a crucial role as a physiological precursor in the biosynthesis of IAA in both plants and microbes (15). Root exudates serve as a naturally occurring reservoir of L-tryptophan for microorganisms residing in the rhizosphere. This influx of L-tryptophan has the potential to stimulate the production of IAA within the rhizosphere (15). Microbial biosynthesis of IAA in soil is enhanced by L-tryptophan from root exudates or decaying cells (16). The process of microbial biosynthesis of IAA in soil is facilitated by the presence of L-tryptophan derived from root exudates or decomposing cells (16).

In the context of sustainable crop production, it is widely recognized that the interactions between plants and microorganisms in the rhizosphere are crucial for optimal plant growth and development (17). Consequently, there is a growing interest in employing biological methods to enhance plant growth and development. There has been extensive research into the trend of PGPR-facilitated plant growth advancement. However, we still don't fully understand how IAA producing rhizobacteria, especially *Bacillus cereus*, affects plant growth. Furthermore, there has been a scarcity of scientific studies examining the growth and productivity of native aromatic rice cultivars in relation to microbial inoculation. The study hypothesized that the inoculation of rhizobacterial isolates (specifically *Bacillus cereus*) capable of producing IAA would have a positive impact on the growth, yield and biochemical characteristics of aromatic rice varieties. Therefore, our current study was aimed to assess the IAA production capability of the chosen strain and examine its impact on the initial growth, yield, metabolite status, soil nutrient status and bioavailability of local aromatic rice cultivars such as BB and GB through pot culture experiments.

Material and Methods

Selection of plant and microbial material

Two local aromatic rice cultivars (GB and BB) were selected and the paddy samples were collected from the Crop Research and Seed Multiplication Farm (CRMF) of The University of Burdwan, West Bengal, India. Seeds were then germinated and maintained in a plant growth chamber (relative humidity 70 to 80 %, temperature 45 ± 5 °C, illumination 270 µE/m^2/s, photoperiod 8 hrs) with microbial treatments. Seedlings (15-day-old) were then transferred to earthen pots (20 per pot) and watered on each alternate day for up to 90 days. After maturing paddy samples were taken out from the plant and dehulled manually. Rice was then powdered (in 20 mesh), sealed in glass vials and kept at room temperature for the analysis. The potent growth-promoting rhizobacteria *Bacillus cereus* (accession no. MCC 4850) was selected as the microbial inoculant and collected from the Molecular Plant Pathology and Fungal Biotechnology Laboratory, Department of Botany, University of Burdwan from June to December 2024. Working cultures were routinely grown at 25 °C on nutrient broth (NB) and stored at -80 °C as stock cultures, using glycerol (20 % w/w) as a cryoprotectant.

Test of IAA-producing ability of the bacteria

The ability of the strain to produce IAA was first tested qualitatively by streaking in a nutrient agar plate supplemented with 0.5 % L-tryptophan. Plates were wrapped and kept in a BOD incubator for 48 hrs at 30 ± 2 °C. After bacterial colonies appeared 5 mL of Van Urk Salkowsky reagent (1 % 0.5 M FeCl3 in 35 % perchloric acid) was added (16). For quantification of the IAA production, the bacterial isolate was grown in NB media and centrifuged (at 12000 rpm) after growth. An equal amount of fresh Salkowsky reagent was added to the culture supernatant. Pink color development suggested IAA production and the optical density of the colored product was measured at 533 nm after 30 minutes and 120 minutes. The quantity of IAA produced was then calculated using a calibration curve made with standard IAA (HIMEDIA). The measurements were given in µM IAA equivalents per mL of liquid culture. Using a non-inoculated medium containing tryptophan as a control, no reaction product was found.

Extraction and purification of IAA for TLC

IAA acid was extracted and purified following the previous method with some modifications. yeast malt dextrose was used for the inoculation of the bacterial strain. After growth broth was centrifuged at 10000 rpm for 15 min three times. The supernatant was taken and mixed with ethyl acetate (1:2). The mixture was mixed vigorously in a cyclomixer for 2 min and kept for 10 min. IAA was extracted from the bottom of the solvent layer with a micropipette. For TLC glass plates were taken and a uniform layer was prepared with silica gel G (Merk, Germany) and calcium carbonate (thickness 0.25 mm). Ethyl acetate: acetone: acetic acid (65:55:10) was used as a solvent system. The extracted IAA and the standard IAA (HIMEDIA) were prepared (10 mg/100 mL) and spotted on plates (20µL) at an equivalent distance (18). The Salkowsky reagent was sprayed on plates and spots with R_f (retardation factor) values identical to authentic IAA were identified. The R_f value was calculated using the following formula:

$$Rf = \frac{A}{B} \qquad \text{(Eqn.1)}$$

Where A= distance spot travels, B= distance solvent travels

Optimization of media and physical factors for IAA production

Auxin production is influenced by tryptophan, vitamins, salt, pH, temperature, carbon supply, nitrogen source and growth phase. Following the modified method of some other workers, several media and physical factors (growth phase, tryptophan concentration, carbon and nitrogen sources and their concentrations) were optimized for IAA production (16, 19).

IAA-producing ability in different stages of growth was detected by inoculating the bacteria in NB media supplemented with 0.5 % tryptophan and turbidity was measured in a spectrophotometer at 600 nm which represents the growth at an interval of 2 hrs. IAA was quantitively estimated in each interval time (16). L-tryptophan being the IAA precursor, has a great impact on IAA production by bacteria. Here, its effect was studied using NB medium supplemented with 0.5 % glucose and L-tryptophan at final concentrations in the medium of 0.05 %, 0.1 %, 0.5 %, 1 % 1.5 % and 2 % then incubated at 30 ± 5 ° under shaking condition (120 rpm) for 2 days. For the carbon source optimization of the media, different sugars of monosaccharide (glucose, arabinose and fructose) disaccharide (lactose, maltose, sucrose) and polysaccharide (cellulose and starch) were supplemented in the minimal media. All sugars were added at 3 % concentration in media along with 0.5 % tryptophan supplementation. For, furthermore investigation of the suitable concentration of carbon source, glucose was supplemented with 0.5 % to 2.5 % concentration in the media. Same as that, for the selection of the best nitrogen source to get the highest amount of IAA, different nitrogen source was used (peptone, tryptone, yeast extract, glycine, ammonium sulfate, sodium nitrate and potassium nitrate) in 0.2 % concentration. Again, tryptone was supplemented with 0.5 % to 2.5 % concentration in the media as peptone was observed to be the most suitable nitrogen source.

Pot culture experiment

Earthen pots (15 cm height and 7.5 cm width) each filled with 2kg soil mixed with sterilized sand at a 3:1 soil/sand ratio, autoclaved for pot culture experiments. Two selected local aromatic rice variety seeds (GB and BB) were surface sterilized and germinated in a plant growth chamber and 15-day-old seedlings were transferred to the earthen pots (20 per pot). All sets were bio-primed at three different phases that is at the germination stage, seedling root treated and soil treated after transplantation each with a replica (20). For soil treatment 20 mL of bacterial cell suspension was added to the autoclaved soil at a cell density of 1.5 X 10^8 cfumL^{-1} and for germinating seeds and seedling treatment roots were dipped in bacterial culture containing pots at a density of 1.5 X 10^8 cfumL^{-1} in 0.1 % carboxy methyl cellulose while un-inoculated pots served as control. After bacterization rice seedlings were planted in the pots and kept in greenhouse conditions (temperature 35±5 °C and relative humidity 70±10 %). To determine the root colonization potential of the inoculated bacteria serial dilution plating technique was done every 15 days using NB agar and the number of viable cells was estimated as colony-forming units (CFU) (21). Irrigation of the plants with sterile distilled water was done every day. The plant was uprooted and seedlings were measured for the shoot and root length and pigment content up to the 15th day.

Plant growth parameters

At the end of the treatment period in the plant growth chamber, the height of the entire plant (including the shoot and root) was measured using a centimeter scale. To measure the root shoot dry weight, fresh samples were dried in a hot air oven at 80 °C for 48 hrs. At the end of pot culture treatment mature plants developed tiller and per plant tiller number was counted.

Germination percentage

The Germination Percentage (GP) of the control sets (without microbial inoculation) and treated (with bacterial inoculation) were calculated by using the following formula (22):

$$GP = \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds in all sets}} \times 100 \qquad \text{(Eqn.2)}$$

Total chlorophyll and carotenoid content

For the estimation of chlorophyll and carotenoid contents by 0.5g fresh leaves homogenized in 80 % acetone. The extract was centrifuged several times (10000 rpm) to become colorless and subsequently analyzed by a UV-VIS spectrophotometer at 645, 663 and 470 nm wavelengths. The equation and specific absorption were used to calculate the concentrations (23):

Total chlorophyll (mg/g FW) =

$(20.2\ A_{645} + 8.02\ A_{663}) \times$ total volume of filtrate / 1000 × tissue weight (Eqn. 3)

Chlorophyll a (mg/g FW) =

$(12.7\ A_{663} - 2.69\ A_{645}) \times$ total volume of filtrate / 1000 × tissue weight (Eqn. 4)

Chlorophyll b (mg/g FW) =

$(22.9\ A_{645} - 4.68\ A_{663}) \times$ total volume of filtrate / 1000 × tissue weight (Eqn. 5)

Total carotenoid (mg/g FW) = $(1000\ A_{470} - 1.82\ C_a - 85.02\ C_b)/198$ (Eqn. 6)

Where, FW-Fresh Weight, A_{645}, A_{663} and A_{470} are absorbance at 645,663 and 470 respectively, C_a -concentration of chlorophyll a, C_b concentration of chlorophyll b (mg/g FW).

