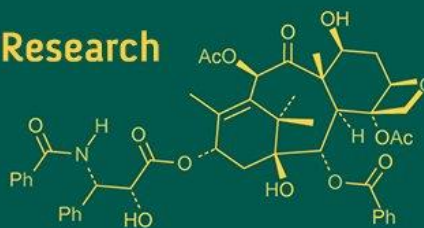


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In vitro evaluation of plant growth promoting endophytic actinobacteria for seedling growth of rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) is one of the important food crops cultivated worldwide, but soil fertility reduction and over-use of chemical fertilizers are limiting rice production. In this context, beneficial Gram-positive bacteria known as plant growth-promoting actinobacteria have emerged due to their benefits to sustain soil health and improve the growth of crops. The present study aimed to investigate the plant growth-promoting property of endophytic actinobacterial isolates on rice (*Oryza sativa* L.) under *in vitro* conditions. Thirty actinobacterial isolates were tested for their effect on germination of seeds, growth of seedlings, biomass and vigor indices. Significant differences were observed between the different isolates for each growth parameter. Germination percentage ranged from 70.00% to 97.50%, with the highest germination recorded in ACB-28 (97.50%), followed by ACB-1, ACB-5, and ACB-15 (92.50%). The longest shoot length was seen in ACB-13 (9.74 cm) and the longest root length in ACB-15 (11.36 cm). The highest root fresh weight was observed in ACB-14 (0.080 g) and maximum shoot fresh weight was recorded in ACB-24 (0.184 g). The parameters of dry weight showed different trends, the highest root dry weight (0.016 g) was obtained at ACB-26 and the maximum shoot dry weight (0.027 g) was recorded at ACB-24 and ACB-27. Higher vigor index I was recorded in ACB-15 (1843.9) while ACB-10 had the highest vigor index II (3229.17). The improved growth of seedlings may be due to plant growth promoting characteristics like production of phytohormones and solubilization of nutrients by actinobacteria. The study identified promising isolates with potential for development as bioinoculants for sustainable rice cultivation.

Keywords: Actinobacteria, endophytes, plant growth promotion, rice, germination, *in vitro* assay

1. Introduction

Rice (*Oryza sativa* L.) is a staple food crop, widely grown in Asia, especially in countries such as India, where it is the backbone of food security (Fukagawa and Ziska, 2019) [9]. The world's population is growing and land for agriculture is decreasing, and there is a strong demand to improve rice productivity in a sustainable way. In long term, conventional agriculture has been known to cause soil degradation, nutrient imbalance, environmental pollution and loss of microbial diversity (Kumar *et al.*, 2022) [14].

The use of beneficial microorganisms as plant growth-promoting microorganisms (PGPM) has gained considerable attention as they play a crucial role in enhancing plant growth through various direct and indirect mechanisms (Aloo *et al.*, 2022) [3]. These include biological nitrogen fixation, solubilization of insoluble phosphates, production of phytohormones like indole-3-acetic acid (IAA), production of siderophore and suppressing plant pathogens (Singh *et al.*, 2025) [27].

The metabolic versatility of actinobacteria in soil microflora, and their ability to survive in various soil environments makes them very important. The plant growth promoting activity of actinobacteria was increasingly studied in recent years and their famous role in the production of antibiotics is well known (Oberhofer *et al.*, 2019) [20]. These microorganisms can promote plant growth by producing growth hormones, help to dissolve nutrients, and inhibit plant pathogens by the synthesis of antifungal and antibacterial compounds (Aguennouz *et al.*, 2025) [1]. Additionally, actinobacteria help maintain soil health by breaking down organic matter and involve in soil nutrient cycles, which increases soil

fertility. They are also good candidates for bioinoculants application in sustainable agriculture because they can colonize plant roots and form symbiotic relationships with plants (Boukhatem *et al.*, 2022) [6].

Although the use of actinobacteria for rice cultivation is still underutilized, systematic evaluation of plant growth promoting properties of actinobacterial isolates is necessary to obtain the maximum potential of these bacteria in rice. The present study was conducted to evaluate the effect of endophytic actinobacteria on seed germination and seedling growth parameters of rice under *in vitro* conditions.

2. Materials and Methods

3.1 Evaluation of *in vitro* Plant Growth-Promoting Traits of endophytic Actinobacterial Isolates

3.1.1 Location of the experiment

The present experiment was conducted at ICAR-Indian Institute of Rice Research, Hyderabad (Telangana), India for evaluating the effect of endophytic actinobacteria on seedling growth of rice plants.

