Rice

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Physiological Basis of Plant Growth Promotion in Rice by Rhizosphere and Endosphere Associated *Streptomyces* Isolates from India

Dhivya P. Thenappan[1,2*], Rakesh Pandey[3], Alkesh Hada[4], Dinesh Kumar Jaiswal[5], Viswanathan Chinnusamy[3], Ramcharan Bhattacharya[6] and Kannepalli Annapurna[2]

Abstract

This study demonstrated the plant growth-promoting capabilities of native actinobacterial strains obtained from different regions of the rice plant, including the rhizosphere (FT1, FTSA2, FB2, and FH7) and endosphere (EB6). We delved into the molecular mechanisms underlying the beneficial effects of these plant-microbe interactions by conducting a transcriptional analysis of a select group of key genes involved in phytohormone pathways. Through in vitro screening for various plant growth-promoting (PGP) traits, all tested isolates exhibited positive traits for indole-3-acetic acid synthesis and siderophore production, with FT1 being the sole producer of hydrogen cyanide (HCN). All isolates were identified as members of the *Streptomyces* genus through 16S rRNA amplification. In pot culture experiments, rice seeds inoculated with strains FB2 and FTSA2 exhibited significant increases in shoot dry mass by 7% and 34%, respectively, and total biomass by 8% and 30%, respectively. All strains led to increased leaf nitrogen levels, with FTSA2 demonstrating the highest increase (4.3%). On the contrary, strains FB2 and FT1 increased root length, root weight ratio, root volume, and root surface area, leading to higher root nitrogen content. All isolates, except for FB2, enhanced total chlorophyll and carotenoid levels. Additionally, qRT-PCR analysis supported these findings, revealing differential gene expression in auxin (*OsAUX1*, *OsIAA1*, *OsYUCCA1*, *OsYUCCA3*), gibberellin (*OsGID1*, *OsGA20ox-1*), and cytokinin (*OsIPT3*, *OsIPT5*) pathways in response to specific actinobacterial treatments. These actinobacterial strains, which enhance both aboveground and belowground crop characteristics, warrant further evaluation in field trials, either as individual strains or in consortia. This could lead to the development of commercial bioinoculants for use in integrated nutrient management practices.

Keywords *Streptomyces* sp., Actinobacterial strains, Plant growth promotion, qRT-PCR, Phytohormone pathways, Integrated nutrient management, *Oryza sativa*

*Correspondence:
Dhivya P. Thenappan
dhivya.thenappan@ag.tamu.edu
[1]Systems Plant Physiology, Texas A&M AgriLife Research and Extension Center, Uvalde, TX 78801, USA
[2]Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[3]Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[4]Divsion of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[5]University School of Biotechnology, Guru Gobind Singh Indraprastha University, New Delhi 110078, India
[6]ICAR-National Institute for Plant Biotechnology, New Delhi 110012, India

Introduction

According to the United Nations, the global population will grow from 7.5 billion to 9.7 billion by 2050, requiring modern agriculture to produce more eco-friendly and sustainable food (Rouphael and Colla 2018). Nearly half the world's population consumes rice (*Oryza sativa* L.), a primary staple food. India is the world's second-largest rice producer in terms of area and quantity (Hada et al. 2020a; Chatterjee et al. 2021). The extensive use of chemical fertilizers, which may negatively impact human health and the environment, is a significant issue related to rice production (Zafar et al. 2012). The demand for agrochemical alternatives has increased interest in using microorganisms for environmentally sustainable agricultural management. Microbial inoculants that act as biofertilizers are biostimulants that promote plant growth by increasing nutrient supply, root biomass or root area, and nutrient uptake capacity (Vessey 2003). Many of the plant growth-promoting (rhizo) bacteria (PGPB or PGPR) that are isolated from plants and crops around the world are used as agricultural inoculants (biofertilizers) (Kloepper et al. 1989; Bhardwaj et al. 2014). Effective bacterial biofertilizers stimulate plant growth and nutrient uptake by fixing atmospheric nitrogen, solubilizing nutrients, sequestering iron through siderophores, and producing volatile organic compounds and phytohormones (Ryu et al. 2003; Beneduzi et al. 2012; Backer et al. 2018).

Actinobacteria of the genus *Streptomyces* are effective rhizosphere and rhizoplane colonizers. They can also colonize the inner tissues of the host plant as endophytes (Sousa and Olivares 2016). Several plant growth-promoting streptomycetes (PGPS) inoculants have been shown to increase biomass in crops such as rice, wheat, sorghum (*Sorghum bicolor*), and tomato (*Solanum lycopersicum*) (Gopalakrishnan et al. 2013; Hu et al. 2020; Zhu et al. 2023). Actinobacteria are thus frequently utilized as bioinoculants (Boukhatem et al. 2022).

One of the suggested mechanisms to explain growth promotion induced by PGPR is phytostimulation, either through the microbial production of phytohormones like auxins, cytokinins (CKs), gibberellins (GAs), and ethylene (ET) or by modulating their homeostasis in plants (Lugtenberg and Kamilova 2009). Most studies on IAA-producing microorganisms have revealed a link between root development, morphology, and IAA production, the major auxin present in plants. Several *Streptomyces* species, including *S. olivaceoviridis* and *S. viridis*, can produce IAA and enhance plant growth by improving seed germination, root elongation, and root dry weight (Khamna et al. 2010). Numerous investigations have documented the production of gibberellins by actinobacterial species, including those producers of gibberellin-like compounds by *Streptomyces olivaceoviridis*, *S. rochei*, and *S. rimosus* cultures, which promoted plant development in wheat and eggplant by influencing the growth parameters of the plant, such as root length and fresh or dry root weight (Rashad et al. 2015). Inoculating plants with cytokinin-producing bacteria boosted shoot growth and reduced the root-to-shoot ratio (Arkhipova et al. 2007). Nevertheless, these hormones have been reported less frequently in strains of actinobacteria.

While the plant growth-promoting effects of *Streptomyces* have been well-established in various greenhouse studies, further research is necessary to comprehend how rice responds to PGPS bacteria at both physiological and molecular levels. Consequently, this study sought to assess the physiological foundation underlying the plant growth-promoting potential of native rhizospheric and endospheric actinobacterial strains of rice. Additionally, the effect of these isolates on the expression of a comprehensive set of marker genes associated with phytohormone pathways involved in rice architecture modifications was examined.

Materials and Methods

Bacterial Strains and Their Molecular Characteristics

The actinobacterial isolates used in the present study were selected from our laboratory's bacterial collection. Isolates FT1, FB2, FTSA2, and FH7 were originally obtained from the rhizosphere, and strain EB6 was derived from the endosphere of paddy fields cultivated with the Indian rice genotype Vardhan. The glycerol stocks of the cultures were revived and purified through successive subculturing on the International Streptomyces Project-2 (ISP2) medium, incubated at 30 °C for 5 days (Ali 2022). The pure cultures were maintained in 30% glycerol (v/v) at -20 °C for future use.

The nearly full-length 16S rRNA gene was amplified in the isolates using the universal primers 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1522 R (5′-AAGGAGGTGATCCAGCCGCA-3′) (Lane 1991), following the PCR conditions described by Thenappan et al. (2022). The nearly complete 16S rRNA gene sequences obtained were analyzed and compared against the EZbiocloud database (https://www.ezbiocloud.net) for potential genus identification (Kim et al. 2012). The nucleotide sequences were then submitted to NCBI GenBank (GenBank accession: MN955410, MN955412, MZ736625, MZ736626, and MZ736627). Furthermore, the sequences of the isolates and associated type strains were aligned in MEGAXI using MUSCLE (Tamura et al. 2013). The phylogenetic tree was built using the maximum likelihood approach (Felsenstein 1981) and the Kimura 2-parameter model (K2+I) in MEGA XI (Kimura 1980). Bootstrap analysis with 1000 replications was used to analyze the tree topology (Felsenstein 1985). Suitable models with the lowest BIC scores (Bayesian Information Criterion)

and the highest AICc values (Akaike Information Criterion, corrected) were selected in MEGA XI.