Total soluble sugars

For this 1g of the leaf sample was taken chopped into pieces and placed in a test tube. To it, 10 mL of HCl (2.5 N) was added and heated in a boiling water bath (100 °C) for 3 hrs. Sodium carbonate is added until effervescence stops. Centrifugation of the solution was done at 10000 rpm (10 minutes) and the supernatant was taken. To 1mL of the supernatant, 1mL of 5 % phenol was added followed by the addition of 5mL concentrated sulphuric acid. After incubation for 30 minutes, absorbance was taken at 490 nm (24). The concentrated acid here converts the complex carbohydrates into simple sugars so a standard solution of glucose (5 to 25 µg/mL) was prepared. The total soluble sugar was thus expressed as mg glucose/g fresh weight (FW).

Total soluble protein

The presence of aromatic amino acids in the proteins is required for the measurement of protein concentration by UV absorption. This approach can be quite accurate when comparing various solutions of the same protein, even though different proteins will have varied amino acid compositions and thus varying molar absorptivities (25). For the experiment reagent A prepared by mixing alkaline sodium carbonate (2 %) and sodium hydroxide (0.1 N). Reagent B was prepared by mixing copper sulfate (0.5 %) and potassium sodium tartrate (1 %). Next Alkaline copper solution was prepared freshly by mixing reagents A and B (50:1). The plant extract (0.2 mL) was mixed with 5 mL of alkaline copper solution and kept for 10 minutes. 0.5 mL of diluted folin-ciocalteau reagent (1:1) was added to the mixture followed by incubation in the dark for 30 minutes. The absorbance was recorded at 740 nm using an ultraviolet-visible spectrophotometer (SHIMADZU UV 19001, Kyoto, Kansai, Japan). Total soluble protein content was calculated using bovine serum albumin (BSA) as a reference protein. A range of concentrations (10 to 100 µg/mL) was made for the preparation of the standard curve of BSA and the absorbance data of the plant samples were plotted against this curve and the quantity was expressed as mg protein/fresh weight (FW).

Total Flavonoid Content (TFC)

For the estimation of flavonoid content, the leaf sample was crushed in 80 % acetone. To 0.5 mL of leaf extract, 4 mL of distilled water and 0.3 mL of $NaNO_2$ (50 %) were added. After 5 minutes of incubation, 0.3 mL $AlCl_3$ (10 %) was mixed thoroughly and incubated for another 5 minutes. Following the addition of 2 mL NaOH (1 M) to it the volume was made up to 10 mL using 95 % ethanol. Absorbance was taken in a UV-VIS spectrophotometer (SHIMADZU UV 19001, Kyoto, Kansai, Japan) at 510 nm using quercetin (QE) as the standard compound. The standard curve of quercetin was prepared with different concentrations (ranging from 100 to 1000 µg/mL) and absorbance data of the samples was analyzed using the standard curve. TFC in the plant samples was represented as the mg quercetin equivalent per gram of the fresh weight (mg QE/g FW) (20).

Total Polyphenol Content (TPC)

The total polyphenol content of the sample was estimated by the folin-ciocalteau reagent which acts as an oxidizer of phenolic compounds (26). Methanolic extracts (1 mL) from rice leaf samples were mixed thoroughly after being added to water: methanol (1:1, 1 mL, v/v), distilled water (3 mL) and newly made folin-ciocalteau reagent (0.5 mL). After 5 min, 1 mL of 5 % weight-to-volume (w/v) sodium carbonate solution was added to the mixture, which was then maintained at 25 °C in the dark for the following 60 min. A UV-VIS spectrophotometer (SHIMADZU UV 19001, Kyoto, Kansai, Japan) was used to determine the absorbance of the samples against blank at a wavelength of 765 nm. Calculation of TPC was made using gallic acid (GAE) as standard. So, a standard calibration curve of gallic acid with a range of concentrations (1000 to 5000 µM/mL) was generated using a straight-line equation. TPC was determined after the calibration curve was examined and the results were expressed as the mM gallic acid equivalent per gram of fresh weight (mM GAE/ g FW).

Statistical analysis

Measurements were made in triplicate for each sample and data were presented for at least three separate examinations of the same extract. SPSS 20.0 was used to conduct statistical studies, including analysis of variance (ANOVA) and Pearson correlation coefficients. The significance of the difference was determined at the 0.05 level.

Results

Test of IAA-production and extraction of IAA by TLC

The production of IAA by the bacterial strain was analyzed using the Salkowski reagent, a well-established colorimetric method for detecting auxin compounds. Upon reagent addition, color development was initially observed at the highest IAA concentration within a few minutes, with the intensity progressively increasing over a period of 30 to 40 min under dark conditions. This gradual intensification of color indicates the presence and stability of IAA within the bacterial extract. To further confirm IAA production, the ethyl acetate extract of the bacterial strain was subjected to TLC. The appearance of distinct bands corresponding to the standard IAA provided additional evidence of auxin synthesis. The chromatographic separation was performed using a solvent system composed of benzene: acetone: acetic acid (65:25:10, v/v/v), which proved to be an efficient medium for resolving IAA. The retention factor (R_f) value of the bacterial IAA was determined to be 0.81, closely matching the R_f value of the standard IAA (0.79), further confirming the identity of the synthesized auxin (Fig. 1).

Optimization of media and physical factors for IAA production

A higher auxin level was obtained at 500 µg/mL tryptophan supplementation in 72 hrs of growth, mannitol being the best -employed sugar whereas yeast extract was the suitable nitrogen source. We also have comprehensively characterized the factors regarding IAA production by *Bacillus cereus* (MCC 4580). The isolate showed maximum production of 8.23 µM/mL at 0.5 % tryptophan supplementation at 18 hrs of growth (Fig. 2, 3). Carbon source greatly affects the overall efficiency of IAA biosynthesis as an energy source and increases the recycling of co-factors in cells. Among several simple to complex sugars (cellulose, starch, sucrose, lactose, maltose, fructose, glucose) cellulose in 0.6 % was best utilized as a carbon source (9.05 µM/mL) followed by lactose (7.08 µM/mL) and sucrose (4.31 µM/mL) (Fig. 4, 5). We replaced the nitrogen source yeast extract with various organic and inorganic nitrogen sources namely lysine, glycine, peptone, potassium nitrate, sodium nitrate and ammonium sulfate (Fig. 6, 7). In this screening, optimum IAA was produced in the presence of 0.4 % sodium nitrate (20 µM/mL).

Pot culture experiment

In the present study, two rice varieties, GB and BB, were fortified with an efficient IAA-producing bacterial strain, *Bacillus cereus*, through a combination of seed coating, root inoculation and soil inoculation to enhance plant growth and development. To ensure effective bacterial colonization, the paddy seeds were first subjected to surface sterilization to eliminate potential contaminants, followed by coating with

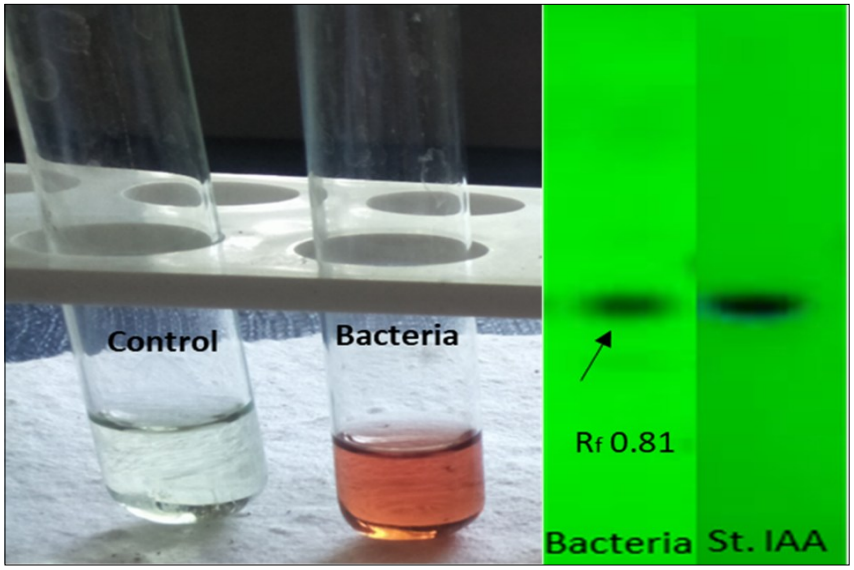

Fig. 1. A-Quantitative IAA production by the selected bacteria; B-TLC plate showing IAA production by the bacteria.