3.1.2 Source of Actinobacterial Isolates

The actinobacterial isolates used in the present study were isolated from rice root samples from the rice field. All isolates were maintained on ISP2 slants at 4 °C and subcultured periodically for further study.

3.1.3 Preparation of Inoculum

Efficient actinobacterial isolates identified from *in vitro* screening were grown in broth medium and incubated at 28±2 °C for 5-7 days. The culture was adjusted to approximately 10⁸-10⁹ CFU mL⁻¹ for seed treatment.

3.1.4 Seed Sterilization

Healthy seeds of rice (*Oryza sativa* L.) were surface sterilized by treating with 70% ethanol for 1 minute followed by 1% sodium hypochlorite for 2-3 minutes. The seeds were then well rinsed with sterile distilled water (3-4 times) to remove traces of the sterilant.

3.1.5 Seed Inoculation

Surface-sterilized seeds were soaked in actinobacterial suspension for overnight. Control seeds were soaked in sterile distilled water.

3.1.6 Germination Test

The overnight soaked seeds were placed on water agar plates and were kept for incubation at 28°C for 7 days. The data such as number of seeds germinated, root length, shoot length, fresh weight, dry weight was collected and germination percentage, vigor indices I and II were also calculated using the formula given below (Pole *et al.*, 2026).

$$\text{Germination(\%)} = \frac{\text{number of seeds germinated}}{\text{number of seeds kept for germination}}$$

$$\text{Vigor index I} = \frac{\text{Germination(\%)}}{\text{seedling length(cm)}} \times 100$$

$$\text{Vigor index II} = \frac{\text{Germination(\%)}}{\text{dry wt. seedlings(g)}}$$

3.1.7 Statistical Analysis

The experiment was designed in a completely randomized design (CRD) with three replications. Analysis of variance (ANOVA) was used to analyze the data and critical difference (CD) was used to compare the treatment means at 5% level of significance (Gomez and Gomez 1984) [10].

4. Results and Discussion

4.1 Germination Percentage (%)

The inoculation with various isolates of endophytic actinobacteria significantly influenced the rice seed germination percentage (Table 1). Germination was between 70.00% and 97.50% in the various treatments. Among the isolates, ACB-28 had the highest germination (97.50%), followed by ACB-1, ACB-5, ACB-15 (92.50%) and ACB-12, ACB-26 (90.00%) which showed good plant growth promoting potential. The control treatment, however, had a lower germination rate of 70.00% similar to that of ACB-21 and ACB-27.

Moderate germination percentages were observed in isolates such as ACB-9 (87.50%), ACB-11 (87.50%), ACB-18 (85.00%) and ACB-4 (82.50%), while the remaining treatments exhibited values between 72.50% and 80.00%. The increased germination of seeds treated with actinobacteria could be attributed to the production of phytohormones like indole-3-acetic acid (IAA), the ability to make nutrients soluble, and boost the seed metabolic activity (Almazrouei *et al.*, 2025) [2]. Similar findings about the increase in the germination percentage with the actinobacterial inoculation was reported by Faddetta *et al.*, 2023 [8] and Zhang *et al.*, 2023 [30].

4.2 Shoot Length (cm)

The growth of rice seedlings in terms of shoot length was statistically different between the treatment and the control, showing the positive effect of actinobacterial isolates on the early plant growth (Table 1). Shoot length was from 4.52 cm to 9.74 cm. The maximum shoot length was recorded in ACB-13 (9.74 cm), followed closely by ACB-27 (9.46 cm), ACB-26 (9.44 cm), ACB-1 (9.26 cm), ACB-29 (9.20 cm), and ACB-28 (9.12 cm). The ability of these treatments to show superior shoot elongation than the control (7.60 cm) indicated that the microbial metabolites stimulated cell elongation and division. Moderately higher shoot lengths were observed in ACB-15 (8.54 cm), ACB-11 (8.64 cm), ACB-12 (8.26 cm), ACB-18 (8.20 cm), and ACB-9 (8.10 cm). Lower shoot growth was found in ACB-7 (4.52 cm), ACB-8 (5.98 cm) and ACB-5 (6.02 cm) which showed comparatively lower growth promoting activity. Such improvement in shoot growth may be due to the production of growth promoting substances, like IAA, Gibberellins, and enhanced nutrient availability by actinobacteria (Boubekri *et al.*, 2022) [5]. Sharma *et al.*, 2023 [26] and Hazarika *et al.*, (2022) [12] also reported the increase in the shoot length when treated with endophytic actinobacteria.

