International Journal of Latest Engineering Research and Applications (IJLERA) ISSN: 2455-7137

Volume – 10, Issue – 07, July 2025, PP – 12-19

Effectiveness of Plant Growth-Promoting Rhizobacteria Mixtures on Growth, Yield, and Quality of VH1 Purple Rice under Field Conditions

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Abstract: Purple rice is a rare and high-value crop recognized for its nutritional and medicinal benefits. This study evaluated the effects of selected Plant Growth-Promoting Rhizobacteria(PGPR) mixtures on the growth, grain yield, and quality of the VH1 purple rice variety under field conditions in Ea Sup District, DakLak Province, Vietnam. Rice plants were treated with different PGPR mixtures combined with three levels of chemical fertilizer application. Treatments involving PGPR mixtures and 50-75% of the recommended chemical fertilizer enhanced chlorophyll a and b contents in rice leaves. As a result, significant improvements were observed in all measured agronomic traits, with fresh biomass, panicle length, number of effective panicles per plant, number of grains per panicle, and grain yield increasing by 15.12-58.81%, 6.78-19.73%, 23.08-137.50%, 0.94-6.09%, and 20.28-123.12%, respectively, when compared to the control treatments. Furthermore, the anthocyanin concentration in VH1 rice ranged from 199.53 to 206.16 mg/100 g, representing a 1.14-fold increase compared to the full-fertilizer treatment without PGPR application. These findings highlight the potential of PGPR-based biofertilizers as an effective and sustainable approach for improving the productivity and quality of purple rice under field conditions.

Keywords: Anthocyanin, biofertilizer, *Enterobacter mori*, PGPR mixtures, purple rice, *Stenotrophomonas maltophilia*, yield.

1. Introduction

Food insecurity and poverty continue to represent major global concerns. According to the Food and Agriculture Organization, a significant portion of the world's population still lacks consistent access to adequate, safe, and nutritious food [1]. These issues are further intensified by a complex set of contributing factors, including climate change, economic instability, rapid industrial expansion, and accelerated urban growth, particularly in developing countries. In this context, there is a critical need to accelerate the transition of agri-food systems toward models that are not only modern, safe, and environmentally sustainable but also economically viable for producers.

The concept of "Plant Growth-Promoting Rhizobacteria" was first introduced by Kloepper to describe beneficial bacteria residing in the rhizosphere that enhance plant growth and productivity [2]. Since then, extensive research has demonstrated that PGPR plays crucial roles in promoting plant development, improving nutrient acquisition, and enhancing tolerance to abiotic stresses. As such, PGPR has emerged as promising biological tools that can complement or reduce the use of synthetic agrochemicals, aligning with the goals of sustainable and eco-friendly agriculture [3], [4], [5], [6].

In Vietnam, PGPR has been applied to a variety of economically important crops, such as black pepper [7],chili [8], and flower[9]. In rice farming, several studies have confirmed their agronomic benefits under local conditions [10], [11], [12]. However, their application in high-value purple rice cultivars-known for their nutritional and medicinal properties-has not been thoroughly explored [13], [14].

Building on some previous experiments conducted under greenhouse condition [15] and field [16], this study further evaluated the effectiveness of selected PGPR mixtures combined with reduced chemical fertilizer under other field condition. The objective was to assess the potential of PGPR in promoting sustainable purple rice cultivation and to provide evidence supporting their integration into low-input rice production systems.

2. Materials and Methods

2.1. Material

The purple rice seedlings used in this study belonged to the VH1 variety, a cultivar developed and cultivated in Vietnam.

Three PGPR strains (*Stenotrophomonas maltophilia* RDL1B41, *Pseudomonas aeruginosa* RDLN43, and *Enterobacter mori*RDLN105) were collected and isolated from some paddy fieldsinDakLakprovince, Vietnam. These strains were identified based on 16S-rRNA gene sequencing and are currently preserved at Tay Nguyen University, Vietnam.

2.2. Methods

2.2.1. Field experiment

Experimental site and soil characteristics: The field experiment was conducted in Ea Sup District, Dak Lak Province, Vietnam (13°09'13.5"N, 107°46'45.7"E). The 0-20 cm surface soil layer was characterized by a pH (KCl) of 4.27, total nitrogen content of 0.07%, available P_2O_5 of 4.48 mg/100 g, available K_2O of 5.94 mg/100 g, exchangeable Ca^{2+} of 5.12 meq/100 g, exchangeable Mg^{2+} of 3.56 meq/100 g, and organic matter content of 3.13%.