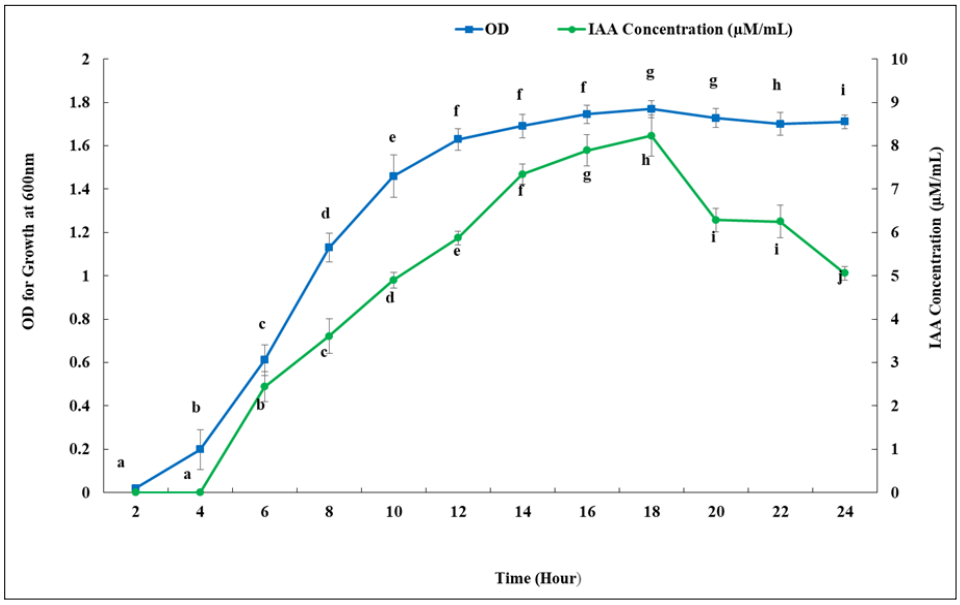

Fig. 2. Optimization of IAA production by the bacteria in different time intervals (2 hrs) of its growth.

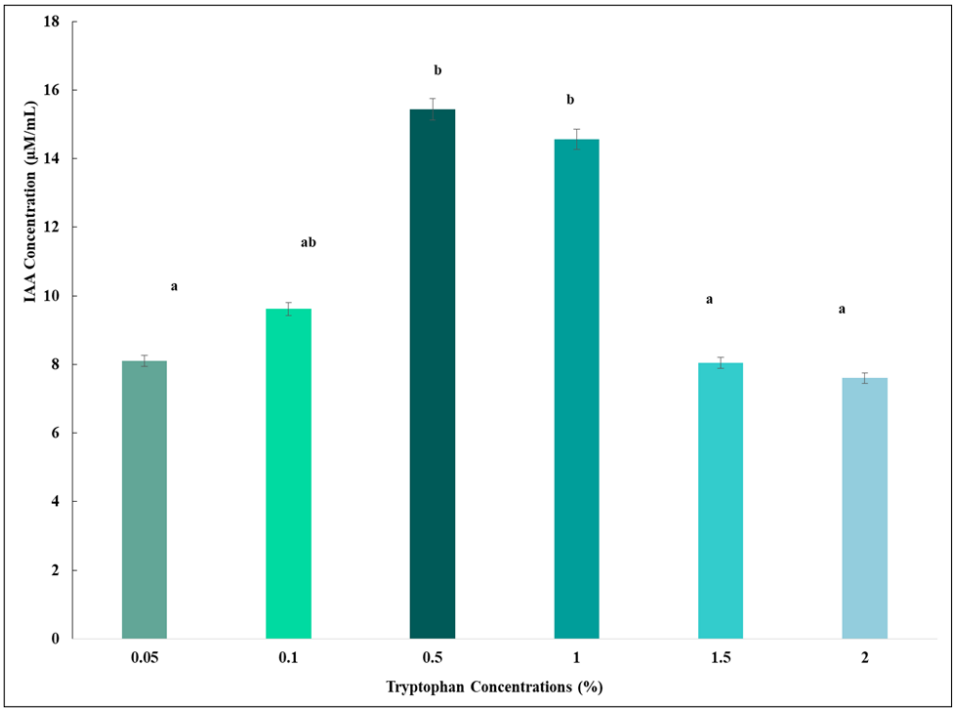

Fig. 3. Quantification of IAA production by the bacteria in different tryptophan concentrations (%).

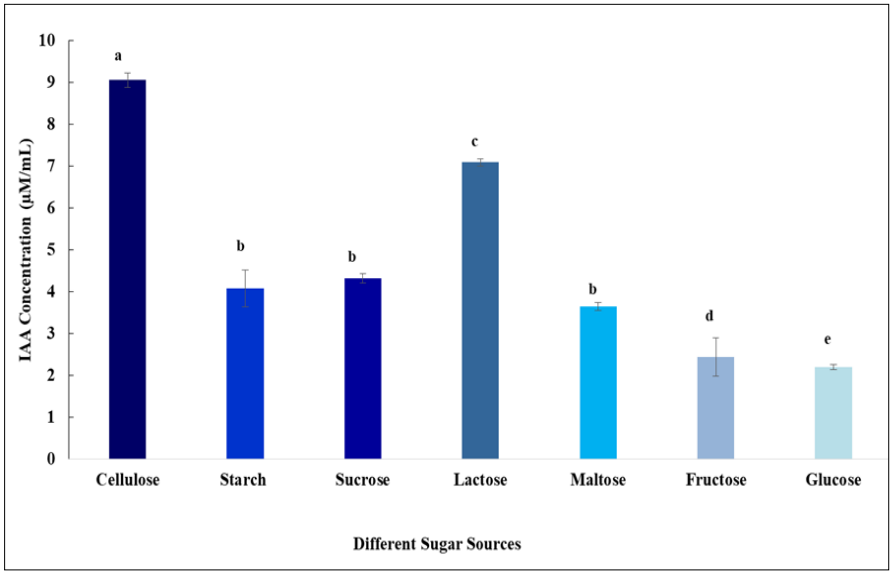

Fig. 4. Quantification of IAA production by the bacteria in different carbon sources in media.

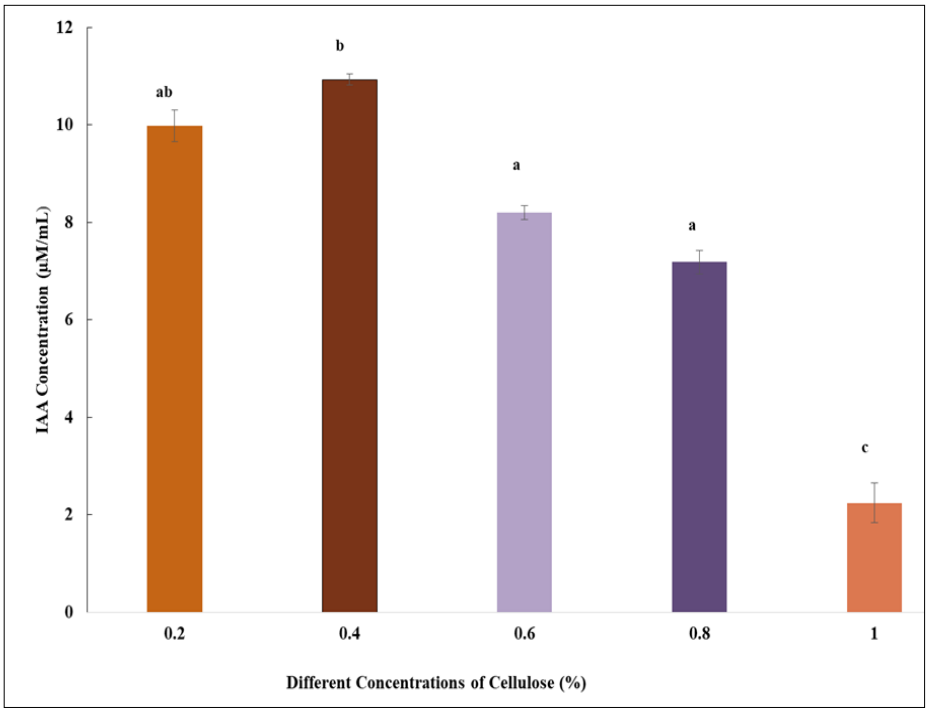

Fig. 5. Quantification of IAA production by the bacteria in different concentrations (%) of selected carbon source (cellulose).

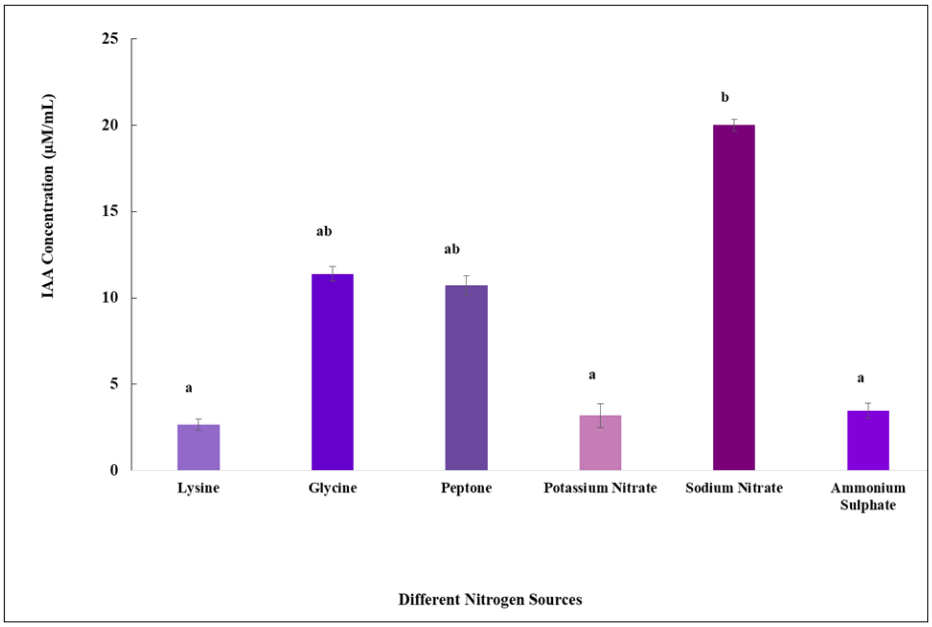

Fig. 6. Quantification of IAA production by the bacteria in different nitrogen sources in the media.