4.3 Root Length (cm)

Root length is an important parameter indicating nutrient uptake efficiency and plant vigour. There was a great difference between the treatments, in which the root length varied from 5.78 cm to 11.36 cm (Table 1). The highest root length was recorded in ACB-15 (11.36 cm), followed by ACB-6 (11.08 cm), ACB-29 (10.66 cm), ACB-30 (10.14 cm), ACB-5 (10.06 cm), and ACB-22 (10.36 cm).

Relatively high root length was obtained for control treatment (11.16 cm), but some actinobacterial isolates gave comparable or better result. Moderate root growth was observed in ACB-26 (9.88 cm), ACB-28 (9.56 cm), ACB-19 (9.62 cm), and ACB-13 (9.08 cm). Lower root lengths were recorded in ACB-4 (5.78 cm), ACB-8 (5.92 cm), and ACB-10 (6.44 cm). The increased root length in actinobacteria-treated plants could be attributed to the production of auxins and siderophores which are responsible for elongation of roots and better nutrient uptake in plants (Hazarika *et al.*, 2022; Mondal *et al.*, 2024) ^[12, 18]. The increase in root length when treated with actinobacteria was previously reported by Govindasamy *et al.*, 2022 ^[11] and Yang *et al.*, 2024 ^[29].

4.4 Fresh Weight Parameters (g)

All the fresh biomass of rice seedlings (root fresh weight (RFW), shoot fresh weight (SFW), and seed fresh weight (SEFW)) were significantly affected by the different actinobacterial isolates, suggesting their participation in boosting early plant growth.

Root fresh weight ranged from 0.017 g to 0.080 g. The highest RFW was recorded in ACB-14 (0.080 g), followed by ACB-2 (0.075 g), ACB-20 (0.066 g), and ACB-25 & ACB-24 (0.057 g). The values were significantly higher than the control (0.035 g), which implies that microbial inoculation has enhanced the ability of the roots to accumulate biomass. ACB-6 and ACB-7 (0.017 g) had lower RFW values (Fig. 1). The other treatments with actinobacteria resulted in an increase in the fresh root weight, which could be explained by the production of phytohormones, such as indole-3-acetic acid (IAA), which stimulate root elongation and lateral root growth. Increased root biomass is associated with increased water and nutrient uptake efficiency (Ashokkumar *et al.*, 2025) ^[4].

The fresh weight of the shoots ranged between 0.069 g and 0.184 g and was broader. The maximum SFW was recorded in ACB-24 (0.184 g), followed by ACB-27 (0.178 g), ACB-26 (0.173 g), and control (0.160 g). Moderate values were observed in ACB-10 (0.154 g), ACB-2 (0.151 g), and ACB-15 (0.150 g). ACB-7 (0.069 g) had the lowest biomass of the shoots (Fig. 2). The differences in fresh weight of shoots among treatments are due to different photosynthetic efficiency and/or nutrient translocation. The higher SFW values observed suggested the better shoot development, which may be attributed to the availability of nutrients such as N and P through microbial activity (Mohamad *et al.*, 2022) ^[16].

The fresh weight of the seeds was from 0.065 g to 0.082 g. The highest SEFW was observed in ACB-8 (0.082 g), followed by ACB-11 and ACB-12 (0.079 g). Lower values were recorded in ACB-15 (0.065 g) and ACB-13 (0.066 g). Lower seed remnant weight suggests greater mobilization of seed sties to seedling growth (Fig. 3). The isolates ACB-24, ACB-26, ACB-27, ACB-14 and ACB-2 showed better results in accumulation of fresh biomass indicating better physiological activity and utilization of nutrients. Patel *et al.*, 2022 ^[23] and Rao *et al.*, 2022 ^[19] had observed the increased fresh biomass of plant when treated with the actinobacteria.

4.5 Dry Weight Parameters (g)

Dry biomass parameters also differed significantly among treatments, such as root dry weight (RDW), shoot dry

weight (SDW) and seed dry weight (SEDW), which indicate differences in structural biomass accumulation.