In vitro plant growth promotion assays

The method outlined by Gordon and Weber (1951) was employed to evaluate IAA production. The actinobacterial isolates were grown in ISP2 broth with 0.2% L-tryptophan and incubated at 30 °C for 6 days with shaking at 150 rpm. After samples were centrifuged at 10,000 rpm for 15 min, 2 ml of Salkowski reagent was added to 1 ml of cell-free supernatant. The development of a pink-red hue indicated the production of IAA. The absorbance of IAA at 530 nm was measured using a spectrophotometer (Labman, India) against a standard curve to determine the quantity of IAA in µg/ml.

The phosphate solubilizing potential of isolates was tested by spot inoculating them on Pikovskaya's agar medium and incubating them for seven days at 30 °C; colonies with clear zones around were termed phosphate solubilizers (Donate-Correa et al. 2005). The isolates were spotted onto a chrome azurol S (CAS) plate and cultured at 30 °C for 5 days for the siderophore production assay (Alexander and Zuberer 1991). The appearance of an orange-yellow halo surrounding the colonies showed that siderophores were detected. Qualitative evaluation of hydrogen cyanide (HCN) generation was done using Lorck's method (1948). Isolates were inoculated onto ISP2 agar plates with 4.4 g of glycine/L. A Whatman No. 1 filter paper soaked in 0.5% picric acid in a 2% sodium carbonate (w/v) solution was placed under the lids of Petri dishes, covered with parafilm, and incubated at 30 °C for 7–10 days. The change in color of the filter paper from yellow to orange-brown indicated the release of HCN.

Seed Germination Assay

Rice seeds (cv. Pusa Basmati 1509) were surface sterilized for 5 min with 2% sodium hypochlorite (v/v) and rinsed four times with sterile distilled water (SDW). Sterilized seeds were immersed in a culture medium (10^8 cells/ml) of each given actinobacterial isolate (EB6, FT1, FTSA2, FB2, and FH7) under investigation and continuously stirred (150 rpm, 6 h). SDW-soaked seeds were used as a control. Ten seeds were placed on each sterile plate using moist filter paper (Whatman No. 1). Plates were incubated at 30 °C for 3 days. Each treatment was replicated three times. On the second day, the percentage of germination was calculated. On the third day, the plumule and radicle lengths were measured.

The vigor index was calculated using the following formula established by Abdul-Baki and Anderson (1973):

Vigor index = germination (%) x total seedling length (cm)

Pot Experiment Assay

For the pot experiment, surface-sterilized rice seeds were soaked for 6 h in actinobacterial cultures (1×10^8 CFU ml^{-1}) prepared in 1% carboxymethyl cellulose (CMC). Ten seeds were planted at a depth of 5 cm in each pot (20 cm x 15 cm x 10 cm) filled with 5 kg non-sterile sandy loam soil (ICAR-Indian Agricultural Research Institute, New Delhi, India) with the following characteristics: pH, 8.21; EC (ds/m), 0.35; organic C, 0.41%; total nitrogen, 250.5 kg ha^{-1}; total phosphorus, 93.15 kg ha^{-1}; total potassium, 436 kg ha^{-1}. The treatments included T1: Uninoculated Control, T2: *Streptomyces* sp. EB6, T3: *Streptomyces* sp. FT1, T4: *Streptomyces* sp. FTSA2, T5: *Streptomyces* sp. FB2, and T6: *Streptomyces* sp. FH7.

Each treatment consisted of five pots set in a completely randomized design (CRD) and watered regularly with tap (non-sterile) water. The pots were kept at 30 °C±2 °C and 90% relative humidity in a greenhouse bench (National Phytotron Facility, ICAR-Indian Agricultural Research Institute, New Delhi, India). Plant samples were taken twice, 30 and 45 days after sowing (DAS). At each harvest, observations were taken on dry matter production (shoot, root, and leaf) and leaf area. The mean of these observations (at 30 and 45 DAS) was used to assess the plant's performance in terms of dry matter production.

The automated root image analysis program WinRhizo (Regent Instruments Inc., Quebec City, Canada) was used to estimate root morphological parameters such as total root length (cm), root surface area (cm^2), average root diameter (mm), number of root points, forks, and total root volume (cm^3), volume and surface area of roots with diameters between 0 and 1 mm (superfine roots), 1–2 mm (fine roots), and larger than 2 mm (thick roots) (de Sousa et al. 2021). Subsequently, shoot height (cm), leaf area, and root and shoot dry weights were oven-dried at 65°C until a constant weight (g) was measured to calculate growth indices (Table S3).

To assess N content in the CHNS/O analyzer, oven-dried plant tissue samples were crushed to pass through a 0.2-mm sieve (CHN-O-RAPID; EuroEA element analyzer, Germany). Photosynthetic pigments such as chlorophyll a and chlorophyll b were quantified using Hiscox and Israelstam's techniques (1979). Furthermore, the carotenoid content was determined using the method of Kirk and Allen (1965).

qRT-PCR Analysis of Phytohormone-Responsive Genes

Total RNA was extracted from 0.1 g of crushed rice leaves (30 days old) using the SV Total RNA isolation system kit following the manufacturer's protocol (Promega, WI, USA). RNA quantity and purity were determined using a NanoDrop-1000 spectrophotometer (Thermo Scientific, MA, USA). Approximately 1 µg of total RNA was reverse

transcribed to cDNA using the Superscript VILO kit (Invitrogen, USA) following the manufacturer's instructions. qRT-PCR was performed in a Realplex2 thermal cycler (Eppendorf, Germany) using the SYBR Green PCR master mix kit (Eurogentec, Liege, Belgium), with *OsActin1* as the reference gene. To assess the specificity of amplification, a melt curve analysis was conducted with a program consisting of 95 °C for 15 s, 60 °C for 15 s, and a slow ramp from 60 to 95 °C. Cycle threshold (Ct) values were obtained using Realplex software (Eppendorf, Germany), and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), normalized with the *OsActin1* gene. Data were generated from three independent biological replicates and three technical replicates, and the analysis was conducted according to the methodology described by Hada et al. (2020b, 2021). The qRT-PCR primers for the genes of the auxin, gibberellic acid, and cytokinin biosynthesis pathways in rice are illustrated in Table S1.

Statistical Analyses

The packages 'ggplot2' and 'factoextra' of R software version 4.0.2 (R Core Team 2019) were used to create bar graphs and PCA biplots. The data was tested for normality using the Shapiro-Wilk test. In the case of a normal distribution, the one-way ANOVA test and post hoc Tukey test were used; in all other cases, the non-parametric Kruskal-Wallis and post hoc Dunn's tests were used. The 'corrplot' package (Wei and Simko 2017) generated correlation matrix plots. All analyses were conducted with $p \leq 0.05$ as the significance level. Further, for statistical validation of qRT-PCR data, one-tailed t-tests and Tukey's HSD tests were conducted on biological replicates ($n = 3$) for each treatment.

Results

Molecular Characterization

The analysis of the nearly full-length 16S rRNA gene sequences (Table 1) revealed that all isolates were members of the genus *Streptomyces* of the family Streptomycetaceae. Strains EB6 and FT1 shared 98.9% sequence similarity (16 nt difference in 1415 and 1414 sites, respectively) with strain *Streptomyces violascens* ISP5183^T, while strains FTSA2 and FH7 shared 98.7% (19 nt difference in 1419 sites) and 98.9% similarity (15 nt difference in 1418 sites) with strain *Streptomyces araujoniae* ASBV1^T. Our study revealed that strain FB2 exhibited a similarity of 98.7% to *Streptomyces longispororuber* NBRC 13488^T (19 nt difference at 1416 sites). A comparison of 16S rRNA identity between the two isolates was also performed using a pairwise BLAST analysis, which indicated that EB6 and FT1 (96.7%), followed by FTSA2 and FH7 (97.2%), did not have nearly identical gene sequences.