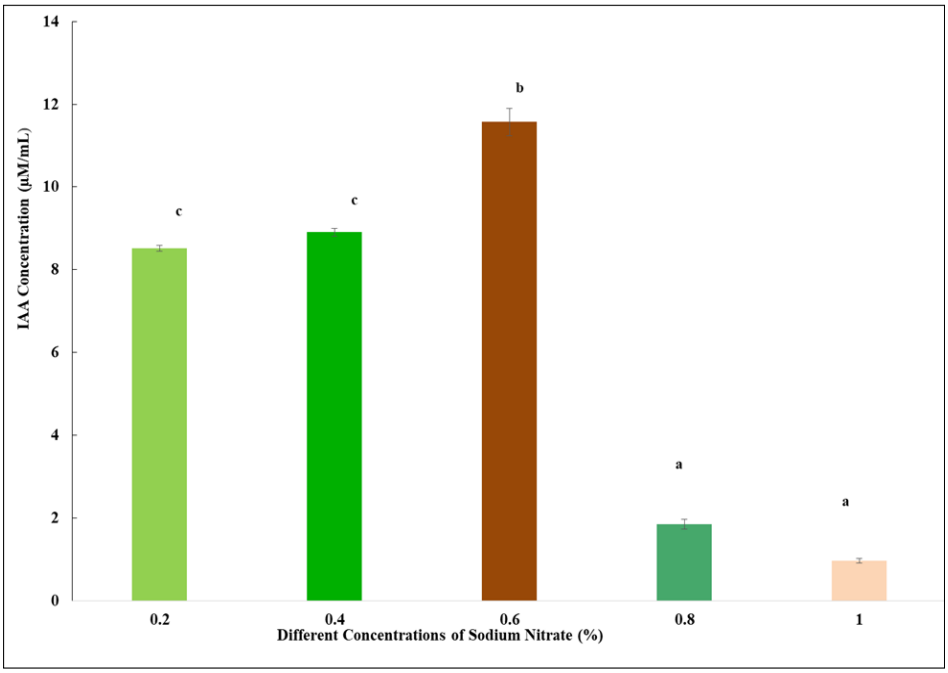

Fig. 7. Quantification of IAA production by the bacteria in different concentrations (%) of selected nitrogen source (sodium nitrate).

the prepared bacterial inoculum to facilitate early-stage microbial association. The second phase of inoculation involved root treatment, wherein 15-day-old nursery seedlings, uprooted for transplantation, were exposed to the bacterial strain through direct root inoculation. This method was designed to promote plant-microbe interactions at a critical early growth stage. Additionally, soil fortification was performed by introducing the bacterial inoculum directly into the potting soil, creating a microbially enriched rhizosphere. To ensure bacterial persistence and continued plant-microbe interaction, soil treatment was repeated at 20-day intervals throughout the experiment. Control pots, which received no bacterial inoculation, were maintained under identical conditions for comparative analysis (Fig. 8).

Plant growth parameters

Consistent with previous findings, the influence of bacterial fortification on plant growth responses was evident, as the rice plants in the treated pots exhibited significant improvements across all measured growth parameters compared to the control group. Upon bacterial inoculation, the two rice varieties demonstrated distinct growth responses. The impact of bacterial production of IAA was particularly pronounced in terms of root and shoot elongation as well as germination rates. In the case of shoot length, the BB variety exhibited a more substantial increase, reaching 1.25 times the length observed in the control plants, whereas the GB variety showed a 1.17-fold increase. Conversely, the enhancement in germination rate was more pronounced in GB, which demonstrated a 1.15-fold increase compared to a 1.11-fold increase in BB. Beyond elongation, the bacterial treatment also stimulated improvements in rootlet formation and shoot branching, which were further reflected in the accumulation of plant dry biomass. Notably, the root dry weight of GB rice increased by 1.38-fold following inoculation, whereas BB exhibited a 1.20-fold increase. Additionally, the number of tillers per plant was significantly enhanced in both rice varieties, further substantiating the beneficial effects of bacterial fortification (Table 1).

Total chlorophyll and carotenoid content

The present study revealed that pigment accumulation, including chlorophyll a, chlorophyll b and carotenoids (expressed in mg/g), reached its peak at 120 days of plant growth. Comparative analysis demonstrated that pigment concentrations were significantly higher in mature plants (120 days old) compared to those at an earlier growth stage (60 days old). A notable increase in pigment content was observed in the GB variety during the later growth stage, where chlorophyll levels exhibited a 1.37-fold increase and carotenoid content increased by 1.5-fold relative to the control. Similarly, in the BB variety, total chlorophyll and carotenoid content were enhanced by 1.2-fold and 1.14-fold, respectively, at 120 days of growth (Fig. 9). These findings suggest that pigment biosynthesis and accumulation are more pronounced in the mature phase of plant development, potentially contributing to enhanced photosynthetic efficiency and overall plant vigor.

Total Soluble Sugar (TSS)

TSS can be used to determine plant productivity. We have observed an increase in total soluble sugar content in seedling leaves indicating the important role of the inoculum which consequently plays an important role in plant metabolism, growth and hormone signaling. Soluble sugars of the plant have the potential to serve as markers for the management of abiotic stress. In the present study, GB variety treated plants yielded 28.42±0.53 mg/g (60 days) and 48.16±0.5 mg/g (120 days) of soluble sugar which is higher than plants without any treatment. A similar pattern of results was found in the BB variety (Fig. 10). High sugar concentration in seedlings may indicate enhanced development and production.

Total Soluble Protein (TSP)

Soluble proteins, the majority of which function as enzymes play a pivotal role in regulating various metabolic processes in plants. The concentration of soluble proteins serves as a critical biochemical indicator for assessing overall metabolic activity and physiological status (27). In the present study,

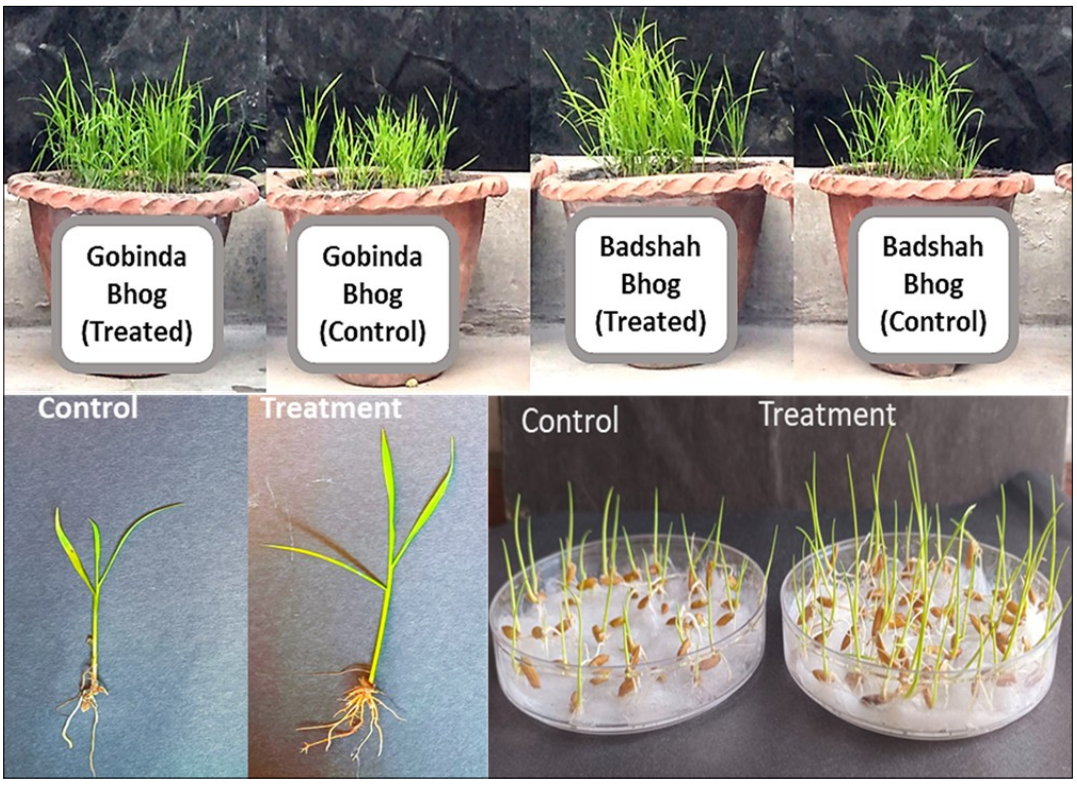

Fig. 8. A-Influence of bacterial inoculation in different rice cultivars in pot culture experiments (20-day-old);
B-Effects in shoot and root growth in the 15-day-old seedlings after inoculation;
C-Rice seeds in the germinating stage in control and bacterial inoculation conditions (1-3-day-old).