Root dry weight ranged from 0.005 g to 0.016 g. The highest RDW was recorded in ACB-26 (0.016 g), followed by ACB-14, ACB-18, ACB-23, and ACB-30 (0.012 g). These were significantly higher than the control (0.005 g), which resulted in better root development and biomass retained (Fig. 4). The growth in root dry matter is a sign of better structural growth and root biomass. This can be correlated with the increased absorption of nutrients, particularly the solubilisation of phosphorus by actinobacteria in plant roots to facilitate increased cell division and growth (Raklami *et al.*, 2023) ^[24].

Shoot dry weight ranged from 0.013 g to 0.027 g. The maximum SDW was observed in ACB-24 and ACB-27 (0.027 g), followed by ACB-22, ACB-15, and ACB-29 (0.023 g). These treatments significantly increased dry matter accumulation over the control (0.024 g). The lowest SDW value was observed in ACB-3 (0.013 g) (Fig. 5). Dry weight of the shoot is an indicator of photosynthetic productivity and assimilate production. The higher SDW values reflect the efficient use of photosynthates for the production of plant biomass, which can be aided by microbial activity, which enhances the uptake of nutrients and hormonal balance (Mohamed *et al.*, 2025) ^[17].

Seed dry weight ranged from 0.002 g to 0.034 g. The highest SEDW was recorded in ACB-24 (0.034 g), followed by ACB-23 (0.031 g), ACB-22 (0.030 g), and ACB-17 (0.032 g). The smallest value was obtained for ACB-10 (0.002 g) (Fig. 6). The higher the seed dry weight, the slower the mobilization of seed reserves, while the lower the more efficient the use for growing seedling is. Efficient translocations of stored reserves into the emerging seedling are shown by lower SEDW values, which leads to improved growth and vigor. The isolates that performed better for dry biomass (ACB-24, 26, 27 and 14) indicated their good plant growth-promoting potential (Mahmoud *et al.*, 2024) ^[15]. The study done by Chinachanta *et al.*, 2023 ^[7] and Xu *et al.* 2022 ^[28] had revealed an increase in the dry weights of plants after being treated with the actinobacterial cultures.

4.6 Vigor Indices

The results of the vigor index (Vigor Index I (VI-I) and Vigor Index II (VI-II)) differed significantly among treatments, reflecting the varying effects of the actinobacterial isolates on the growth and establishment of seedlings. Vigor Index I (VI-I) based on germination percentage and total seedling length varied significantly among treatments. The results of the VI-I index showed the best performance in ACB-28 (1821.3), ACB-15 (1843.9), ACB-1 (1645.6) and ACB-26 (1738.8) which demonstrated better seedling elongation and establishment. The VI-I value of the control was lower (1313.2) in comparison (Table. 1). The increased value for these treatments may be attributed to better germination and increased root and shoot growth which may be related to the production of phytohormones (e.g., indole-3-acetic acid (IAA)) by these microbes and improved nutrient solubilization. Other treatments like ACB-7 (896.1) and ACB-8 (986.0) had lower VI-I values, indicating relatively less growth in seedlings (Saho *et al.*, 2024; Padilha *et al.*, 2022) ^[18, 22].

Additionally, Vigor Index II (VI-II) showed significant difference, combining germination percentage with seedling dry weight. The highest VI-II value was recorded in ACB-

10 (3229.2), followed by ACB-6 (2434.2) and ACB-1 (2312.5). These relatively low values, however, were seen in ACB-25 (1056.3) and ACB-14 (1250.0) (Table. 1). The higher VI-II values suggest that the plant can convert seed

reserves into biomass efficiently and its metabolic activity is high (Sharma *et al.*, 2023) [26]. Similar increase in the vigor indices when treated with the actinobacteria were also reported by Kavya *et al.*, 2025 [13].

Table 1: Effect of endophytic Actinobacterial inoculations on growth parameters of rice