Table 1 In vitro screening for plant growth promoting (PGP) characteristics of actinobacterial isolates from rice rhizosphere and endosphere

Isolate	Length (bp)	Similarity (%)	Closest type strain	GenBank Accession No.	Plant growth-promoting traits					Seed germination tests	
					IAA (µg/ml)		Siderophore	P solubilization	HCN	Germination (%)	Vigor Index
					Trp+	Trp-					
EB6	1491	98.87	*Streptomyces violascens* ISP5183	MN955412	26.08 ± 0.66^{a}	6.62 ± 0.30^{bc}	7.25 ± 0.25^{c}	-	-	97.8 ± 2.22^{ns}	658.9 ± 27.52^{ab}
FT1	1483	98.87	*Streptomyces violascens* ISP5183	MN955410	24.22 ± 1.15^{ab}	13.96 ± 1.51^{a}	9.75 ± 0.25^{b}	-	+	97.8 ± 2.22^{ns}	706.7 ± 12.02^{a}
FTSA2	1477	98.66	*Streptomyces araujoniae* ASBV-1	MZ736625	19.05 ± 1.11^{b}	5.91 ± 0.91^{bc}	10.75 ± 0.75^{b}	-	-	100^{ns}	507.8 ± 42.51^{bc}
FB2	1483	98.66	*Streptomyces longispororuber* NBRC 13488	MZ736626	23.91 ± 1.67^{ab}	5.28 ± 2.21^{c}	17.0 ± 1.5^{a}	-	-	95.6 ± 2.22^{ns}	705.5 ± 69.67^{a}
FH7	1478	98.94	*Streptomyces araujoniae* ASBV-1	MZ736627	27.10 ± 0.72^{a}	13.09 ± 2.01^{ab}	11.75 ± 0.25^{b}	-	-	97.8 ± 2.22^{ns}	374.44 ± 31.76^{a}

Note: Seed germination data are expressed as mean ± standard error. Different letters indicate significant differences at $p < 0.05$ by Tukey's HSD test. ns = non-significant

Phylogenetic Analysis

By the maximum likelihood method of phylogenetic analysis of 16S rRNA gene sequences, strains FT1 and EB6 formed separate clusters, with the latter forming a monophyletic clade with *S. violascens* ISP 5183^{T}, *S. daghestanicus* NRRL B-5418^{T}, and *S. albidoflavus* DSM 40455^{T} (Fig. 1). Similarly, strains FTSA2 and FH7 formed an independent clade with the closest relatives, such as *S. rhizosphaericola* 1AS2c^{T} and *S. araujoniae* ASBV-1^{T}; strain FB2, on the other hand, formed a cluster with *S. nigra* 452^{T} with high bootstrap values in all three phylogenetic studies (Fig. 1).

In vitro Plant Growth Promotion Assay

The PGP traits of the actinobacteria are summarized in Table 1. All the isolates showed diverse abilities to synthesize IAA, with strains FH7 (27.10±0.72 µg ml^{-1}) and FTSA2 (19.05±1.11 µg ml^{-1}) confirming the highest and lowest IAA production in medium supplemented with 0.2% tryptophan, respectively. In the absence of tryptophan, FH7 and FT1 strains produced more IAA (13.09±2.01 and 13.96±1.51 µg ml^{-1}, respectively), and FB2 strains produced the least (5.28±2.21 µg ml^{-1}). In the CAS medium, all identified *Streptomyces* isolates produced siderophores, with FB2 recording the maximum orange halo (17±1.5 mm) and FT1 producing the least (7.25±0.25 mm). Tri-calcium phosphate solubilization was found to be absent in all isolates. Only the FT1 isolate tested slightly positive for HCN production (Fig. S1).

Seed Germination Bioassay

Seed bacterization treatment with the specified isolates for three days resulted in significant changes ($p<0.05$) in seedling length and seed vigor index. However, no significant changes in germination percentage were observed. Isolate FT1 exhibited 100% germination, but isolate FTSA2 marginally decreased seed germination (95.6%). In terms of seed vigor index, isolates FTI and FB2 exhibited about the same effect as the control, whereas isolates EB6, FTSA2, and FH7 exhibited significant adverse

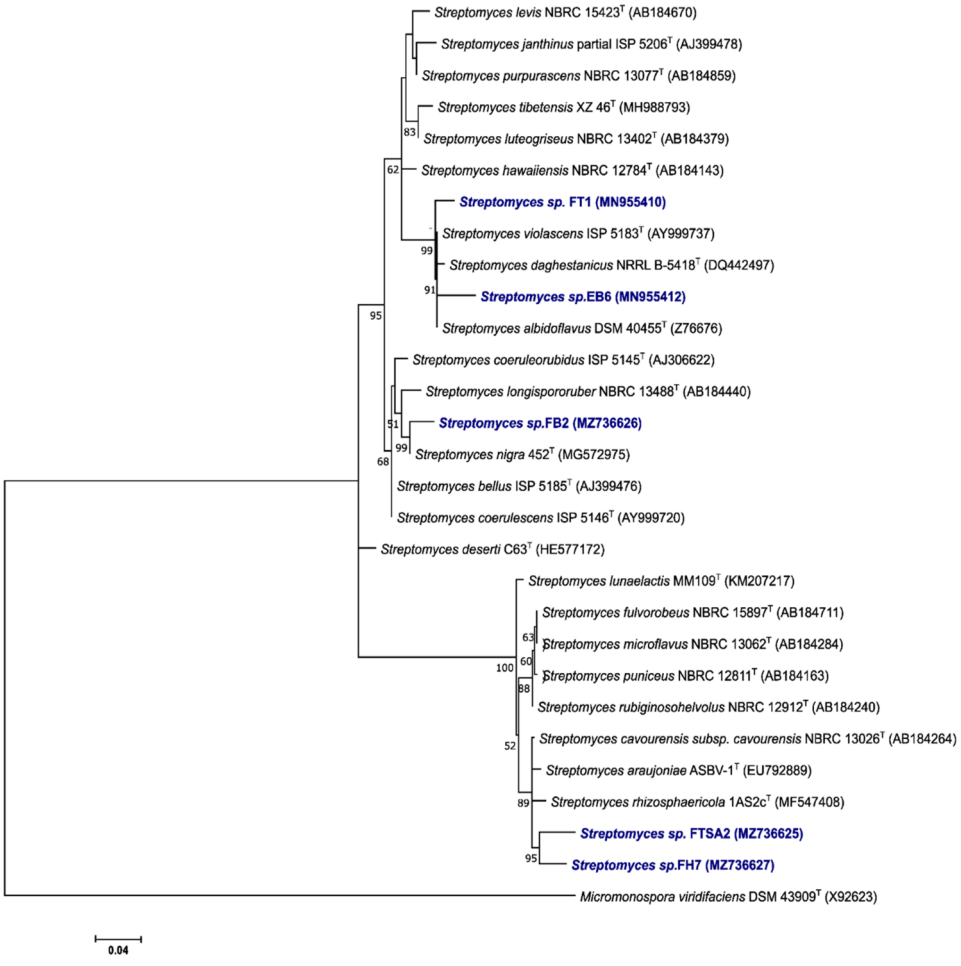

Fig. 1 Maximum likelihood tree based on the 16S rRNA showing the phylogenetic position of isolates within the genus *Streptomyces*. The type strain *Micronomospora viridifaciens* DSM43909 ^{T} was used as the outgroup. Numbers at branching points are percentage bootstrap values based on 1,000 replications. The scale bar shows 0.04 nucleotide changes per site

effects by -6.4, -27.9%, and 46.8%, respectively (Table 1; Table S2).

Effect of Actinobacterial Isolates on rice above- and belowground Growth

Shoot dry Matter and leaf Morphological Traits

Treatment with strains FTSA2, FB2, and EB6 substantially enhanced leaf dry weight (LDW), shoot dry weight (SDW), and total dry weight (TDW), whereas strain FH7 showed a reduction in these parameters. Strain FTSA2 increased leaf dry weight and shoot dry weight, resulting in a 30.7% rise in total dry weight ($p<0.05$). Strain FB2 followed with an 8.3% increase, while strain FT1 increased total biomass by 1.5% (Table S3; Fig. S1; Fig. 2A, B).

Plants inoculated with strain FTSA2 exhibited the highest shoot C and N content, with leaf N content increasing by 46.7% (Table S6; Fig. 2H). Although not statistically significant, FTSA2 showed an increase in leaf carbon content, consistent with their overall substantial improvement in leaf dry weight (LDW). Concerning photosynthetic pigments, the maximum total chlorophyll content observed in plants treated with the strain FTSA2 was 33.1% higher, whereas FB2-treated plants showed lower total pigments than the untreated control (Table S4; Fig. 2G).

