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Effectiveness of Rhizobacteria on the Growth, Yield and Diseases of VH1 Purple Rice Cultivated under Plastic-House Conditions

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Abstract: Seven rhizobacterial strains isolated from the rhizosphere soil of purple rice cultivated in Dak Lak Province, Vietnam, were evaluated for their effects on the growth and yield of the VH1 purple rice variety under plastic-house conditions. The experiment was conducted using a completely randomized design (CRD) with three replications and nine treatment formulas. The results showed that all seven strains increased the photosynthetic pigment contents in rice leaves at the flowering stage. These rhizobacteria also strongly stimulated the growth of VH1 purple rice, resulting in increases of 11.53-19.71% in the number of tillers per plant, 13.96-37.43% in flag leaf area, and 6.03-34.61% in root length compared with the two control treatments. Moreover, the inoculated strains enhanced disease tolerance, leading to actual yields ranging from 69.31 to 88.36 quintals per hectare, which were 1.41-2.14 times higher than the controls. Overall, *Stenotrophomonas maltophilia* RDL1B41, *Pseudomonas aeruginosa* RDLN43, and *Enterobacter mori* RDLN105 were identified as the most promising rhizobacterial strains, with potential application in biofertilizer development for sustainable rice cultivation.
Keywords: Diseases, growth, plastic-house conditions, purple rice, rhizobacteria, yield.

1. Introduction

The VH1 purple rice (*Oryza sativa* L.) is a specialty cultivar developed in Vietnam in 2007. Through continuous breeding and selection, VH1 has been recognized for its outstanding nutritional and medicinal properties [1]. In recent years, the rising demand for purple rice has coincided with the global trend toward functional and health-promoting foods [2], [3]. However, realizing the full agronomic potential of this variety remains challenging, as its cultivation still depends heavily on chemical fertilizers and pesticides [4], [5]. To address these challenges, Vietnam's rice sector is gradually transitioning toward a "green agriculture" model that emphasizes environmentally friendly and resource-efficient production practices[6]. Within this framework, the use of plant growth-promoting rhizobacteria (PGPR) has emerged as a promising biological approach to reduce chemical dependency and promote sustainable rice cultivation.

PGPR constitute a group of beneficial bacteria that inhabit the rhizosphere and enhance plant growth through several direct and indirect mechanisms. These include atmospheric nitrogen fixation, solubilization of insoluble phosphates, synthesis of phytohormones such as indole-3-acetic acid (IAA), secretion of siderophores, and suppression of phytopathogens through antibiotics, cell wall-degrading enzymes, or competition for nutrients and colonization sites [7],[8], [9], [10], [11], [12], [13], [14], [15], [16]. Numerous studies have demonstrated the effectiveness of PGPR in improving rice growth, productivity [17],[18], [19], [20], [21], [22], [23], [24], [25] and increase the resistance and tolerance of plants [26], [27], [28], [29], [30].

Although the roles of PGPR have been extensively documented in conventional rice systems, their application in high-value purple rice cultivars, particularly VH1variety, remains poorly explored. Therefore, this study was conducted to evaluate the effectiveness of seven rhizobacterial strains on the growth, yield, and disease incidence of VH1 purple rice cultivated under plastic-house conditions. Specifically, the objectives were to (i) assess their effects on growth-related traits, (ii) evaluate disease tolerance,and (iii) determine the actual yield. By integrating physiological and agronomic observations, this study aims to identify promising PGPR strains suitable for sustainable VH1 rice production systems.

2. Materials and Methods

2.1. Materials

Variety: The purple rice variety VH1 was obtained from Vinh Hoa Science and Technology Company Limited, Vietnam.

PGPR strains: Seven PGPRstrains were employed, including *Stenotrophomonas maltophilia* RDL1B41, *Pseudomonas aeruginosa* RDLB49, *Serratia nematodiphila* RDLB73, *Enterobacter mori* RDLB74, *Pseudomonas aeruginosa* RDLN43, *Enterobacter mori* RDLN105, and *Serratia marcescens* RDLN186. These strains were collected and isolated from the rhizosphere of purple rice cultivated in Vietnam. Identification was performed based on partial 16S rRNA gene sequences (~1500 bp) using BLASTn searches for species-level confirmation.