Experimental design: A two-factor randomized complete block design (RCBD) with 12 treatment combinations (4 × 3) was employed and replicated three times.

Factor 1: PGPR mixture treatments

V0: Nutrient broth (NB) medium (control)
V1: *Stenotrophomonas maltophilia*RDL1B41 + *Pseudomonas aeruginosa*RDLN43
V2: *Pseudomonas aeruginosa*RDLN43 + *Enterobacter mori*RDLN105
V3: *Stenotrophomonas maltophilia*RDL1B41 + *Enterobacter mori*RDLN105

Factor 2: Chemical fertilizer levels ($N + P_2O_5$)

P1: 100% of the recommended dose
P2: 75% of the recommended dose
P3: 50% of the recommended dose

Inoculum preparation: Preparation of inoculants was performed by propagating selected isolates in NB medium at 30◦C, 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 different PGPR mixture treatments for 1 hour, germinated in the dark, and then transplanted into pots, following the method described by Saengsanga [17]. At 21 days after sowing, the seedlings were dipped in the corresponding PGPR suspensions for 15 minutes before transplanting into the field, as described by Purwanto & Wijonarko [18].

Crop management and fertilization: All management practices followed the VH1 purple rice cultivation protocol. Fertilizers were applied at the following rates per hectare: 85 kg N, 70 kg P_2O_5, and 110 kg K_2O.

Data collection:At the flowering stage, chlorophyll a and chlorophyllb in the leaves were determined using a spectrophotometer following the method described by Yoshida et al. [19]. Morphological traits, including fresh biomass (g/plant), panicle length (cm), number of effective panicles per plant, number of grains per panicle, and grain yield (quintals/ha), were recorded following QCVN 01-55:2011/BNNPTNT of Vietnam's National Technical Regulation on Testing for Value of Cultivation and Use of Rice Varieties[20].

2.2.2. Laboratory quality analysis

All laboratory analyses were performed at Tay Nguyen University, Vietnam.

Preparation of grains: VH1 grains were harvested from the field were dried to a moisture content of less than 14%, dehulled , vacuum-sealed, and stored at 4 °C until analysis.

Data collection: Anthocyanin content (mg/100 g) was determined following the pH differential method outlined by Giusti & Wrolstad [21].

2.3. Data analysis

All datasets were processed and statistically analyzed using Microsoft Excel 2021 and SAS 9.1 software. A two-way analysis of variance (ANOVA) was performed, and differences among means were considered statistically significant at $p < 0.05$ and $p < 0.01$.

3. Results and Discussion

3.1. Effects of PGPR mixtures and chemical fertilizer levels on leaf chlorophyll accumulation of the VH1 purple rice variety

According to the data presented in Table 1, the application of PGPR mixture treatments considerably enhanced chlorophyll content in the leaves of VH1 purple rice at the flowering stage. The mean chlorophyll concentrations in the leaves of VH1 plants treated with V1 (*Stenotrophomonas maltophilia* RDL1B41 + *Pseudomonas aeruginosa* RDLN43), V2 (*Pseudomonas aeruginosa* RDLN43 + *Enterobacter mori* RDLN105), and V3 (*Stenotrophomonas maltophilia* RDL1B41 + *Enterobacter mori* RDLN105)were significantly higher than those of the V0 control (NB medium), ranging from 2.26 to 2.36 mg/g for chlorophyll a and from 1.12 to 1.17 mg/g fresh leaf for chlorophyll b ($p < 0.05$).Notably, even under a 25% reduction in the recommended chemical fertilizer rate, these inoculated treatments maintained elevated chlorophyll levels (2.21 mg/g for chlorophyll a and 1.11 mg/g for chlorophyll b), which were significantly greater than those of the uninoculated formulas under full fertilizer application ($p < 0.01$).

Further analysis across all fertilizer levels confirmed that rhizobacterial inoculation consistently improved chlorophyll accumulation in the VH1 purple rice variety compared to the respective controls. Chlorophyll a concentration in PGPR mixtures-treated plants ranged from 2.06 to 2.45 mg/g, while chlorophyll b ranged from 1.03 to 1.22 mg/g. The most pronounced improvement was observed in the VH1 purple rice plants treated with the V3 treatment (*Stenotrophomonas maltophilia* RDL1B41+ *Enterobacter mori* RDLN105). The application of the V3 PGPR mixture, combined with a 25-50% reduction in chemical fertilizer relative to the standard recommendation, led to a significant increase in leaf chlorophyll content by 25.26-34.62% compared to the uninoculated control receiving 100% fertilizer ($p < 0.01$).