No statistically significant differences ($p>0.05$) were observed between the treatments and the control for leaf area (LA; Fig. 2D), plant height, specific leaf area (SLA; Fig. 2E), total plant carbon per unit leaf area (Fig. 2F), leaf area ratio (LAR; Fig. 2I), and root:shoot (R:S) ratio (Table S7). Nonetheless, leaf area was observed to increase in all strains compared to the control, excluding FH7. The leaf weight ratio increased only in strains EB6 and FH7. The specific leaf area increased in all strains; total plant carbon per unit leaf area increased in FB2, FT1, and EB6-treated plants; and the leaf area ratio increased in all strains except FB2 (Table S5).

Root dry Matter and Morphological Traits

Strain FTSA2 considerably lowered root morphological features such as root weight ratio (RWR; −16.3%), total root length (TRL; −36.2%), root length ratio (RLR; −51.0%), and specific root length (SRL; −37.1%). Furthermore, the number of tips (NTips), root surface area, root:shoot ratio, and the number of forks were also decreased. However, it significantly improved leaf area to root length ratio (LA/RL; +106.5%), plant nitrogen content per unit root dry weight (N content/RDW; +33.9%), and root diameter (RD; +23.3%).

In contrast, FB2 and FT1 increased root dry weight (+17.7% and +16.3%, respectively). Notably, FB2 positively influenced most root morphological features, including total root length (+18.5%), root surface area (+40%), root diameter (+12.4%), root volume (+53.2%), and number of forks (+16.0%), in comparison to control plants. Strain FT1 showed a similar trend for the root mentioned above features, but to a lesser amount than FB2, except for RLR and Ntips, which increased by 13.4% and 62%, respectively. Strain FT1 demonstrated a non-significant rise ($p>0.05$) in root weight and root:shoot ratios. Although all treatments increased the length, surface area, and volume of thick roots, rice plants inoculated with FT1 and FB2 produced more fine and superfine roots than control plants. While EB6 and FH7 drastically decreased the majority of belowground root properties, they increased leaf area/root length by 36.4% and 54.6%, respectively (Tables S7–S10; Fig. 2J–P; Fig. S2).

Because strains FT1 and FB2 produced a superior total root system, FB2 substantially increased C (+123%) and N (+28.5%) content in roots (Table S6). Except for FH7, which displayed a decrease in C and N content in both the root and shoot, the remaining strains increased ($p<0.05$) total nitrogen uptake and total carbon assimilated in comparison to the control. In terms of total carbon partitioning, FB2 excelled in the root zone (24.9%), while FTSA2 outperformed in the shoot zone (91.3%) (Table S11; Fig. 2C, L).

The maximum total chlorophyll and carotenoid content for photosynthetic pigments were reported with the strain FTSA2 at 33.1% and 41.4%, respectively. In contrast, strain FB2 dramatically decreased the total pigments in treated plants compared to the control. Overall, strain FB2 exhibited a moderate to pronounced response to both above- and below-ground characteristics, whereas strain FH7 dramatically reduced both above- and below-ground characteristics and displayed the lowest total biomass. Strain FTSA2, which showed the highest proportion of dry matter in the shoots (89.1%), outperformed other treatments in total dry biomass (Table S11).

Principal Component Analysis (PCA)

Principal component analysis (PCA) examined the relationship between the measured parameters and treatments. Based on this multivariate analysis, the first two principal components, PC1 and PC2, explained 91.3% of the total variation and were utilized to construct the PCA biplot for the aboveground growth metrics. PC1 accounted for 59.1% of the phenotypic variation and was substantially linked with shoot and total dry weight, leaf area, and shoot nutritional characteristics. The second principal component (PC2) explained 32.2% of the phenotypic variability, with leaf characteristics (leaf area ratio, specific leaf area, leaf weight ratio, specific leaf weight, and pigments) and plant height accounting for the majority. Along the PC1 and PC2 axes, the bacterial

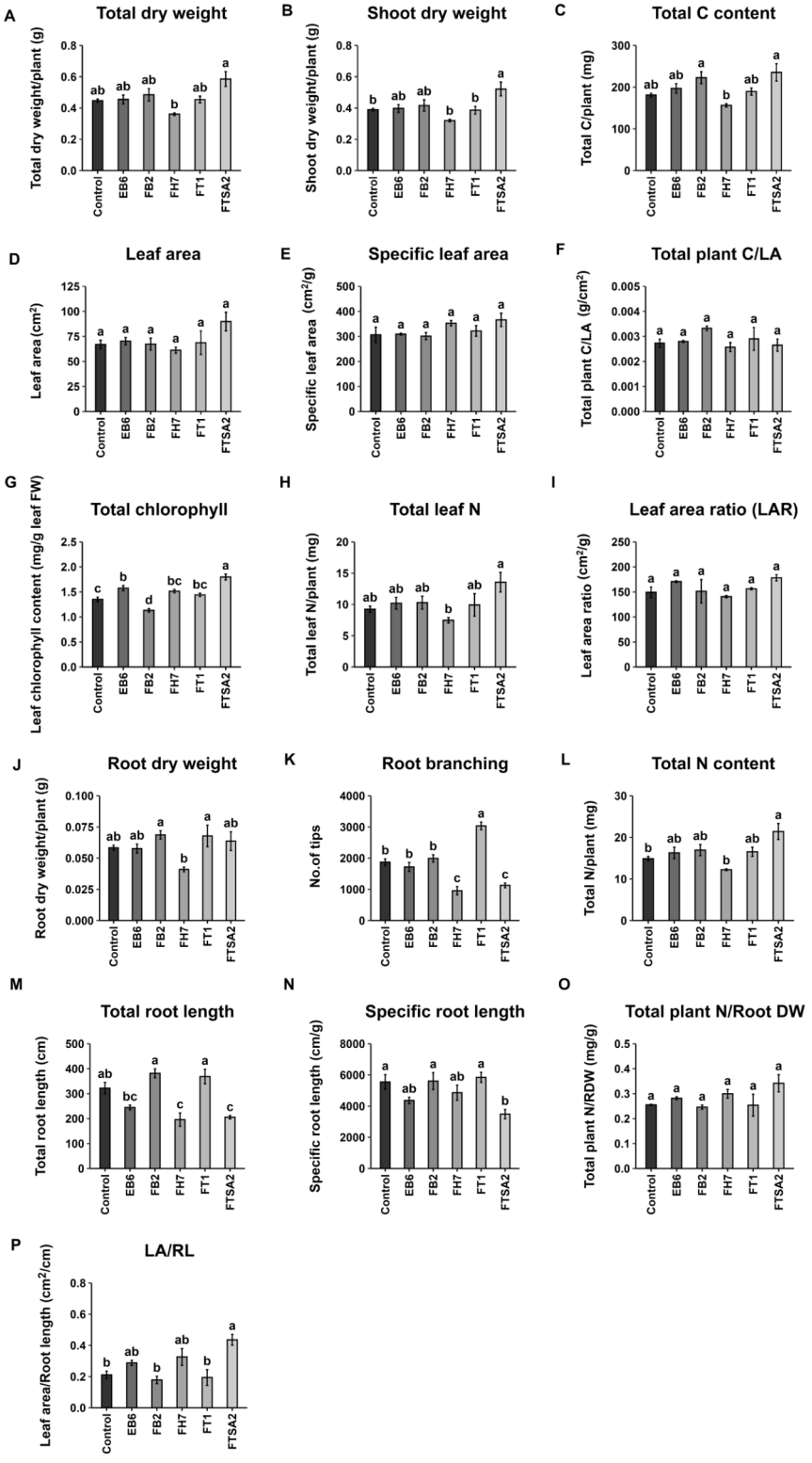

Fig. 2 Effect of *Streptomyces* strains on above-ground (**A**–**I**) and below-ground (**J**–**P**) plant growth parameters in rice. Values were expressed as mean ± standard error. Different letters indicate significant differences at $p < 0.05$ by Tukey's HSD test. **A**: total dry weight; **B**: shoot dry weight; **C**: total C content; **D**: leaf area; **E**: Specific leaf area; **F**: total plant C/LA; **G**: total chlorophyll; **H**: total leaf N; **I**: leaf area ratio (LAR); **J**: root dry weight; **K**: total root length (TRL); **L**: specific root length (SRL); **M**: number of root tips; **N**: total N content; **O**: total plant N/Root DW; **P**: leaf area/root length (LA/RL)

treatments were clearly distinguished from the control in the biplot. While the non-inoculated control and FH7 treatments in the lower and upper left quadrants exhibited lower shoot dry weight and nutrient content, the treatments in the right quadrants displayed greater shoot, total dry weight, and nutrient content, highlighting the performance of FTSA2, FB2, and EB6. The FT1 strain, located close to the center of the quadrant, produced intermediate outcomes but a better shoot system than the uninoculated control (Fig. 3A).