2.2. Methods

Experimental site: The experiment was conducted at Tay Nguyen University, Vietnam.

Experimental soil: Soil samples used in this study were collected from Krong Bong District, Dak Lak Province, Vietnam.

Table 1: Physicochemical characteristics of the experimental soil

Characteristics	Unit	Results
pH_{KCl}		4.24
Total nitrogen (N)	%	0.11
Available P_2O_5	mg/100 g soil	9.62
Available K_2O	mg/100 g soil	6.33
Exchangeable Ca^{2+}	mg/100 g soil	5.17
Exchangeable Mg^{2+}	mg/100 g soil	3.73
Organic matter(OM)	%	3.24

Experimental design: The experiment was arranged in a completely randomized design (CRD) with three replications. The total number of plants used in the experiment was 729, with nine treatments.

CT1: Sterile distilled water (Control 1)
CT2: Nutrient Broth (NB) medium (Control 2)
CT3: *Stenotrophomonas maltophilia* RDL1B41
CT4: *Pseudomonas aeruginosa* RDLB49
CT5: *Serratia nematodiphila* RDLB73
CT6: *Enterobacter mori* RDLB74
CT7: *Pseudomonas aeruginosa* RDLN43
CT8: *Enterobacter mori* RDLN105
CT9: *Serratia marcescens* RDLN186

Inoculum preparation: Inoculants were prepared by propagating the selected isolates in NB medium at 30°C and 150 rpm, and adjusted to optical density 10^9 CFU/mL to inoculation.

Seed germination and seedling inoculation: Surface-sterilized rice seeds were soaked in nine different treatments for 1 hour, germinated in the dark, and then transplanted into pots, following the method described by Saengsanga [20]. After 21 days of sowing, seedlings were uprooted and dipped in the respective suspension for 15 min before transplanting into pots (30 x 45 cm) under plastic-house conditions, as described by Purwanto *et al.* [22].

Crop management and fertilization: All management practices followed the Vinh Hoa 1 herbal rice cultivation protocol. Fertilizers were applied at the rates of 85 kg N, 70 kg P_2O_5, and 110 kg K_2O per hectare [31].

Data collection:At the flowering stage, chlorophyll a, chlorophyll b và carotenoid in rice leaves were determined using a spectrophotometer following Yoshida *et al.*[32].Morphological traits and major diseaseswere recorded according toQCVN 01-55:2011/BNNPTNT of Vietnam's National Technical Regulation on Testing for Value of Cultivation and Use of Rice Varieties[33]. Recorded parameters included the number of tillers per plant, flag leaf area, root length, actual yield, and incidences of major rice diseases (leaf blast, neck blast, sheath blight, brown spot, and bacterial leaf blight).

2.3. Data analysis

All datasets were statistically analyzed using Microsoft Excel 2021 and SAS 9.1 software. One-way analysis of variance (ANOVA) was performed, and the significance of differences between means was determined using Duncan's Multiple Range Test at significance levels of $p< 0.05$ and $p< 0.01$.

3. Results and Discussion

3.1. Effects of PGPR on photosynthetic pigment contents in the leaves of VH1 purple rice cultivated under plastic-house conditions

The seven selected rhizobacterial strains showed positive effects on the accumulation of photosynthetic pigments in the leaves of VH1 purple rice grown under plastic-house conditions. The results presented in Table 1 indicate that, at the flowering stage, the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Ccar) in leaves from most rhizobacterial treatments were significantly higher than those in the two control treatments, with differences being statistically significant at $p < 0.01$.

Rhizobacterial inoculation exerted a marked influence on chlorophyll a content in VH1 purple rice leaves. Among all treatments, *Stenotrophomonas maltophilia* RDL1B41 (CT3) exhibited the most pronounced effect, reaching 2.44 mg g^{-1} fresh leaf weight. This was followed by *Enterobacter mori* RDLN105 (CT8) (2.28mg g^{-1}), and *Pseudomonas aeruginosa* RDLN43 (CT7 (2.26mg g^{-1}). The lowest chlorophyll a contents were observed in the two control treatments (CT1 and CT2), ranging from 1.16 to 1.67 mg g^{-1}, which were up to 2.10 times lower than those in the rhizobacterial treatments.