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Vigor Index I	Vigor Index II
Control	70.00	7.60	11.16	373.4	1521.74
ACB-1	92.50	9.26	8.52	520.58	2312.5
ACB-2	77.50	7.24	9.02	476.75	1937.5
ACB-3	75.00	7.28	9.42	449.1	2027.03
ACB-4	82.5	7.46	5.78	623.3	1875
ACB-5	92.50	6.02	10.06	575.25	2434.21
ACB-6	77.50	7.32	11.08	421.2	1890.24
ACB-7	72.50	4.52	7.88	584.68	1479.59
ACB-8	82.50	5.98	5.92	693.28	1718.75
ACB-9	87.50	8.10	8.40	530.3	1715.69
ACB-10	77.50	7.88	6.44	541.33	3229.17
ACB-11	87.50	8.64	8.06	524.55	2034.88
ACB-12	90.00	8.26	6.04	629.37	2195.12
ACB-13	80.00	9.74	9.08	425.3	1250
ACB-14	80.00	8.22	7.24	517.46	1509.43
ACB-15	92.50	8.54	11.36	465.33	1712.96
ACB-16	82.50	6.22	7.44	604.1	1964.29
ACB-17	80.00	6.90	6.92	579.01	1355.93
ACB-18	85.00	8.20	7.66	536.06	1770.83
ACB-19	72.50	7.18	9.62	431.55	1686.05
ACB-20	72.50	8.34	7.38	461.33	1647.73
ACB-21	70.00	7.52	7.12	478.14	1666.67
ACB-22	80.00	6.62	10.36	471.14	1230.77
ACB-23	80.00	7.82	8.16	500.63	1403.51
ACB-24	75.00	8.12	8.62	448.03	1056.34
ACB-25	72.50	7.42	11.28	387.7	1510.42
ACB-26	90.00	9.44	9.88	465.8	1764.71
ACB-27	70.00	9.46	8.40	392.04	1428.57
ACB-28	97.50	9.12	9.56	522.08	1805.56
ACB-29	72.50	9.20	10.66	365	1188.52
ACB-30	82.5	8.06	10.14	453.3	1556.6
SE(m)	6.26	0.57	0.98	94.89	119.71
CD (P = 0.5)	24.34	2.12	3.61	369.04	465.54

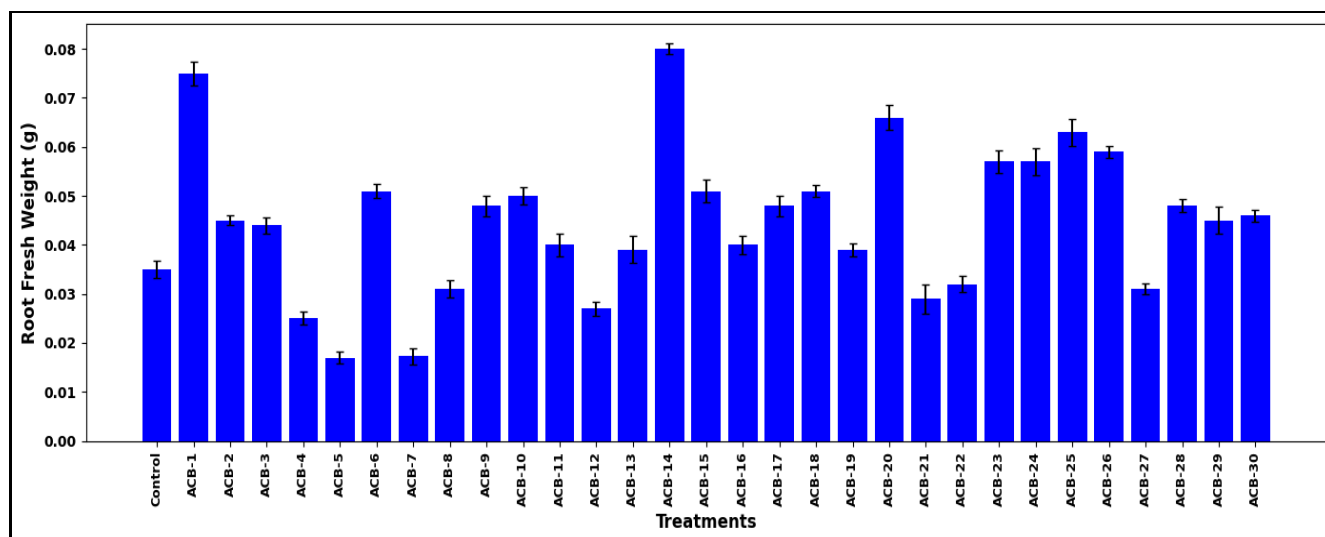


Fig 1: Effect of endophytic Actinobacterial inoculations on root fresh weight of rice