For belowground growth characteristics, the first two dimensions of PCA explained 91.6% of the total variation, with principal component 1 (PC1) explaining 66.9% of the variation and principal component 2 (PC2) accounting for 24.7% of the variation. While PC2 is linked to root dry weight, leaf area/root length, root:shoot ratio, and root morphology traits (root length, specific root length, root diameter, thick roots), PC1 was primarily represented by the other root morphology characteristics examined in this study. Both dimensions led to the distribution of inoculation treatments over the quadrants, with strains FB2 and FT1 in the upper left quadrant exhibiting greater root system development than the control (Fig. 3B).

All results obtained for the tested actinobacterial strains were included in the correlation analysis, confirming the PCA-generated data. The findings of the correlation analysis between the above- and below-ground growth metrics of the treated plants are depicted in Fig. 4A and B. Significant positive relationships ($p<0.05$, shown in blue) were discovered between plant biomass (leaf, shoot, and total dry weight), leaf area, leaf nutrients, and photosynthetic pigments for aboveground characteristics. In addition, a negative correlation ($p<0.05$, shown in red) was seen between plant height and photosynthetic pigments, leaf weight, and leaf area ratios. For belowground characteristics, root morphology demonstrated strong positive relationships ($p<0.05$, shown in blue) between root morphological variables, including total root length, root surface area, root volume, number of tips and forks, and several root classes of length, surface area, and volume. Moreover, root morphological features demonstrated a positive association with root dry weight. However, it showed a negative correlation ($p<0.05$, shown in red) with the leaf area/root length ratio. In addition, root weight ratio, root length ratio, and specific root length were negatively correlated with the leaf area/root length ratio but positively correlated with the root:shoot ratio. There was a positive and significant correlation between root dry weight and root macronutrients, root carbon, and root nitrogen ($p<0.05$).

Gene Expression Studies

The relative expression ($\log_2$ fold change) of the identified genes in Pusa Basmati 1509 inoculated with the selected strains was evaluated by qRT-PCR. First, the expression patterns of genes involved in auxin biosynthesis were investigated. Except for FT1, the expression level of *OsYUCCA1*, a member of the YUCCA family of genes involved in IAA biosynthesis, was significantly elevated in all the isolates, with FH7 exhibiting the most significant upregulation (4.31-fold increase) (Fig. 5A). A mixed

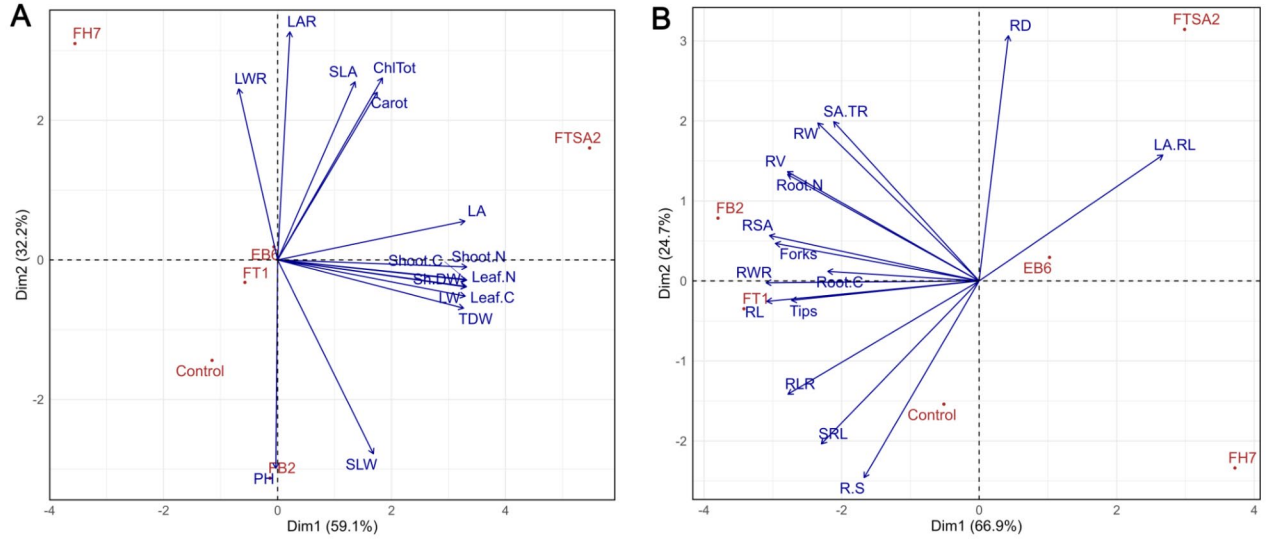

Fig. 3 A biplot display of principal component analysis (PCA) of the above (**A**) and below (**B**) ground parameters analyzed in *Streptomyces-treated* rice plants. **A**: LWR: leaf weight ratio; LAR: leaf area ratio; SLA: specific leaf area; ChlTot: total chlorophyll; LA: leaf area; Shoot N: shoot nitrogen; TDW: total dry weight; SLW: specific leaf weight; PH: plant height. **B**: RD: root diameter; L.TR: length of thick roots; SA.TR: surface area of thick roots; V.TR: volume of thick roots; V.FR: volume of fine roots; L.FR: length of fine roots; SA.FR: surface area of fine roots; SA.SFR: surface area of superfine roots; RSA: root surface area; RWR: root weight ratio; RL: total root length; R:S: root-to-shoot ratio; RV: root volume; RW: root weight; LA.RL: leaf area to root length