Similarly, chlorophyll b content in the leaves of inoculated plants was also significantly higher than in the control treatments (CT1: sterile distilled water; CT2: NB medium). The highest Chl b contents were recorded in treatments with *Stenotrophomonas maltophilia* RDL1B41 (CT3) and *Enterobacter mori* RDLN105 (CT8) with values ranging from 1.24 to 1.35 mg g^{-1}, which were significantly greater than those of two controls ($p < 0.01$).

Regarding carotenoid content, leaves from rhizobacterial treatments showed values ranging from 0.86 to 1.15 mg g^{-1} at the flowering stage. The treatment with *Stenotrophomonas maltophilia* RDL1B41 (CT3) again produced the highest carotenoid accumulation, exceeding other bacterial treatments by 15.00-33.72% and the two controls by 53.33-79,69%. The differences among treatments were statistically significant at the 99% confidence level. The next highest carotenoid levels were recorded in *Enterobacter mori* RDLN105 (CT8) and *Pseudomonas aeruginosa* RDLN43 (CT7), representing increases of up to 56,25% compared with the sterile-water control (C1).

These findings are consistent with the report of Sarin *et al.* (2025) [34], who demonstrated that PGPR inoculation significantly enhances chlorophyll accumulation in potted rice plants.

Table 2: Photosynthetic pigment contents in the VH1 purple rice leaves at the flowering stageas affected by PGPR under plastic-house conditions

Treatment		**Photosynthetic pigment contents (mg g^{-1} fresh leaf weight)**		
		Chlorophyll a	**Chlorophyll b**	**Carotenoid**
CT1	Control 1	1.16^{e}	0.71^{e}	0.64^{d}
CT2	Control 2	1.67^{d}	0.92^{d}	0.75^{cd}
CT3	*Stenotrophomonas maltophilia* RDL1B41	2.44^{a}	1.35^{a}	1.15^{a}
CT4	*Pseudomonas aeruginosa* RDLB49	2.05^{bc}	1.05^{cd}	0.91^{b}
CT5	*Serratia nematodiphila* RDLB73	2.19^{ab}	1.21^{ab}	0.99^{b}
CT6	*Enterobacter mori* RDLB74	1.82^{cd}	0.93^{d}	0.86^{bc}
CT7	*Pseudomonas aeruginosa* RDLN43	2.26^{ab}	1.21^{ab}	$1{,}00^{b}$
CT8	*Enterobacter mori* RDLN105	2.28^{ab}	1.24^{ab}	1.00^{b}
CT9	*Serratia marcescens* RDLN186	1.99^{bcd}	1.12^{bc}	0.94^{b}
	p	**	**	**
	CV%	*6.73*	*5.60*	*5.81*

*Values are the means of three replicates. **: highly significant difference at p< 0.01. Different superscript letters within the same column indicate significant differences according to Duncan's test.*

3.2. Effects of PGPR on growth-related traits of the VH1 purple rice variety cultivated under plastic-house conditions

Beyond enhancing photosynthetic pigment contents, the seven PGPR strains also significantly influenced the growth performance of the VH1 purple rice cultivar under plastic-house conditions, as shown in Fig. 1 and Table 3.

The number of tillers in VH1 purple rice across all treatments ranged from 3.40 to 4.07 tillers per plant. In the rhizobacterial inoculation treatments, the number of tillers increased by 11.53-19.71% compared with the two control treatments (CT1 and CT2), and the differences were statistically significant at $p < 0.05$. The treatments that produced the highest number of tillers were CT3 (*Stenotrophomonas maltophilia* RDL1B41), CT7 (*Pseudomonas aeruginosa* RDLN43), and CT8 (*Enterobacter mori* RDLN105).