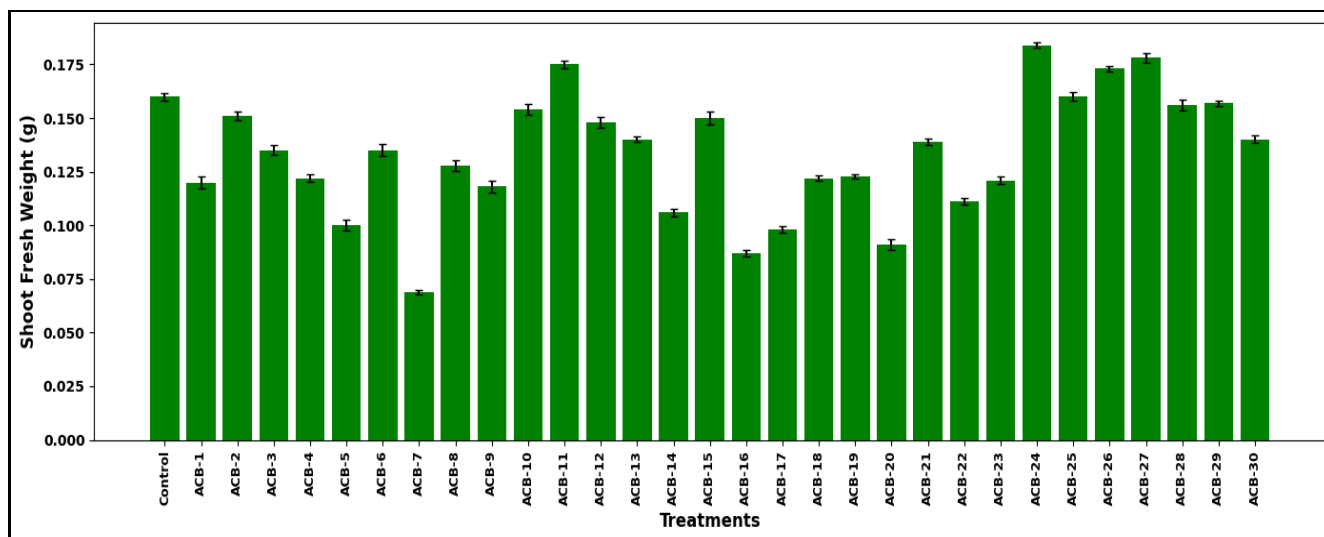


Fig 2: Effect of endophytic Actinobacterial inoculations on shoot fresh weight of rice

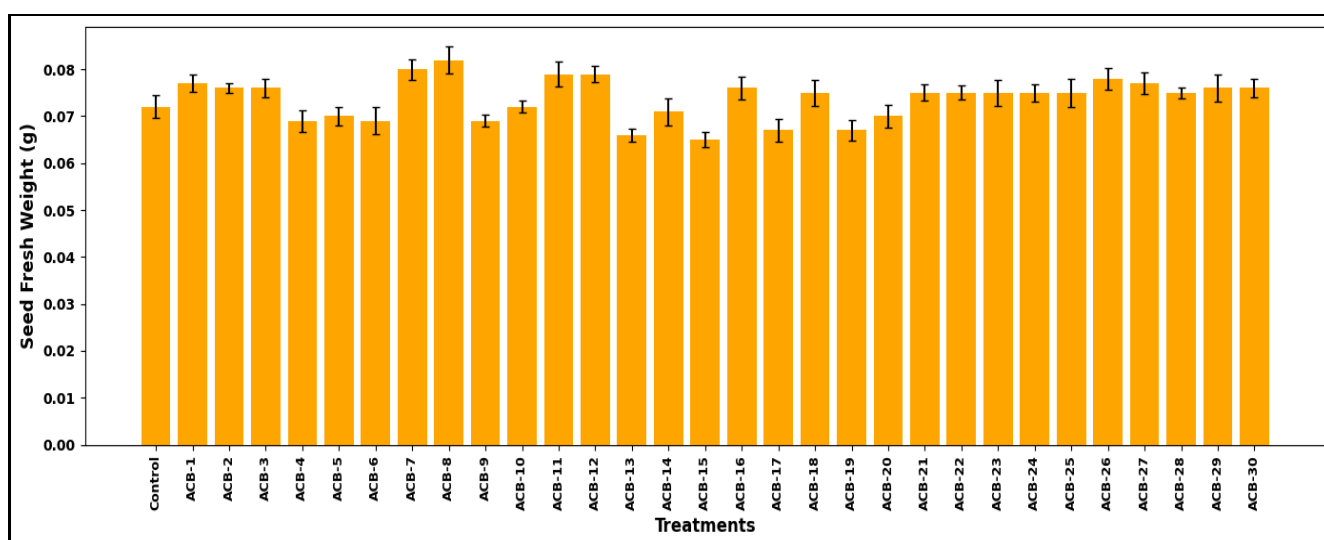


Fig 3: Effect of endophytic Actinobacterial inoculations on seed fresh weight of rice