Table 1. Various growth parameters of GB and BB rice after bacterial inoculation (growth parameters at 120 days)

Rice varieties	Sets	Germination percentage (%)	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)	Tiller No. per plant
Gobinda Bhog	Control	83.33±0.94^{a}	9.9±0.95^{a}	70.5±4.30^{a}	4.4±0.03^{a}	7.8±0.05^{b}	2.5±0.5^{b}
	Treated	96.66±1.07^{b}	19.6±1.30^{b}	82.6±5.45^{b}	6.1±0.33^{a}	8.32±0.02^{a}	6.8±0.5^{a}
Badshah Bhog	Control	86.66±1.84^{a}	11.2±0.88^{a}	80.1±2.72^{b}	4.9±0.42^{a}	6.5±0.11^{b}	3.1±0.5^{b}
	Treated	96.66±2.00^{b}	14.9±2.15^{ab}	99.7±5.36^{c}	5.9±0.16^{a}	8.96±0.02^{a}	6.4±0.5^{a}

[Different letters indicate statistically significant differences between groups (mean ± SD, n = 3, p < 0.05)]

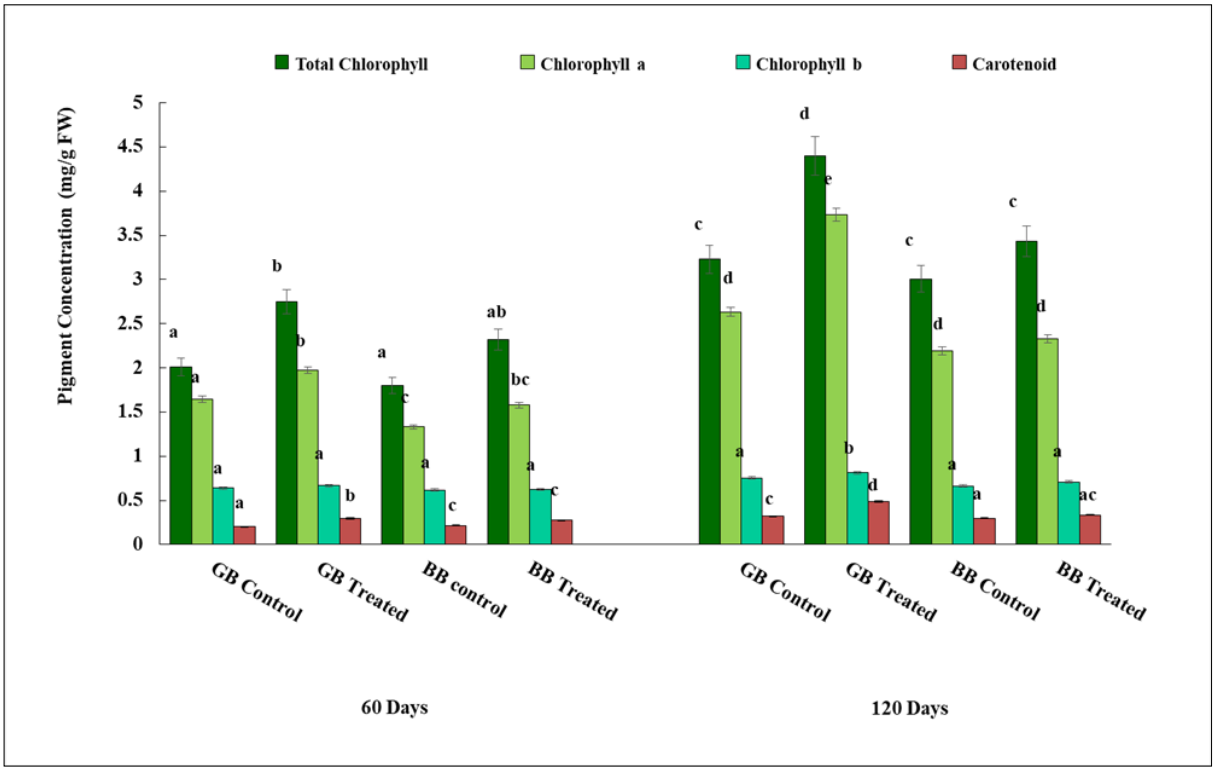

Fig. 9. Estimation of photosynthetic pigments (µg/g FW) from leaves after 60 days and 120 days of inoculation.

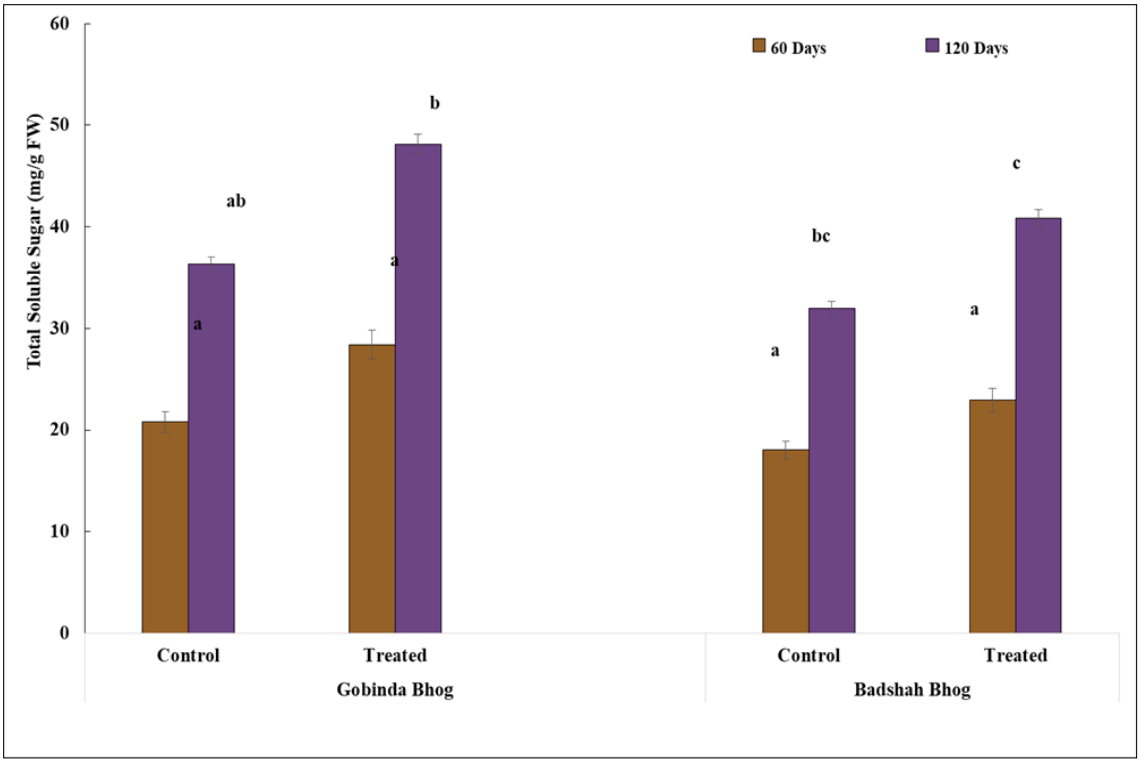

Fig. 10. Estimation of TSS content (mg/g FW) in leaves in the treated and untreated sets after 60 days and 120 days of inoculation.

bacterial fortification led to a significant increase in soluble protein content in the treated leaves of both rice varieties compared to the untreated control. Among the treated plants, the BB variety exhibited the highest mean soluble protein concentration, reaching 15.55 ± 0.65 mg/g, whereas GB-treated plants recorded a slightly lower yet substantial increase (14.9 ± 0.55 mg/g). Notably, all treated groups demonstrated statistically significant differences from the control, indicating a positive impact of bacterial inoculation on protein metabolism and overall plant health (Fig. 11).

Total Flavonoid Content (TFC)

Plants with high flavonoid and polyphenol content have stronger defense mechanisms that enable them to withstand harsh and stressful climatic conditions. Plants with high polyphenolic and flavonoid content have stronger defense mechanisms that enable them to withstand harsh and stressful climatic conditions. Here TFC was measured in terms of mg quercetin equivalents per gram FW and it was increasingly higher at the later stages of development in both varieties (1.79±0.03 mg QE/g FW in BB and 1.32±0.05 mg QE/g FW in GB 120-days-old treated set). Bacterial inoculation enhanced flavonoid production in plant leaves compared to un-inoculated leaves (28) (Fig. 12).