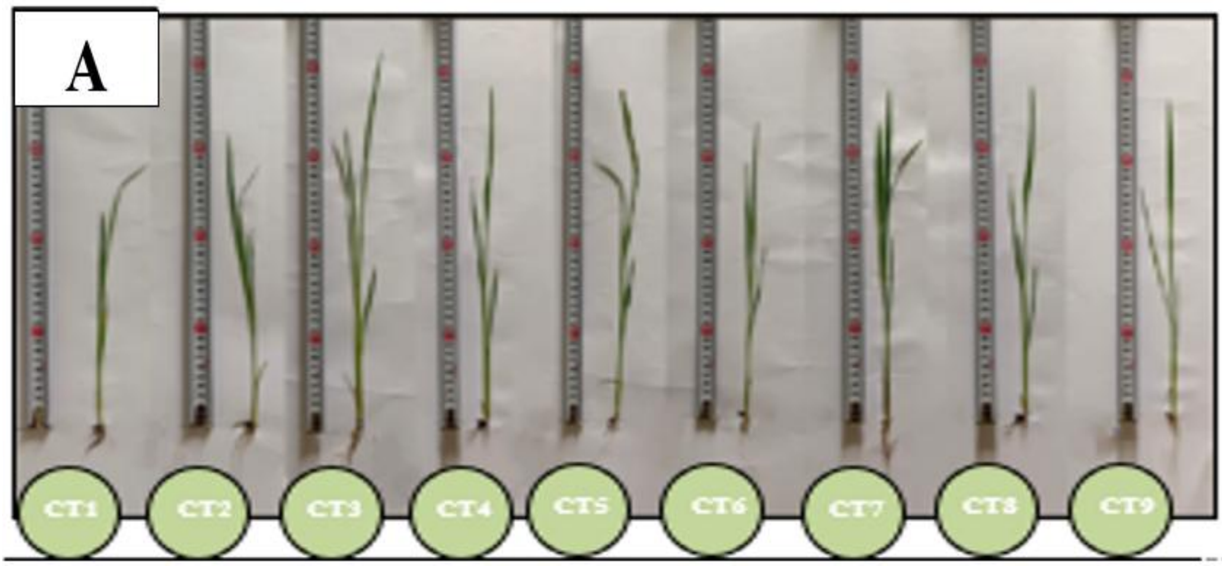
After full panicle emergence, the flag leaf area of VH1 purple rice varied from 43.51 to 48.61 cm² per leaf among the rhizobacterial treatments. Among them, treatment CT3 (*Stenotrophomonas* *maltophilia*RDL1B41) recorded the largest flag leaf area, followed by CT8 (*Enterobacter mori* RDLN105) and CT7 (*Pseudomonas aeruginosa* RDLN43). Notably, although the flag leaf area of plants inoculated with *Enterobacter mori* RDLB74 (CT6) was the smallest among the PGPR-treated groups, it was still 23.01% higher than that of the control treatments, and the difference was statistically significant ($p< 0.05$; Table 3). Compared with previous studies, the flag leaf area of VH1 purple rice observed in this experiment was considerably higher. Truong *et al.* (2017) [35] reported that the flag leaf area of the VH1 variety grown in Quang Ngai ranged only from 33.80 to 34.60 cm² per leaf. These results indicate that rhizobacterial inoculation contributed to a substantial increase in flag leaf area compared with conventional cultivation conditions.

Moreover, VH1 plants treated with the seven PGPR strains exhibited root lengths ranging from 26.37 to 31.00 cm, representing significant increases compared with the control treatments ($p< 0.01$). Notably, treatment CT8 (*Enterobacter mori* RDLN105) showed the most pronounced effect, with roots 1.06-1.18 times longer than those in other rhizobacterial treatments and 1.25-1.35 times longer than in the control treatments. This was followed by treatments CT7 (*Pseudomonas aeruginosa* RDLN43) and CT3 (*Stenotrophomonas maltophilia* RDL1B41), which also promoted substantial root development in VH1 purple rice.