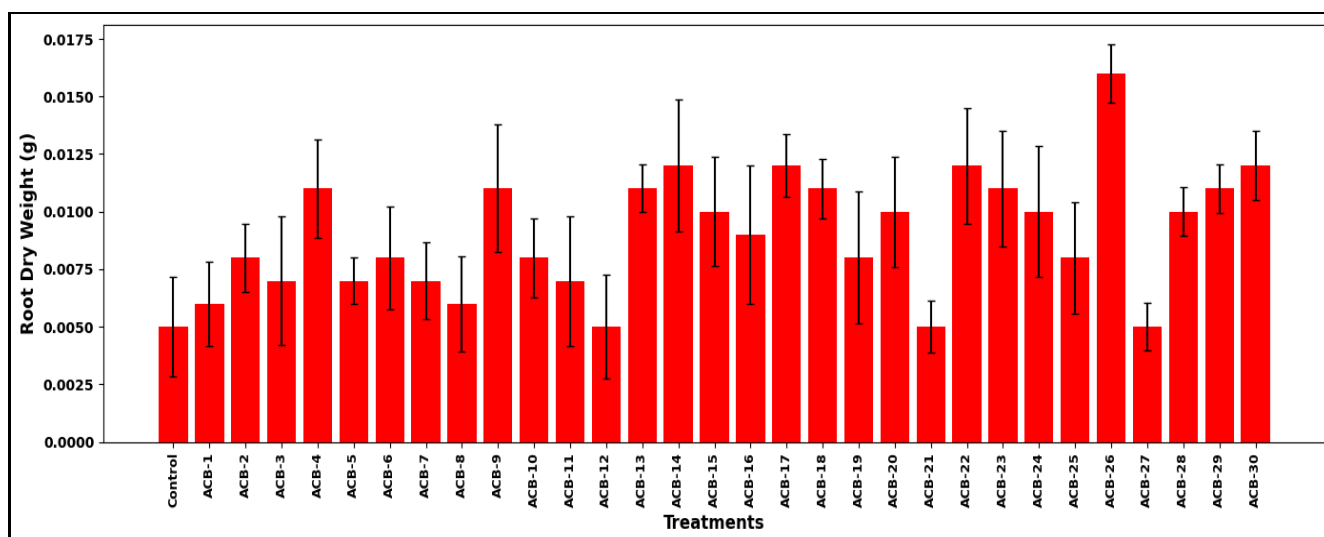


Fig 4: Effect of endophytic Actinobacterial inoculations on root dry weight of rice