Comparable findings have been documented by Purwanto & Suharti [22], who demonstrated that the application of a PGPR consortium markedly increased chlorophyll content in rice leaves, primarily through enhanced nitrogen uptake and assimilation in the plant. The consistency between this study and previous findings confirms that rhizobacterial consortia can effectively promote pigment accumulation in rice even under reduced fertilizer input.

Table 1: Chlorophyll content in the VH1 purple rice leaves at the flowering stage as affected by PGPR mixtures and chemical fertilizer levels

Chlorophyll content	PGPR mixture(V)	Chemical fertilizer level (P) (%of the recommended dose)			Mean (V)
		P1 (100%)	P2 (75%)	P3 (50%)	
Chlorophyll a(mg/g fresh leaf)	V0	1.82^{e}	1.61^{f}	1.43^{g}	1.62^{C}
	V1	2.32^{b}	2.40^{ab}	2.06^{d}	2.26^{B}
	V2	2.21^{c}	2.39^{ab}	2.23^{c}	2.28^{B}
	V3	2.22^{c}	2.45^{a}	2.42^{a}	2.36^{A}
	Mean (P)	2.14^{B}	2.21^{A}	2.04^{C}	
	CV = 1.56%				
Chlorophyll b (mg/g fresh leaf)	V0	0.95^{ef}	0.87^{f}	0.77^{g}	0.86^{C}
	V1	1.15^{abc}	1.18^{ab}	1.03^{de}	1.12^{B}
	V2	1.09^{cd}	1.18^{ab}	1.12^{bc}	1.13^{B}
	V3	1.11^{bcd}	1.22^{a}	1.19^{ab}	1.17^{A}
	Mean (P)	1.07^{A}	1.11^{A}	1.03^{B}	
	CV = 3.30%				

Values are the means of three replicates. Different superscript letters within the same column indicate significant differences according to LSD or Duncan's test. Significance was determined at $p < 0.05$ for PGPR mixture (V), $p < 0.01$ for chemical fertilizer level (P), and $p < 0.01$ for V*P interaction.

3.2. Effects of PGPR mixtures and chemical fertilizer levels on growth and yield-related traits of the VH1 purple rice variety

Beyond enhancing leaf chlorophyll accumulation, PGPR mixtures also had a substantial positive impact on the growth and yield-related traits of the VH1 purple rice variety under field conditions as shown in Fig. 1 and Table 2. Across all fertilizer levels, VH1 plants treated with PGPR mixtures (V1, V2, and V3) exhibited 282.00-333.50 g/plant of fresh biomass, 25.20-26.88 cm of panicle length, 6.40-7.60 effective panicles per plant, and 131.00-133.65 grains per panicle, indicating significant increases compared to the control treatment without inoculation (V0) at the same fertilizer levels ($p < 0.01$).

The morphological traits of VH1 rice also showed particularly strong responses to rhizobacterial inoculation under reduced fertilizer conditions (Fig. 1 and Table 2). The V3 treatment (*Stenotrophomonas maltophilia*RDL1B41 + *Enterobacter mori*RDLN105) consistently outperformed other PGPR mixtures, especially at 75% of the recommended fertilizer dose (P2). Under this condition, V3-treated plants recorded the highest values for fresh biomass (333.50 g/plant), panicle length (26.88 cm), effective panicles per plant (7.60 panicles), and number of grains per panicle (133.65 grains), corresponding to increases of 2.98-46.15% compared to the full-fertilizer control. Even at 50% fertilizer input (P3), the V3 treatment maintained superior morphological performance relative to the uninoculated control, indicating a strong compensatory effect of PGPR under nutrient-limited conditions.

In previous research, Nguyen et al. [16] demonstrated that PGPR inoculation enhanced the growth performance and productivity of purple rice by up to 50.00% compared to the control treatment without rhizobacterial mixtures and with 100% chemical fertilizer application.