Table 3: Morphological traits of the VH1 purple rice as affected by PGPR under plastic-house conditions

Treatment		Morphological traits		
		Tillers/plant	**Flag leaf area (cm^2/leaf)**	**Root length (cm)**
CT1	Control 1	3.40^{c}	35.37^{c}	23.03^{d}
CT2	Control 2	3.47^{bc}	38.18^{c}	24.87^{cd}
CT3	*Stenotrophomonas maltophilia* RDL1B41	3.87^{abc}	48.61^{a}	29.07^{abc}
CT4	*Pseudomonas aeruginosa* RDLB49	3.93^{abc}	46.36^{ab}	28.30
CT5	*Serratia nematodiphila* RDLB73	3.87^{abc}	44.99^{ab}	27.07^{abcd}
CT6	*Enterobacter mori* RDLB74	3.87^{abc}	43.51^{b}	26.37^{bcd}
CT7	*Pseudomonas aeruginosa* RDLN43	4.00^{ab}	47.52^{ab}	29.13^{ab}
CT8	*Enterobacter mori* RDLN105	4.07^{a}	48.48^{a}	31.00^{a}
CT9	*Serratia marcescens* RDLN186	3.93^{abc}	47.16^{ab}	28.93^{abc}
	p	*	**	**
	CV%	*7.97*	*3.51*	*5.80*

Values are the means of three replicates.: significant difference at p< 0.05; **: highly significant difference at p< 0.01. Different superscript letters within the same column indicate significant differences according to Duncan's test.*