Total Polyphenol Content (TPC)

Polyphenolics are widely recognized for their protective role in plants, primarily through their antioxidative properties and Reactive Oxygen Species (ROS) scavenging mechanisms. Their accumulation in plant tissues serves as a crucial defense strategy against various environmental stressors, thereby contributing to improved stress tolerance and overall plant health. In the present study, a one-way ANOVA analysis of total polyphenol content revealed a statistically significant increase in treated plants compared to the control. Among the tested varieties, the GB rice at 120 days of growth exhibited the highest polyphenol accumulation, reaching 2680 ± 10.55 mM GAE/g FW, a substantial elevation relative to the control group (Fig. 13). This marked increase in polyphenol content suggests that bacterial fortification may enhance the plant's intrinsic antioxidant defense system, potentially improving resilience to oxidative stress and environmental challenges.

Discussion

Plant development and production as a whole are significantly influenced by the availability of nutrients in the soil. The majority of the essential minerals in the soil must be absorbed by plants. The soil bacteria can boost their bioavailability for plants (29). IAA has a major role in controlling a variety of elements of plant growth and development. It participates in a variety of processes, including the promotion of seed germination, plant cell division and differentiation, the beginning of the production of lateral and adventitious roots, the development of root hair and the improvement of plant stress tolerance (16, 18). IAA serves no purpose in bacterial cells; however, it might be used to demonstrate the relationship between plants and microbes (30). Therefore, this study aims to optimize IAA production by the rhizospheric bacteria *Bacillus cereus* and its inoculation in aromatic rice plants with a goal of further use of this metabolite in the field to ensure its availability for plants.

In this study, the selected strain *Bacillus cereus* MCC4850 exhibited significant IAA production, as quantified through a quantitative assay and further confirmed by TLC. Consistent with previous reports indicating *Bacillus cereus* as a potent IAA producer, our findings corroborate its capability in auxin biosynthesis (11, 31). It was found that silica gel chromatography is an effective method for extracting, separating and identifying both natural and synthetic indole

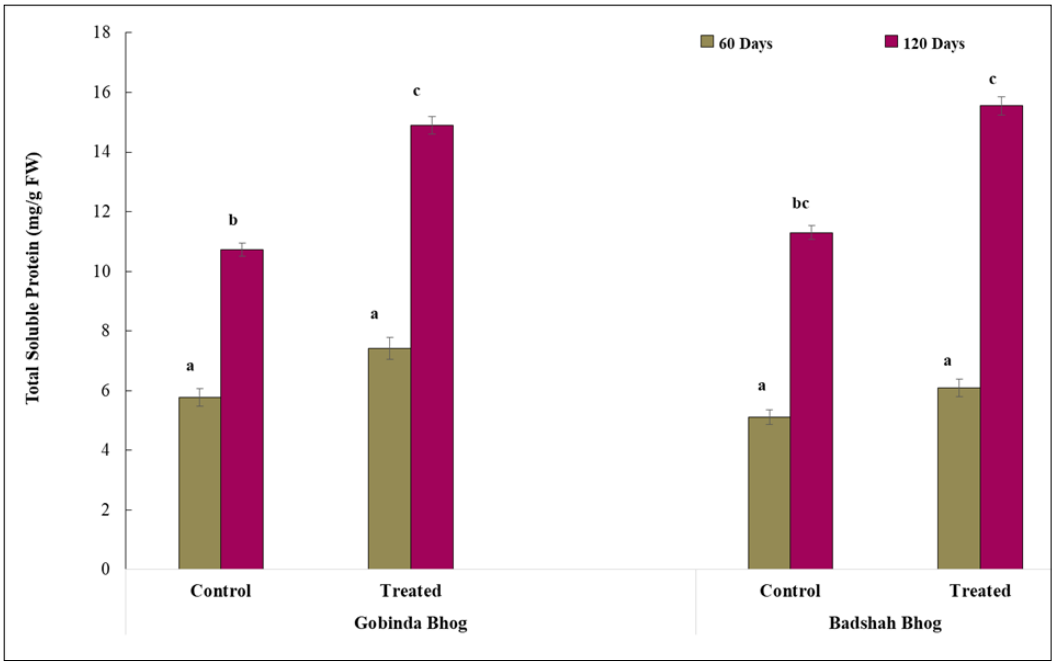

Fig. 11. Estimation of TSP content (mg/g FW) in leaves in the treated and untreated sets after 60 days and 120 days of inoculation.

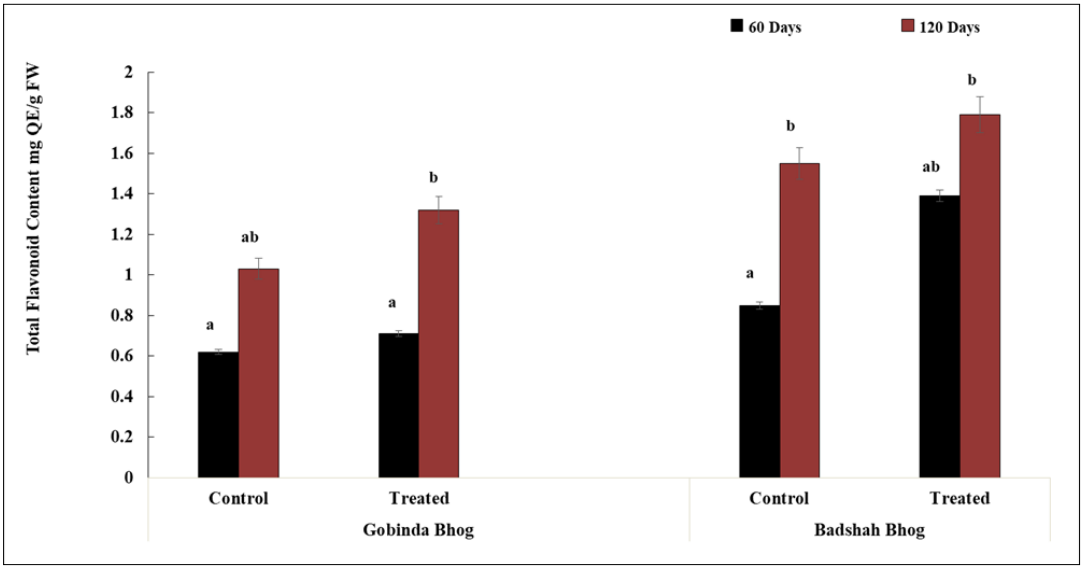

Fig. 12. Estimation of TFC (mg QE/g FW) in leaves in the treated and untreated sets after 60 days and 120 days of inoculation.

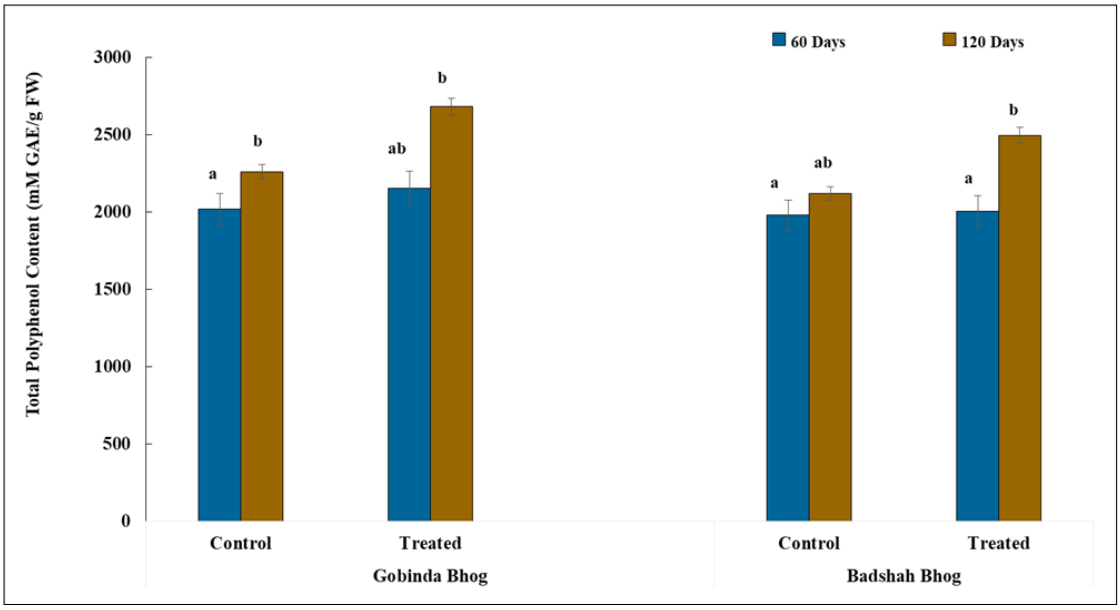

Fig. 13. Estimation of TPC (mM GAE/g FW) in leaves in the treated and untreated sets after 60 days and 120 days of inoculation.

compounds. So, we have further confirmed the IAA produced through TLC using the solvent system benzene, acetone and acetic acid. Although to detect IAA in *Azotobacter* sp. and *Pseudomonas* sp., previous researchers employed the solvent system chloroform: ethyl acetate: formic acid "5:3:2 (v/v/v)" resulting in an R_f value of 0.57 (18). The homogeneity of the partially purified auxins has been checked by TLC which also gave an R_f value of 0.81 in the solvent system isopropanol: water "30:20 (v/v)" (32). R_f value was 0.66 in the solvent system isopropanol: ammonia: water "80:10:10 (v/v/v)" in the case of *Bacillus licheniformis* MML2501 has been confirmed by using Ehrlich's reagent (33).