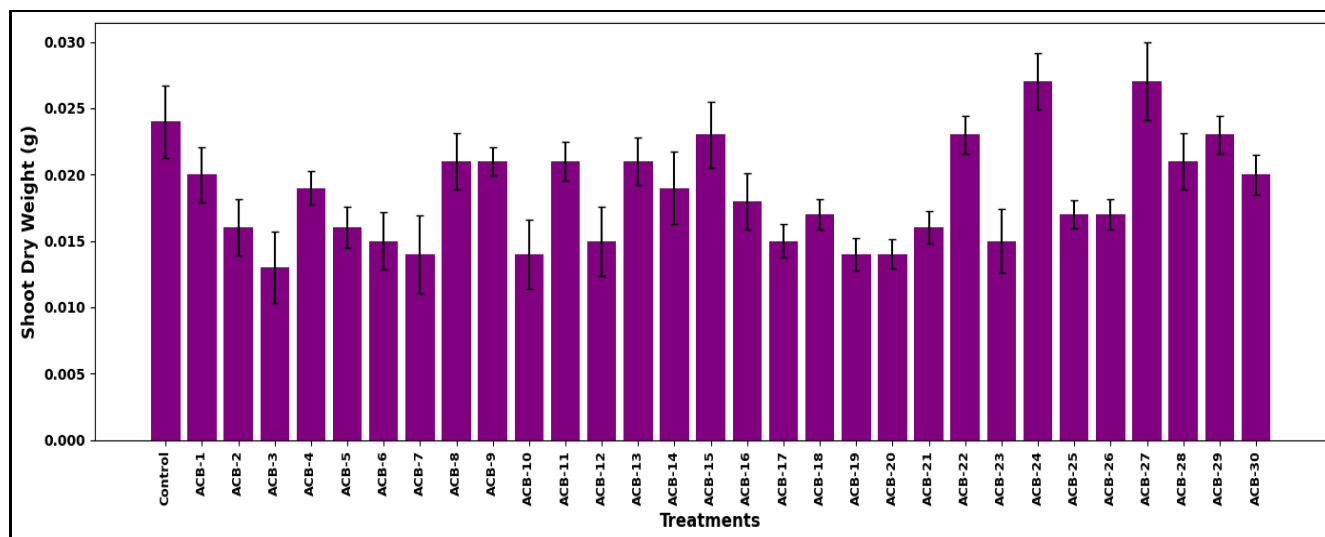


Fig 5: Effect of endophytic Actinobacterial inoculations on shoot dry weight of rice

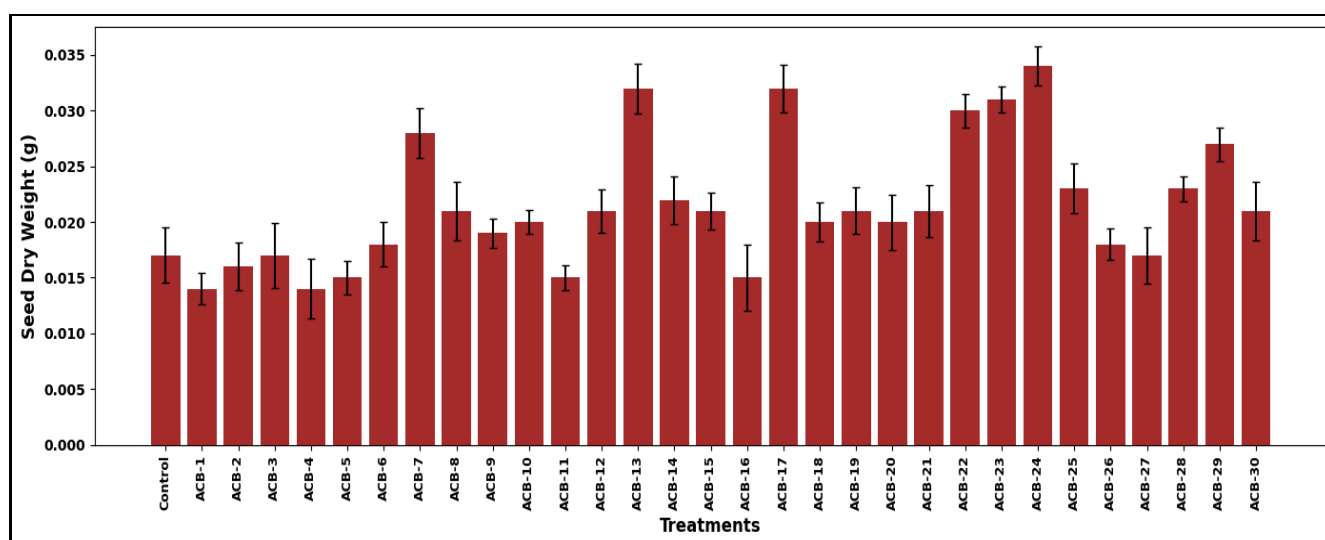


Fig 6: Effect of endophytic Actinobacterial inoculations on seed dry weight of rice

Conclusion

The present study revealed the significant potential of endophytic actinobacterial isolates that supports the germination and early seedling growth of rice under *in vitro* conditions. There were significant differences between the isolates in terms of germination percentage, length of shoot and roots, biomass production and vigor index, reflecting the different plant growth promoting potentials of actinobacteria. The isolates showing better growth in various growth parameters were ACB-28, ACB-15, ACB-24, ACB-26 and ACB-27 which were found to have strong potentiality as beneficial bioinoculants for rice cultivation among the isolates evaluated. The enhanced growth observed in inoculated seedlings may be attributed to the ability of actinobacteria to produce phytohormones, improve nutrient availability, and stimulate plant metabolic activities. The findings highlight the importance of exploiting indigenous actinobacterial resources for sustainable agriculture and reducing dependence on chemical fertilizers.

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