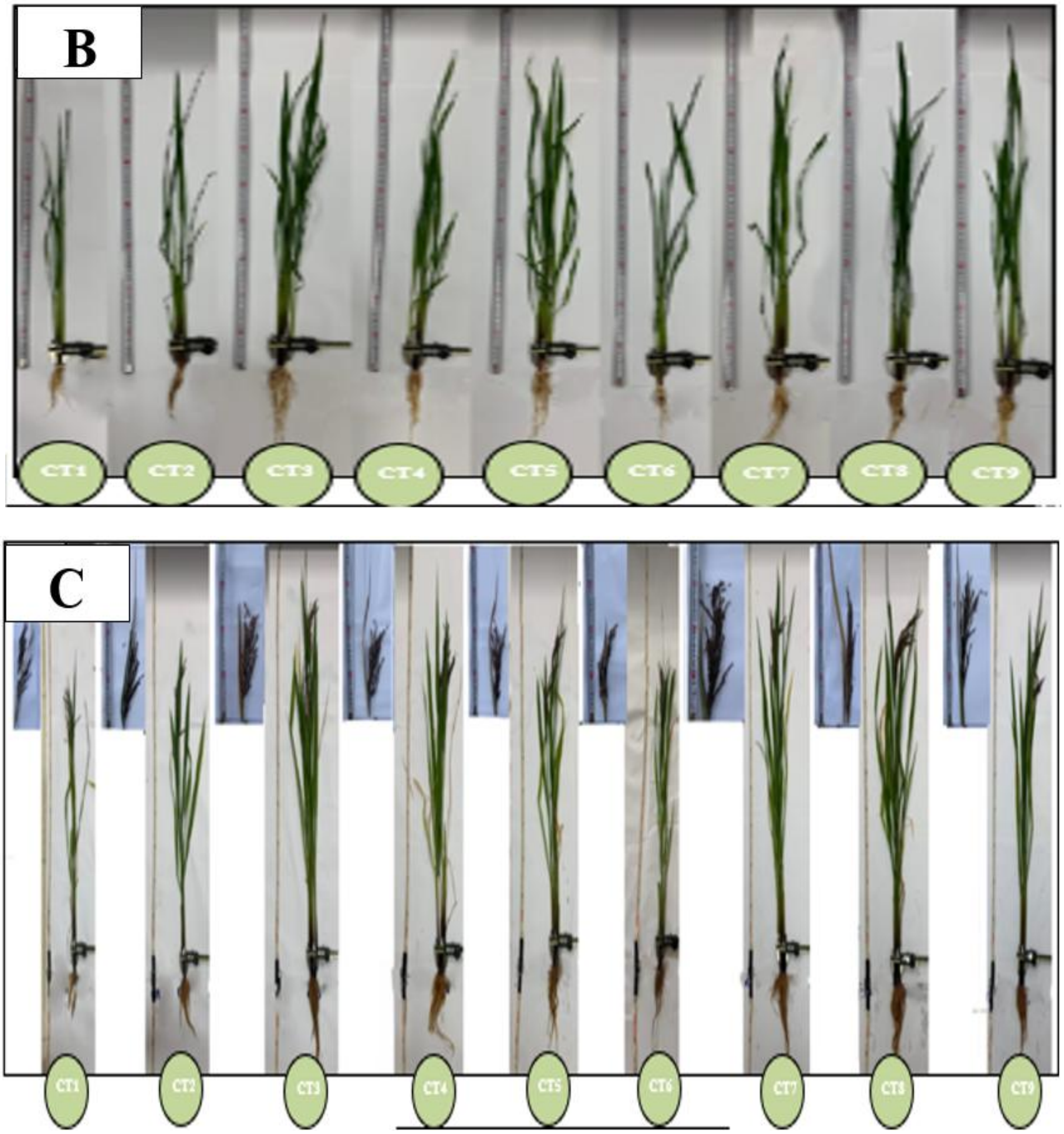


Figure 1: Comparative morphological traits of 9 treatment strains

A: Plant morphology at 14 days after the first inoculation; B: Plant morphology at two months after the first inoculation; C: Plant morphology at four months after the first inoculation.

3.3. Effects of PGPR on disease incidence of the VH1 purple rice variety cultivated under plastic-house conditions

During the growth and development stages, VH1 purple rice plants were affected by several diseases.

As shown in Table 4, four major diseases were observed during the experimental period: leaf blast, neck blast, sheath blight, and bacterial leaf blight, with severity scores-ranging from 0-1, 0-1, 0-3, and 0-3, respectively.

The results also revealed that VH1 purple rice plants inoculated with selected rhizobacterial strains exhibited enhanced disease resistance, with disease severity levels limited to scores between 0 and 1. Espcecially, plants treated with *Stenotrophomonas maltophilia* RDL1B41, *Pseudomonas aeruginosa* RDLN43 and *Enterobacter mori* RDLN105 showed almost no visible symptoms of infection.

Table 4: Effects of rhizobacterial inoculation on disease incidence in the VH1 purple rice variety under plastic-house conditions

Treatment	**Disease severity rating values**				
	Leaf blast	**Neck blast**	**Sheathblight**	**Brown spot**	**Bacterial leaf blight**
CT1 (Control 1)	1	0-1	3	0	3
CT2 (Control 2)	0-1	0-1	1-3	0	3
CT3	0	0	0	0	0
CT4	0-1	0	0-1	0	0
CT5	0	0	0	0	0
CT6	0-1	0-1	0-1	0	0-1
CT7	0	0	0	0	0
CT8	0	0	0	0	0
CT9	0-1	0	0-1	0	1