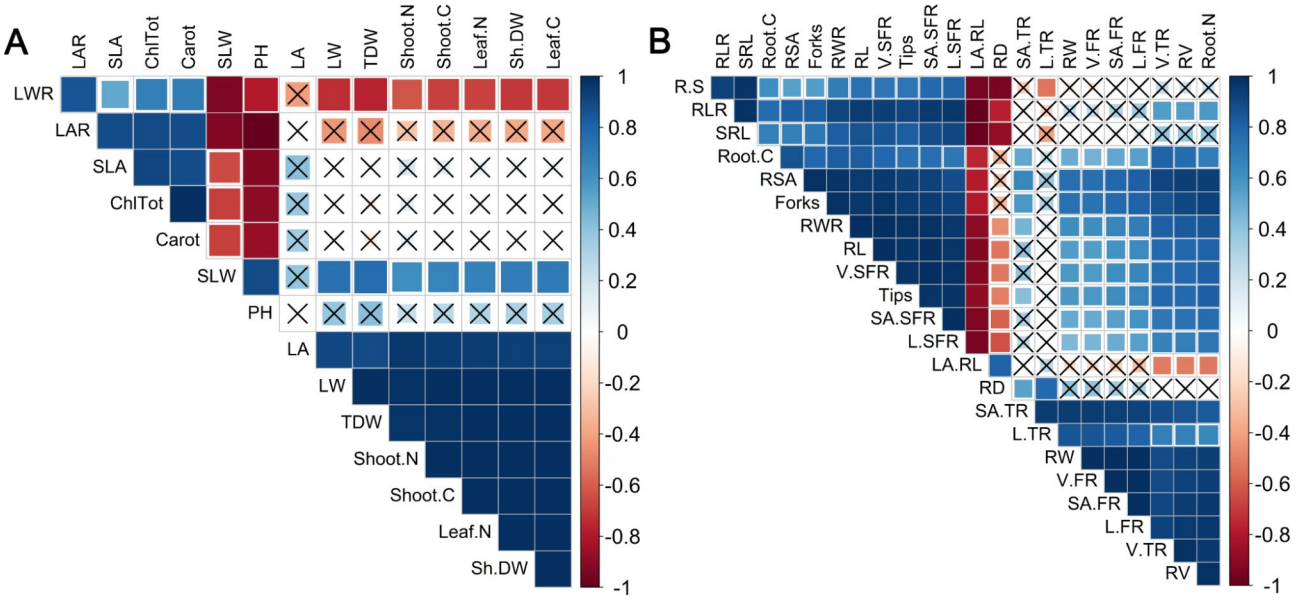

Fig. 4 Corr-plot representing Pearson's correlation analysis between the variables in (**A**) above and below (**B**) ground characteristics. Here, the size of the square is proportional to the absolute value of correlation coefficients, whereas their color represents the value in positive or negative. A box with a cross indicates non-significant correlations ($p < 0.05$). A) LWR: leaf weight ratio; LAR: leaf area ratio; SLA: specific leaf area; ChlTot: total chlorophyll; Carot: carotenoids; SLW: specific leaf weight; PH: plant height; LA: leaf area; LW: leaf dry weight; TDW: total dry weight; Shoot N: shoot nitrogen; Shoot C: shoot carbon; Leaf N: leaf nitrogen; Sh.DW: shoot dry weight. B) R:S: root-to-shoot ratio; RLR: root length ratio; SRL: Specific root length; Root C: root carbon; Root N: root nitrogen; RSA: root surface area; RWR: root weight ratio; RL: total root length; RV: root volume; RW: root dry weight; RD: root diameter; LA.RL: leaf area to root length; L.TR: length of thick roots; SA.TR: surface area of thick roots; V.TR: volume of thick roots; L.FR: length of fine roots; SA.FR: surface area of fine roots; V.FR: volume of fine roots; L.SFR: length of superfine roots; SA.SFR: surface area of superfine roots; V.SFR: volume of superfine roots

pattern of expression was observed for *OsYUCCA3*, another member of the YUCCA family in rice (Fig. 5B). No significant change in *OsYUCCA3* gene expression was observed in EB6-, FTSA2-, and FT1-treated samples. In contrast, it was significantly up- and down-regulated by FB2 and FH7, respectively. The genes involved in auxin influx carrier and auxin signaling, *OsAUX1* and *OsIAA1*, were considerably upregulated with inoculation of all studied strains, except FT1, which down-regulated *OsIAA1* with no significant difference compared to the control (Fig. 5C, D). In the GA biosynthetic pathway, *OsGA20ox-1*, the gene encoding the gibberellin 20-oxidase enzyme in GA biosynthesis, was significantly induced in response to FH7 (1.28-fold increase), whereas the remaining strains non-significantly suppressed the gene expression. While *OsGID1*, the gene encoding the gibberellic acid receptor in rice, was stimulated by most strains, highest in FB2 (3.43-fold increase), its expression was shown to be down-regulated by FT1 (fold decrease of −0.68) (Fig. 5E, F). We also analyzed the transcriptional responses of two genes involved in cytokinin production, *OsIPT3* and *OsIPT5*. While *OsIPT3* was found to be elevated by more than one-fold increase following inoculation with FH7, EB6, and FT1, *OsIPT5* was found to be upregulated by all the strains except FTSA2 (−1.41-fold decrease) (Fig. 5G, H).

Discussion

In recent years, the use of actinobacteria in agriculture has expanded due to their potential action as PGPR and their widespread distribution in plants. Rhizospheric and endophytic actinobacteria from rice have been employed in studies to combat crop diseases and enhance rice growth (Naik et al. 2009; Gao et al. 2021; Saikia and Bora 2021). In the present study, the plant growth regulation effect of five native actinobacterial isolates from rice rhizosphere and endosphere niches was studied.

The in vitro assessment of plant growth-promoting characteristics, such as IAA production, showed that adding tryptophan to the bacterial broth significantly increased IAA production, similar to the findings of Spaepen et al. (2011). Thus, all the tested isolates preferred a tryptophan-dependent IAA production pathway. The production of IAA varies among species and strains (19–27 µg/ml) and is affected by culture conditions, growth stage, and substrate availability. This result is consistent with those of Khamna et al. (2010) and Djebaili et al. (2020), who showed that the IAA production levels of rhizospheric actinomycetes varied from 11.03 to 144 µg/ml and 7.44 to 21.4 µg/ml, respectively. Also, all the isolates produced siderophores, as indicated by the formation of an orange halo zone on the CAS agar medium. It has been observed that *Streptomyces* sp. from rhizosphere soil produces siderophores that boost

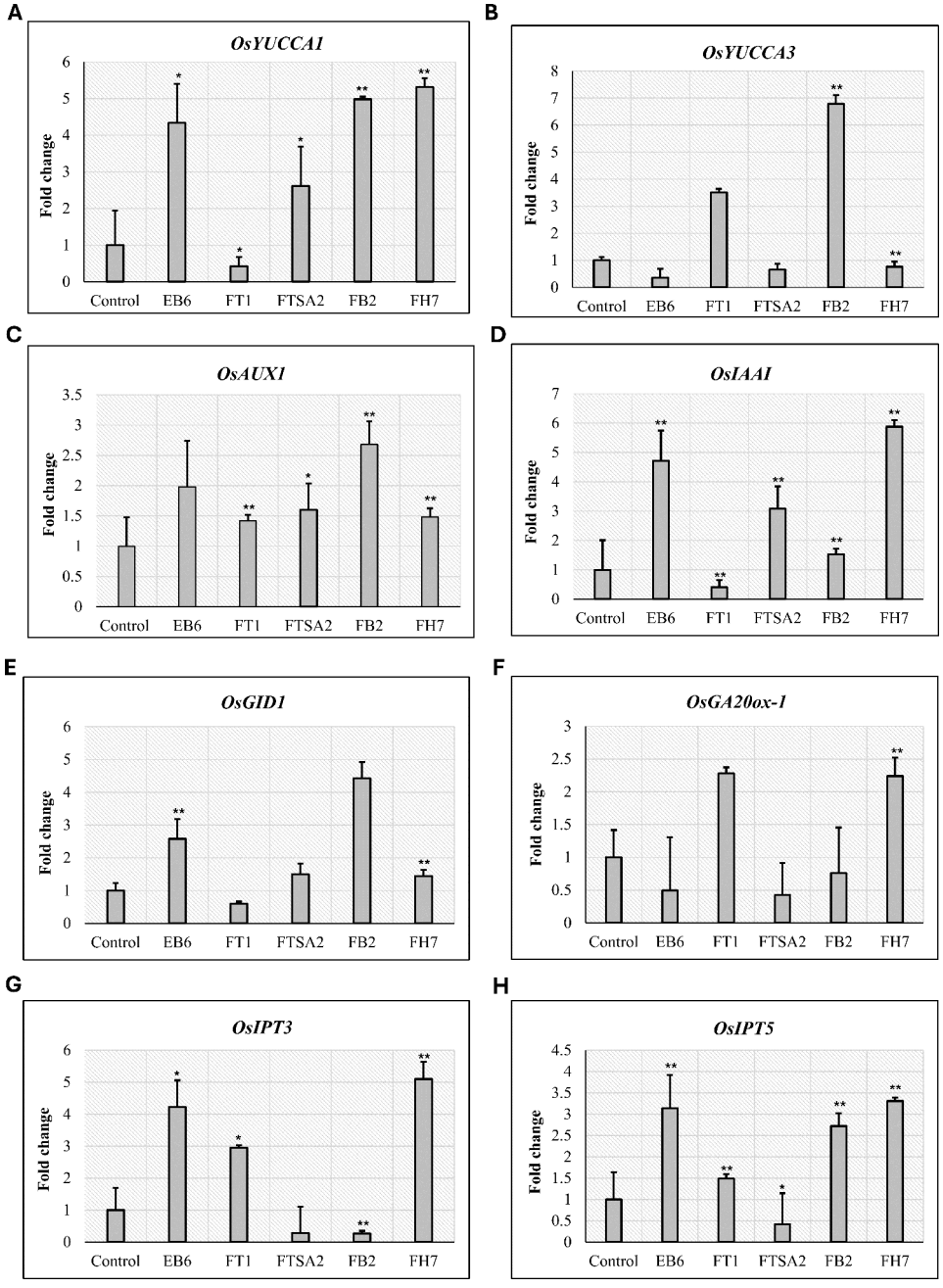

Fig. 5 Effect of actinobacterial inoculation on the expression levels of genes involved in phytohormone metabolism, transport, and signaling in rice (**A**–**D**): auxin; (**E**, **F**): GA; (**G**, **H**): CK. The *actin* (*Os03g0718100*) gene was used as the reference gene. Bars represent the mean ± SE of $n = 3$. Significant differences between control and treated samples are indicated in an asterisk (*). The sign * represents $p \leq 0.05$, and ** represents $p \leq 0.01$