IAA production by *Bacillus cereus* (MF693119.1) is influenced by incubation time, tryptophan concentration and carbon and nitrogen sources (12). In the present experiment also the change in media condition and physical factors influenced the amount of IAA production. The selected strain reached its maximum growth and auxin production at 18 hrs of inoculation. Similar works reported the bacteria to reach its stationary phase after 14 hrs of growth and IAA is produced during the stationary phase as a by-product of secondary metabolism (34). Alternatively, studies have reported that after 96 hrs of growth, *Bacillus siamensis* and *Bacillus subtilis* produce higher amounts of auxin (35).

Different bacteria produce different amounts of IAA depending upon the nutrient source used in the media (31). In our findings, the bacteria preferred the complex sugar cellulose as its preferred carbon source to improve IAA production. However, our results disagree with the other findings where *Bacillus cereus* prefers glucose as a carbon source (12). Again, sucrose was the most preferred source for IAA production by *Pseudomonas putida* (36), bacteria isolated from *Stevia reboudiana* used dextrose most efficiently as a carbon source (37) and in other cases, the microbes best utilize mannitol to produce *in-vitro* IAA (18). So, we can state that the optimal carbon source for IAA production is bacterial strain-dependent.

Again, the selected strain in our study utilized inorganic N_2 sodium nitrate as the preferred nitrogen source. Similarly, ammonium chloride was also chosen by *Pseudomonas fluorescence* to synthesize IAA (38). However, this study also does not agree with most of the other reports where organic nitrogen source was maximum utilized by bacterial isolates to produce optimum IAA. The highest IAA production by *Bacillus subtilis* was reported in the presence of 1.5 % glycine and *Bacillus siamensis* preferred tryptone for maximum IAA production (16). Considering our study and previous works it can be said that the optimal nitrogen source for IAA production is also bacterial strain-dependent.

Growing plants with naturally occurring beneficial soil microbes for increased productivity and soil health has become a more sustainable, economical and environmentally responsible alternative. We looked at the effects of microbial application multiple times on soil nutrient status and plant health parameters (growth and biochemical parameters) because we assume that single inoculation or only one-time application of bacterial inoculants, either as seed or seedling coating or soil enrichment, may not result in a successful impact due to spatial and temporal reasons including competence with native microorganisms (39). Here we have observed a significant enhancement in terms of all growth parameters by applying the IAA-producing bacterial strain (*Bacillus cereus*) in both the aromatic rice varieties (GB and BB). Previous studies have shown that treatment of rice plants with *Bacillus cereus* NMSL88 grew root and shoot lengths by 34.19 % and 36.81 %, respectively (40). Similar to our study, reports suggest that co-inoculation of *Trichoderma* and *Pseudomonas* affected tiller count positively in two rice varieties namely PB1612 and CO51 (20). Zn-solubilizing *Bacillus cereus* has influenced yield, tiller number, panicle length also plant height in Basmati-385 and Super Basmati rice varieties (41). In contrast to our study, *Bacillus cereus* YN917 positively affects root shoot length and dry weight but has no role in seed germination (42). IAA produced by bacteria enhances ethylene signalling which has a role in seed germination and a small change in ethylene-to gibberellin ratio can negatively affect seed germination.

Carotenoid is the accessory pigment and chlorophyll-a and chlorophyll-b are two examples of photosynthetic pigments that are necessary for photosynthesis. Together, they make up the light-harvesting complex, which is vital for the survival of the plant. Chlorophyll content is a direct indicator of plant health status and carotenoids provide a variety of functional tasks that are important and connected to the photosynthetic activity of plants, interactions with the environment and as intermediates of phytohormones and bioactive mediators (20). In our experiment, the pigment status of the rice varieties has been greatly improved in the earlier (60-days-old) and later stage (120-days-old) of growth after bacterial treatment. Researchers found that *Bacillus* species increased the amount of carotenoid and chlorophyll in several plants (7). Our investigation follows previous works where co-inoculation of bacteria (*Pseudomonas fluorescens*) and fungi (*Trichoderma asperellum*) improves pigment constituent in CO51 and PB1612 rice varieties (20). Rice plants inoculated with *Bacillus cereus* have improved chlorophyll content in the presence of Cd stress (43). Chlorophyll and carotenoid contents have been significantly enhanced by the inoculation of AMF (arbuscular mycorrhizal fungi) and rhizobacteria on ryegrass (*Cupressus arizonica*) (44). Both chlorophyll and carotenoid in rice leaves have improved by the application of *Bacillus subtilis* and *Saccharomyces cerevisiae* (27). The higher pigment content in plants inoculated with the bacterial strains could be associated with their ability to fix nitrogen and *Bacillus cereus* can fix nitrogen as shown in previous works (11).

Various bacterial strains alter certain inherent plant mechanisms, like total soluble sugar, at various intensities (45). Here in our investigation also the strain *Bacillus cereus* has shown its effect on plants' soluble sugar content. 120-day -old treated plants of both varieties resulted in a better sugar content than the uninoculated control plants. Similarly, an increase in soluble sugar content in rice leaves treated with *Bacillus* sp. has also been reported by other researchers (46, 20). The effectivity of *Bacillus cereus* in the growth of soybean, maize, rice and wheat also showed the effect on biologically active substances including soluble sugar content (35).

Similarly, the soluble protein content in the leaves has been improved by the application of our selected strain. The detailed proteosome analysis in rice seedlings after *Bacillus cereus* inoculation revealed that *Bacillus cereus* NMSL88 treatment could upregulate 22 different proteins whereas downregulated 9 proteins (40). Foliar spray of *Bacillus subtilis*, *Bacillus thuringiensis and Bacillus megaterium* in pot experiment enhanced the leaf protein in chickpea plants under moisture stress (47). Similar to our findings soluble protein content has been increased over time after inoculated with *Bacillus subtilis* and *Saccharomyces cerevisiae* in rice plants (27). In other recent studies, *Bacillus cereus* DW019 inoculation has significantly increased soluble protein in Tomatoes (48). Plants in pot experiments face several stresses at the mature stage and it is indisputable that proteins and amino acids protect plant cells from oxidative damage this could be the reason for an increased protein content in leaves.

Secondary metabolites like flavonoids and phenols produced by plants enhance IAA production in the rhizospheric bacteria and in response to that IAA producing bacteria positively affects the production of these metabolites (49). In the current study, the IAA-producing *Bacillus cereus* has positively affected flavonoid and polyphenol content in both aromatic rice plants. A similar result was reported when the application of *Trichoderma* and *Pseudomonas* in two rice varieties namely PB1612 and CO51 enhanced flavonoid and polyphenol content post-inoculation (20). Polyphenols are naturally occurring compounds that mainly include multiple phenol (aromatic alcohol) rings. However, certain phenolic compounds are produced in plants in response to pathogenic attacks or stress (50). So, enhancement of polyphenol content after *Bacillus cereus* inoculation provides resistance in rice plants against foreign invaders.

Conclusion

The findings of the present study suggest that the selected bacterial strain can produce a significant amount of IAA in a tryptophan-supplemented medium. IAA production parameters were screened and optimized for these isolates using different media components. The research showed that the isolate used carbon, tryptophan and incubation time differently to get the highest output. The current research also shows that bacterial IAA can increase the germination percentage, root-shoot length, dry weight and tiller number in GB and BB plants. This characteristic shows that they play a role in improving plant growth performance through early preparedness against forthcoming environmental stress and that they work well as bio-inoculants. It will ease the way for organic farming and reduce the usage of chemical fertilizers if harnessed properly and carefully.

Acknowledgements

The authors thank the University Science Instrumentation Centre (USIC) and Crop Research and Seed Multiplication Farm (CRSMF), The University of Burdwan, West Bengal, India, for the SEM study of the bacteria and pot experiments, respectively.