3.4. Effects of PGPR on actual yield of the VH1 purple rice variety cultivated under plastic-house conditions

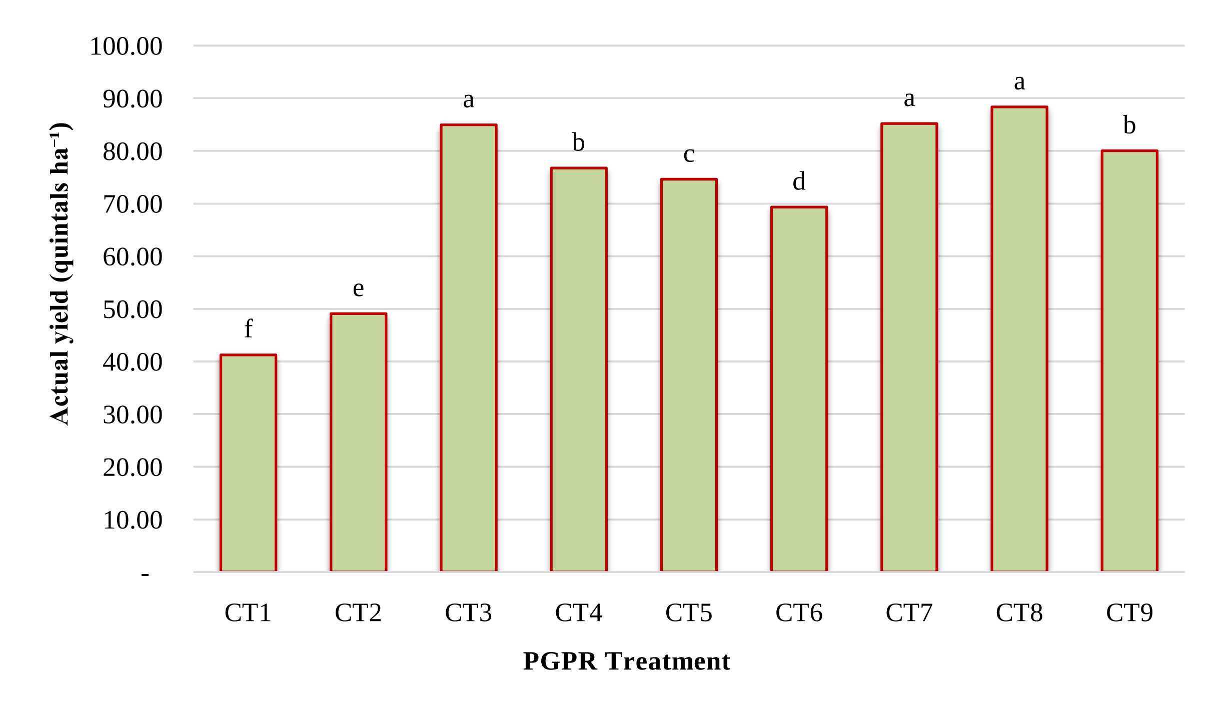


Figure 2: Actual yield of the VH1 purple rice variety as affected by PGPR cultivated under plastic-house conditions

Different superscript letters above the bars indicate statistically significant differences according to Duncan's multiple range test ($p < 0.01$).

The VH1 purple rice plants exhibited healthy and vigorous growth under the positive influence of the inoculated rhizobacterial strains. Furthermore, the plants showed enhanced resistance to the major diseases commonly affecting purple rice. Consequently, the actual grain yield of VH1 purple rice grown in pots under rhizobacterial treatments increased significantly compared with the controls, with differences being statistically significant at $p< 0.01$ (Fig. 2). The actual yields obtained from PGPR-treated plants ranged from 69.31 to 88.36 quintals ha^{-1}. The highest yields were recorded in treatments CT8 (*Enterobacter mori* RDLN105), CT7 (*Pseudomonas aeruginosa* RDLN43) and CT3 (*Stenotrophomonas maltophilia* RDL1B41). Although CT6 (*Enterobacter mori* RDLB74) produced the lowest yield among the rhizobacterial treatments, it was still 1.41-1.68 times higher than the control treatments, with differences statistically significant.

Overall, VH1 purple rice cultivated under plastic-house conditions with rhizobacterial inoculation achieved yields within the potential range recommended by the seed producer (5.50-10.00 tons per hectare). Compared with previous studies of Truong *et al.* (2017) [35] and Nguyen (2017) [36] the yield of VH1 observed in this experiment was markedly higher than that of non-inoculated plants, confirming the positive impact of the rhizobacterial strains in improving the actual productivity of VH1 purple rice.

4. Conclusion

This study demonstrated that the inoculation of plant growth-promoting rhizobacteria (PGPR) significantly improved the physiological and agronomic performance of the VH1 purple rice variety under plastic-house conditions. The seven tested strains enhanced photosynthetic pigment accumulation, vegetative growth, and root development, while simultaneously reducing the incidence of major rice diseases. Among them, *Stenotrophomonas maltophilia* RDL1B41, *Pseudomonas aeruginosa* RDLN43, and *Enterobacter mori* RDLN105 consistently produced the most pronounced effects, leading to actual yield increases of up to 2.14 times compared with the controls. The findings highlight the potential of these rhizobacterial strains as effective biofertilizer candidates for sustainable rice cultivation. Their application could not only reduce the reliance on chemical fertilizers and pesticides but also contribute to maintaining high yield and quality in high-value purple rice production systems.

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