plant growth by building a complex with iron (Fe^{3+}) in the rhizosphere, rendering iron inaccessible to phytopathogens and inhibiting their growth. On the other hand, none of the isolates could solubilize phosphate in Pikovskaya's agar medium. A siderophore-producing endophytic Streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105) yielded comparable results (Rungin et al. 2012). Previous studies demonstrated that the phosphate solubilizing bacteria chelate iron from Fe-P complexes in the soil by converting insoluble inorganic forms of phosphate into soluble forms *via* the secretion of organic acids or siderophore-like compounds (Hamdali et al. 2008; Ben Farhat et al. 2009; Rungin et al. 2012). This suggests that the selected isolates in this study may use the siderophore-mediated phosphate solubilization mechanism to promote plant development. However, this must be validated through in vitro experiments. Similarly, the isolates did not exhibit HCN

synthesis, which has been associated with the biocontrol mechanism (Keel 1997), except for FT1, which produced negligible amounts of HCN.

Effect of Streptomyces Strains on rice seed Germination

Coating of seeds with plant beneficial microbes (PBM) provides precise application of inoculum at the seed-soil interface (Scott 1989), ensuring that the PBMs are readily accessible at germination and early plant developmental phases, boosting healthy and speedy establishment and increasing crop yield (Colla et al. 2015). In this seed germination bioassay, all treatments resulted in a germination percentage greater than 95% compared to the control, and none of the *Streptomyces* strains had any phytotoxic effect on rice seeds (cv. Pusa Basmati 1509). However, the decline in seed vigor index in the treatments with FTSA2 and FH7 was significant. This is explained by the fact that plant roots either operate as filters of rhizosphere bacteria adhering to root surfaces (Reinhold-Hurek et al. 2015) or by the overproduction of IAA by the plant growth-promoting bacteria-induced stress hormone ethylene in plants, resulting in lower colonization, as reported in rice treated with endophytic *Klebsiella pneumoniae* S2 (Shabanamol et al. 2018).

Streptomyces Strains Display Different Effects on Plant Growth Physiology

The study explores the physiological basis of plant growth promotion in *Streptomyces* strains based on their (i) enhancement in total dry matter production, (ii) root proliferation and N uptake, (iii) carbon partitioning, and (iv) changes in the expression of phytohormone-related pathways augmenting root-shoot growth. It provides a framework for understanding the effects of these strains on plant growth (Fig. 6). These strains, isolated from the rice rhizosphere and endosphere, have been acclimated to the native ecology of the host plant, which may make their effects more consistent (Meldau et al. 2012).

Dry Matter Production and Partitioning

An increase in total dry matter production is the most significant factor influencing plant growth performance (McDonald et al. 1996). The distribution of dry matter between the root and the shoot, however, might differ (Marcelis 1996; Hunt and Lloyd 2008). The study evaluated the performance of different treatments in terms of dry matter production and dry matter partitioning to roots and shoots. Results showed three categories of responses in treated plants: increased biomass production (FTSA2, EB6, FB2, and FT1), increased partitioning to roots (FB2 and FT1), and no growth promotion (FH7).

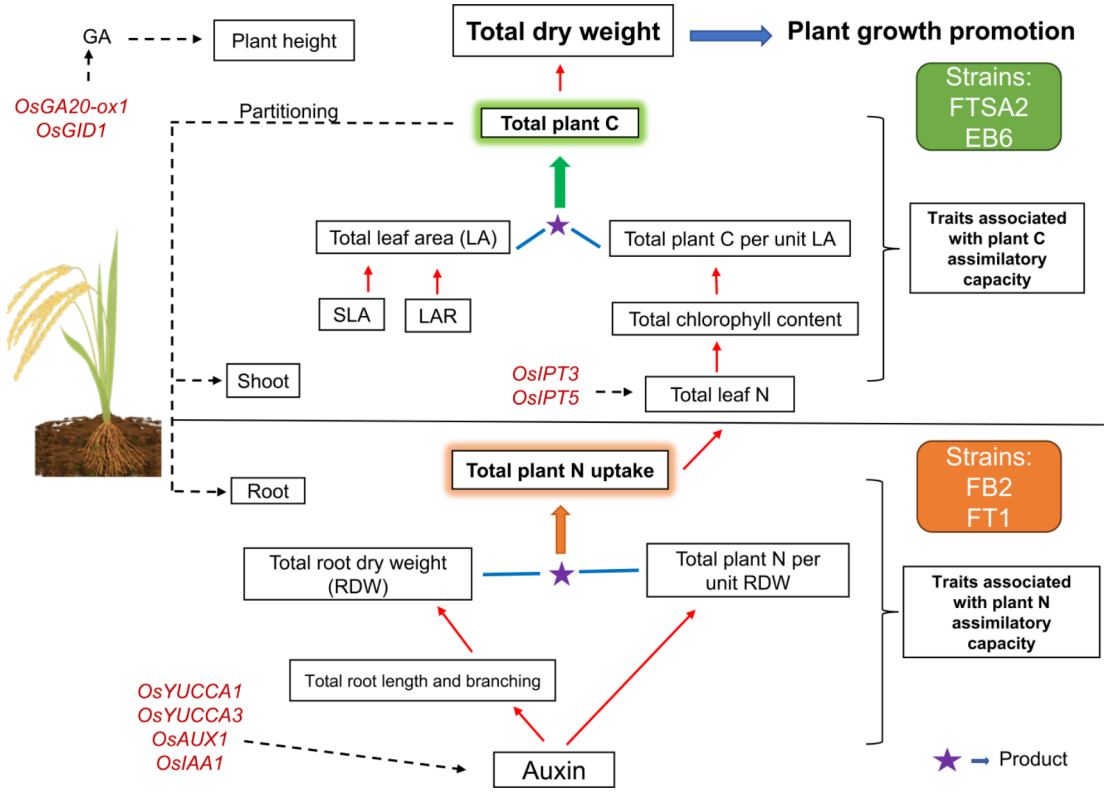

Fig. 6 The conceptual framework underlying plant growth promotion by *Streptomyces* strains

a. Inoculation with strains FTSA2 and EB6 may have higher total biomass and lesser partitioning to roots

In the FTSA2-inoculated roots, higher root thickness resulted in a slightly higher allocation of biomass to roots (9.1%). Plant N uptake is a product of total root production (in terms of root dry matter and length) and the assimilatory capacity of roots (in terms of total plant N per unit root dry matter) (Lawlor 2002). In FTSA2-treated plants, higher total plant N uptake in roots was a result of increased total plant N uptake per unit root dry weight and improved root dry biomass functioning together, corroborating with other findings by Shaharoona et al. (2008) and Nguyen et al. (2019). This assimilated N led to a rise in leaf N that can lead to increased photosynthesis (Evans et al.1983; Osaki et al. 1995). Plant carbon assimilation is a function of the photosynthetic capacity of the leaf and total leaf area production (Watson 1952). More capacity for carbon fixation in FTSA2-treated plants can also be associated with their higher leaf area (LA), the increase in specific leaf area (SLA), leaf area ratio (LAR), and higher leaf area to root length (LA/RL), which also suggested a higher proportion of carbon was partitioned to leaves. The C assimilatory capacity of the leaf is also related to higher chlorophyll content and leaf N content, which may be associated with higher Rubisco content (Parry et al. 2013). This was supported by our findings, which showed that the shoot and root received 91.3% and 8.7% increases in carbon allocation, respectively, compared to the control. So, among other treatments, the FTSA2 strain enhanced shoot carbon, increasing shoot and total dry matter.

The strain EB6 also showed higher biomass production than the control. However, it varied from FTSA2-treated plants in that it exhibits a little increase in plant height and a lesser decline in carbon partitioning in roots. Overall, we observed that root endophyte inoculation shifted plant resource allocation patterns without impacting the accumulation of total plant biomass (Henning et al. 2016). Thus, treatment with FTSA2 and EB6 strains improved the total biomass through root traits associated with carbon assimilation and partitioning to shoot, although partitioning to roots (RWR) decreased in both.