Authors' contributions

NP designed the methodology, analyzed the data and prepared the manuscript; BM analyzed thoroughly, added revisions and comments and edited the manuscript; SR helped in the statistical analysis of the data; SD conceptualized and supervised the research and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Verma DK, Srivastav PP. Extraction, identification and quantification methods of rice aroma compounds with emphasis on 2-acetyl-1-pyrroline (2-AP) and its relationship with rice quality: A comprehensive review. Food Rev Int. 2022;38(2):111-62. https://doi.org/10.1080/87559129.2020.1720231
2. Abbas R, Rasul S, Aslam K, Baber M, Shahid M, Mubeen F, et al. Halotolerant PGPR: A hope for cultivation of saline soils. J King Saud Univ Sci. 2019;31(4):1195-201. https://doi.org/10.1016/j.jksus.2019.02.019
3. Prodhan ZH, Shu Q. Rice aroma: A natural gift comes with price and the way forward. Rice Sci. 2020;27(2):86-100. https://doi.org/10.1016/j.rsci.2020.01.001
4. Verma DK, Thakur M, Srivastav PP. Biochemistry and molecular aspects of 2-acetyl-1-pyrroline biosynthesis in rice (*Oryza sativa* L.): A review. Isr J Plant Sci. 2020;67(3-4):129-43. https://doi.org/10.1163/22238980-bja10012
5. Salvadi N, Mir MI, Bee H. Plant growth promoting and biosurfactant activity of rice rhizosphere bacteria. Biochem Cell Arch. 2023;23(1):463. https://doi.org/10.51470/bca.2023.23.1.463
6. Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, El Enshasy H. Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints and prospects. Sustainability. 2021;13(3):1140. https://doi.org/10.3390/su13031140
7. Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj DJ. *Bacillus* species in soil as a natural resource for plant health and nutrition. J Appl Microbiol. 2020;128(6):1583-94. https://doi.org/10.1111/jam.14506
8. Wang Y, Zhang H, Zhang Y, Fei J, Xiangmin R, Peng J, et al. Crop rotation-driven changes in rhizosphere metabolite profiles regulate soil microbial diversity and functional capacity. Agric Ecosyst Environ. 2023;358:108716. https://doi.org/10.1016/j.agee.2023.108716
9. Kumar P, Sharma S, Tripathi VN. Quorum sensing in bacteria of rice rhizospheres from Chhattisgarh, India. Bioinformation. 2023;19(2):199-205. https://doi.org/10.6026/97320630019199
10. Zhang F, Xu N, Zhang Z, Zhang Q, Yang Y, Yu Z, et al. Shaping effects of rice, wheat, maize and soybean seedlings on their rhizosphere microbial community. Environ Sci Pollut Res. 2023;30(13):35972-84. https://doi.org/10.1007/s11356-022-24835-3
11. Fierer N. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol. 2017;15(10):579-90. https://doi.org/10.1038/nrmicro.2017.87
12. Shah S, Wu T, Xu D, Li L, Liu Y, Yang X, et al. Nitrogen application and water regime affects archaeal community structure and functional genes for nitrification and denitrification in a paddy

soil. Agric Ecosyst Environ. 2021;310:107300. https://doi.org/10.1016/j.agee.2021.107300

13. Arora P, Dixit S, Gupta A, Verma M, Dubey A. Co-cultivation with other PGPR or phytopathogen improves the 2-acetyl-1-pyrroline production and biofilm formation in *Bacillus subtilis* HAK7 isolated from aromatic rice (*Oryza sativa* L.). Arch Microbiol. 2020;202(3):549-58. https://doi.org/10.1007/s00203-019-01759-6

14. Vaishnav A, Shukla AK, Sharma A, Kumar R, Choudhary DK. Endophytic bacteria in plant salt stress tolerance: Current and future prospects. J Plant Growth Regul. 2019;38(2):579-94. https://doi.org/10.1007/s00344-018-9860-2

15. Kumar A, Verma JP, Sharma S, Maheshwari DK. Reduction in dose of chemical fertilizers and growth enhancement of rice (*Oryza sativa* L.) by PGPR under the system of rice intensification (SRI). Commun Soil Sci Plant Anal. 2011;42(5):593-610. https://doi.org/10.1080/00103624.2011.549059

16. Thapa S, Kumar A. Impacts of inoculation of plant growth promoting rhizobacteria (*Bacillus amyloliquefaciens*) on growth and productivity of rice in Nepal. Arch Agron Soil Sci. 2020;66(9):1266-79. https://doi.org/10.1080/03650340.2019.1673549

17. Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, et al. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci. 2017;8:172. https://doi.org/10.3389/fpls.2017.00172

18. Egamberdieva D, Wirth SJ, Shurigin VV, Hashem A, Abd_Allah EF. Endophytic bacteria improve plant growth, symbiotic performance of chickpea (*Cicer arietinum* L.) and survival under stress conditions. PLoS One. 2017;12(5):e0180887. https://doi.org/10.1371/journal.pone.0180887

19. Radhakrishnan R, Hashem A, Abd-Allah EF. Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. Front Physiol. 2017;8:667. https://doi.org/10.3389/fphys.2017.00667

20. Zhang N, Yang D, Kendall J, Borrás-Hidalgo O, Fan X, Wang Q, et al. *Bacillus subtilis* Ydj3 upregulates ascorbate peroxidase and catalase to enhance rice tolerance to salinity and oxidative stress. Plant Cell Rep. 2022;41(2):487-504. https://doi.org/10.1007/s00299-021-02799-7

21. De Vrieze M, Pandey P, Bucheli TD, Varadarajan AR, Ahrens CH, Weisskopf L. The impact of 2,4-diacetylphloroglucinol on the wheat microbiome under laboratory and greenhouse conditions. ISME J. 2019;13(3):509-20. https://doi.org/10.1038/s41396-018-0280-4

22. Suyal DC, Yadav A, Shukla P, Goel R. PGPR mediated regulation of gene expression and antioxidant enzyme activities in plants for alleviation of abiotic stresses. In: Meena VS, editor. Role of rhizospheric microbes in soil. Singapore: Springer. 2018:145-68. https://doi.org/10.1007/978-981-10-8402-7_7

23. Ngoma L, Ahmad F. Ecophysiology of plant growth promoting bacteria. Sci Hortic. 2012;136:1-14. https://doi.org/10.1016/j.scienta.2011.12.006

24. Seneviratne G, Rajakaruna RMJJK, Seneviratne KA, Madawala HMJS, Kulasooriya SA. Soil microbial communities in organic and conventional farming systems in tropical conditions: a case study. Appl Soil Ecol. 2010;46(3):210-6. https://doi.org/10.1016/j.apsoil.2010.08.002

25. Goswami D, Dhandhukia P, Patel P, Thakker JN. Screening of PGPR from saline desert of Kutch: growth promotion in *Arachis hypogaea* by *Bacillus licheniformis* A2. Microbiol Res. 2014;169(1):66-75. https://doi.org/10.1016/j.micres.2013.06.003

26. Radhakrishnan R, Khan AL, Lee IJ. Endophytic fungal interactions with plants and their contribution to salt tolerance. In: Ahmad P, Azooz MM, Prasad MNV, editors. Ecophysiology and responses of plants under salt stress. New York: Springer. 2013:153-69. https://doi.org/10.1007/978-1-4614-4747-4_7

27. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003;255(2):571-86. https://doi.org/10.1023/A:1026037216893

28. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261-7. https://doi.org/10.1128/AEM.00062-07

29. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:590-6. https://doi.org/10.1093/nar/gks1219

30. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852-7. https://doi.org/10.1038/s41587-019-0209-9

31. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581-3. https://doi.org/10.1038/nmeth.3869

32. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550. https://doi.org/10.1186/s13059-014-0550-8

33. Dixon P. VEGAN, a package of R functions for community ecology. J Veg Sci. 2003;14(6):927-30. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

34. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package. R package version 2.5-7;2020. https://CRAN.R-project.org/package=vegan

35. R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2021. https://www.R-project.org/

36. Wickham H, Averick M, Bryan J, Chang W, McGowan LDA, François R, et al. Welcome to the tidyverse. J Open Source Softw. 2019;4(43):1686. https://doi.org/10.21105/joss.01686

37. Liaw A, Wiener M. Classification and regression by random Forest. R News. 2002;2(3):18-22. https://CRAN.R-project.org/doc/Rnews/

38. Breiman L. Random forests. Mach Learn. 2001;45(1):5-32. https://doi.org/10.1023/A:1010933404324

39. Aitchison J. The statistical analysis of compositional data. London: Chapman & Hall; 1986.

40. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome datasets are compositional: and this is not optional. Front Microbiol. 2017;8:2224. https://doi.org/10.3389/fmicb.2017.02224

41. Gloor GB, Macklaim JM, Fernandes AD. Displaying variation in large microbiome data sets: a plot of log-ratios. Microbiome. 2016;4(1):68. https://doi.org/10.1186/s40168-016-0208-3

42. Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PLoS One. 2013;8(7):e67019. https://doi.org/10.1371/journal.pone.0067019

43. Nearing JT, Douglas GM, Comeau AM, Langille MGI. Denoising the denoisers: an independent evaluation of microbiome sequence error-correction approaches. Peer J. 2018;6:e5364. https://doi.org/10.7717/peerj.5364

44. Callahan BJ, Wong J, Heiner C, Oh S, Theriot CM, Gulati AS, et al. High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. Nucleic Acids Res. 2019;47(18):e103. https://doi.org/10.1093/nar/gkz569

45. Navas-Molina JA, Peralta-Sánchez JM, González A, McMurdie PJ, Vázquez-Baeza Y, Xu Z, et al. Advancing our understanding of the human microbiome using QIIME. Methods Enzymol. 2013;531:371-444. https://doi.org/10.1016/B978-0-12-407863-5.00019-8

46. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 2018;6(1):90. https://doi.org/10.1186/s40168-018-0470-z

47. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852-7. https://doi.org/10.1038/s41587-019-0209-9

48. Gloor GB, Reid G. Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. Can J Microbiol. 2016;62(8):692-703. https://doi.org/10.1139/cjm-2015-0821

49. Egozcue JJ, Pawlowsky-Glahn V. Groups of parts and their balances in compositional data analysis. Math Geol. 2005;37(7):795-828. https://doi.org/10.1007/s11004-005-7381-9

50. Aitchison J, Egozcue JJ. Compositional data analysis: where are we and where should we be heading? Math Geol. 2005;37(7):829-50. https://doi.org/10.1007/s11004-005-7383-7

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.