Furthermore, gene expression studies revealed that plants treated with EB6 and FTSA2 strains may have produced fewer auxin molecules due to auxin homeostasis. This resulted in an increase in the expression of genes for the influx carrier (*OsAUX1*) and IAA biosynthesis (*OsYUCCA1*), but the levels were insufficient to counteract the action of the repressor (*OsIAA1*). Studies suggest that nitrate concentrations and auxin homeostasis in plants interact on multiple levels (Guan 2017). Thus, we propose that shoot-derived auxin stimulates crown roots and nitrate transporters, leading to increased nitrate uptake and root dry matter (Guo et al. 2005; Yamamoto et al. 2007; Poupin et al. 2016; Hsu et al. 2021). In GA signaling, while *OsGA20-ox-1* gene transcript abundance was down-regulated in both treatments, GID1 gene transcript abundance was up-regulated in EB6 rice plants. This suggests that exogenous gibberellins produced by bacterial isolates may have upregulated GIDI. Subsequently, the 26S proteasome pathway degrades the GA-GID1-DELLA complex, activating the GA response. While both treatments showed down-regulation of GA biosynthesis genes, *OsIPT3* and *OsIPT5* were up-regulated in EB6 plants and down-regulated in FTSA2 plants. It is conceivable that KNOTTED-like homoeobox (KNOX) proteins regulate the ratio of CK to GA in rice plants by suppressing GA20ox-1 and activating IPT genes (Jasinski et al. 2005). Overexpression of IPT genes in EB6 rice plants might increase shoot nitrogen content. In FTSA2 plants, shoot-derived auxin probably slightly inhibited KNOX function, causing a decrease in the CK:GA ratio. Nevertheless, bacterial effects on the concentration of all phytohormones (IAA, GA, and CK) in plants should be confirmed by direct measurements.

b. Inoculation with strains FB2 and FT1 may have moderate to higher total biomass and more significant partitioning to roots

The study found that an increase in total dry weight (TDW) was linked to increased root dry weight (RDW) and partitioning to roots (RWR) in strains FB2 and FT1. This is due to improved root architectural and morphological characteristics, such as total root length, branching volume, surface area, and diameter. The increase in root length ratio (RLR) improves nutrient (total N) and water uptake by increasing root surface area (increased soil area explored by finer roots) in conjunction with a moderate to marginal increase in fine and superfine roots (Eissenstat 1992). The high metabolic cost for root growth in early plant development may increase shoot growth once the plant is established, compensating for the initial cost of plant/bacteria protocooperation (Lynch and Brown 2012; de Sousa et al. 2021). The leaf nitrogen concentration increased with total chlorophyll content in FT1-treated leaves (Gholizadeh et al. 2017), whereas the results of FB2 treatment are inconclusive due to decreased chlorophyll *b*. The partitioning of more C to roots in FB2 and FT1 compared to the control resulted in increased root dry matter.

Plants treated with FB2 and FT1 strains showed a high level of IAA production due to the overexpression of both the YUCCA genes in FB2 and moderate upregulation of *OsYUCCA3* gene in FT1. This led to an increase in the expression of *OsAUX1* in both strains. However, in FT1-treated plants, *OsYUCCA3* and *OsAUX1* were

slightly upregulated, allowing less free auxin to enter cells. Like FTSA2 and EB6, increased shoot-derived auxin concentrations improved root characteristics. Our results were confirmed by the finding that volatile compounds produced by *Bacillus amyloliquefaciens* SQR9 promoted lateral root formation in *Arabidopsis*, which involved the auxin signaling system, polar auxin transport, and (YUCs)-mediated auxin synthesis (Li et al. 2021). In FB2-treated plants, host-derived GAs or bacterially synthesized GAs led to the degradation of DELLA repressor proteins, resulting in auxin maxima concentration. This stimulated KNOX-mediated downregulation of *OsIPT3* and upregulation of *OsGA20ox-1* genes, resulting in increased biomass allocation to shoots and a slight increase in total dry weight. Both gibberellin-producing and non-producing PGPR stimulate shoot growth and induce GA biosynthetic gene expression, supporting this notion (Kang et al. 2014; Lee et al. 2015). *OsIPT3* and *OsIPT5*, which synthesize CK, are slightly more upregulated than *OsGA20-ox1*, which would have reduced shoot elongation but not dry biomass.

c. Inoculation with Strain FH7 may have a Negative Effect on Total Biomass and Plant Growth

The FH7 strain, despite producing more IAA in vitro, did not increase plant biomass above or below ground, contributing to its poor growth response in treated plants. The regulation of IAA production in PGPR liquid culture is substantially different from that of natural soil due to the presence of environmental factors, soil properties, root exudates, and soil microbial interactions (Spaepen and Vanderleyden 2011). In treated plants, the leaf morphology (leaf area ratio, specific leaf area, leaf weight ratio, and total chlorophyll) improved due to increased plant nitrogen per unit root dry matter, resulting in increased carbon allocation to roots. However, this caused a decrease in root dry weight because growth respiration uses some of the carbon (C) partitioned to roots to produce energy to convert into new biomass (Weraduwage et al. 2015). As this strain has reduced seed vigor in the germination tests, an additional possibility is the formation of strain-mediated inhibitory secondary metabolites.

FH7-treated plants showed poor root trait performance due to overexpression of the *OsIAA1* auxin repressor gene, while increased shoot N and total chlorophyll content were produced by KNOX-mediated upregulation of IPT genes. The apparent discrepancy between IAA biosynthesis gene expression profiles and root traits may indicate that gene expression levels do not necessarily translate to phenotypic traits, which is consistent with Zhang et al. (2007) reporting lower auxin accumulation in *Bacillus subtilis* GB03-exposed leaves despite increased *ASA1* expression.

Conclusion

The current study aimed to select plant growth-promoting actinobacterial isolates from the rice environment that were both efficient in promoting plant growth and could contribute to enhanced nutrient uptake. Our findings highlight the potential of native *Streptomyces* strains, particularly FTSA2 and FB2, as effective bioinoculants for sustainable agriculture. These strains exhibited superior plant growth promotion (PGP) potential, with FTSA2 enhancing total dry matter production and FB2 stimulating root development. The observed enhancements in plant growth were linked to improvements in both nitrogen (N) and carbon (C) assimilation capacities, with these strains significantly increasing N assimilation capacity in roots and associated C assimilation in shoots. This was accompanied by notable increases in key root traits such as total root length and surface area, as well as enhanced leaf area per plant and increased partitioning to roots. Furthermore, our study sheds light on the role of plant hormones in mediating these growth-promoting effects. We observed an upregulation of phytohormone biosynthesis-related genes, including *OsYUCCA1*, *OsYUCCA3*, *OsIPT3*, and *OsIPT5*, suggesting a potential mechanism by which these strains modulate plant growth and development. These PGP strains, particularly FB2 and FT1, which demonstrated the ability to increase belowground biomass, could be particularly advantageous in soils with a low nitrogen (N) supply. These strains effectively increase the root surface area and volume of soil foraged by the root, resulting in greater nutrient uptake and growth-promoting effects. Thus, strains FTSA2 and FB2 can be promising bioinoculants for enhancing rice growth and nutrient uptake. Further research is necessary to elucidate the underlying mechanisms of plant growth promotion and optimize the application of these strains in the field.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12284-024-00732-w.

Supplementary Material 1

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Author Contributions
DPT, RP, VC: Conceptualization, Supervision and Funding Acquisition. DPT and AH: Data curation, Investigation, Methodology. KA and VC: Funding acquisition. RP, VC, and RB: Resources and Project administration. DPT and AH: Software run and Formal analysis. AH and DKJ: Validation. DPT: Writing original draft. RP, AH, DPT and DKJ: Writing – review & editing.

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Data Availability
The near full-length 16 S rRNA gene sequences of the actinobacterial isolates reported in this study were submitted to GenBank under the accession numbers MN955410, MN955412, MZ736625, MZ736626, MZ736627.

Declarations

Competing Interests
The authors declare no competing interests.

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