Table 2: Morphological traits of the VH1 purple rice variety as affected by PGPR mixtures and chemical fertilizer levels

Morphological traits	**PGPR mixture (V)**	**Chemical fertilizer level (P) (% of the recommended dose)**			**Mean (V)**
		P1 (100%)	**P2 (75%)**	**P3 (50%)**	
Fresh biomass (g/plant)	**V0**	244.97^{f}	222.02^{g}	210.00^{h}	225.66^{D}
	V1	310.00^{c}	329.53^{a}	282.00^{e}	307.18^{B}
	V2	293.45^{d}	318.60^{b}	295.50^{d}	302.52^{C}
	V3	295.47^{d}	333.50^{a}	330.50^{a}	319.82^{A}
	Mean (P)	285.97^{B}	300.91^{A}	279.50^{C}	
CV = 1.25%					
Panicle length (cm)	**V0**	23.60^{bc}	23.20^{c}	22.45^{c}	23.08^{B}
	V1	25.80^{a}	26.35^{a}	25.20^{ab}	25.78^{A}
	V2	25.20^{ab}	26.00^{a}	25.20^{ab}	25.47^{A}
	V3	25.20^{ab}	26.88^{a}	26.40^{a}	26.16^{A}
	Mean (P)	24.95	25.61	24.81	
CV = 3.07%					
Number of effective panicles/plant	**V0**	5.20^{bc}	4.20^{cd}	3.20^{d}	4.20^{B}
	V1	7.33^{a}	7.60^{a}	6.40^{ab}	7.11^{A}
	V2	6.60^{a}	7.20^{a}	6.80^{a}	6.87^{A}
	V3	6.40^{ab}	7.60^{a}	7.40^{a}	7.13^{A}
	Mean (P)	6.38^{AB}	6.65^{A}	5.95^{B}	
CV = 8.65%					
Number of grains/ panicle	**V0**	129.78^{ab}	126.87^{ab}	125.98^{b}	127.54^{B}
	V1	133.20^{ab}	133.30^{a}	131.00^{ab}	132.50^{A}
	V2	131.45^{ab}	133.10^{ab}	132.00^{ab}	132.18^{A}
	V3	132.32^{ab}	133.65^{a}	132.55^{ab}	132.84^{A}
	Mean (P)	131.69	131.73	130.38	
CV = 2,08%					

Values are the means of three replicates. Different superscript letters within the same column indicate significant differences according to LSD or Duncan's test. Significance was determined at $p < 0.05$ for PGPR mixture (V), $p < 0.01$ for chemical fertilizer level (P), and $p < 0.01$ for V*P interaction.

Figure 1: Comparative morphology of 12 purple rice panicles subjected to 12 treatment combinations

3.3. Effects of PGPR mixtures and chemical fertilizer levelson grain yield of the VH1 purple rice variety

To more accurately assess the effectiveness of the rhizobacterial mixtures on the VH1 purple rice variety under field conditions, grain yield at the maturity stage was monitored (Fig. 2). The use of rhizobacterial mixtures contributed to marked improvements in traits associated with purple rice yield. As a result, the grain yield of VH1 purple rice treated with PGPR mixtures achieved a mean value ranging from 113.84 to 120.51 quintals/ha. Three treatments with PGPR inoculation (V1: *Stenotrophomonas maltophilia* RDL1B41 + *Pseudomonas aeruginosa* RDLN43, V2: *Pseudomonas aeruginosa* RDLN43 + *Enterobacter mori* RDLN105, V3: *Stenotrophomonas maltophilia* RDL1B41 + *Enterobacter mori* RDLN105) produced significantly higher yields than the control treatment without inoculation (V0: NB medium only) across all fertilizer levels, which indicated a statistically significant difference according to Duncan's multiple range test ($p < 0.01$).

The highest grain yield was recorded in the V3P2 treatment (128.36 quintals/ha), followed by V1P2 treatment (127.83 quintals/ha) and V3P3 treatment (123.26 quintals/ha). Notably, the V3 treatment-*Stenotrophomonas maltophilia* RDL1B41 + *Enterobacter mori* RDLN105 combined with a 25-50% reduction in recommended N + P_2O_5 fertilizer-resulted in a 1.44- to 2.23-fold increase in grain yield compared to the uninoculated controls (57.53-85.65 quintals/ha), and this improvement was statistically significant ($p < 0.01$). Thus, this field experiment indicated that the PGPR mixture, particularly V3, can reduce the required chemical fertilizer dose by up to 50% while maintaining high productivity of the VH1 purple rice under field conditions, thereby lowering production costs.

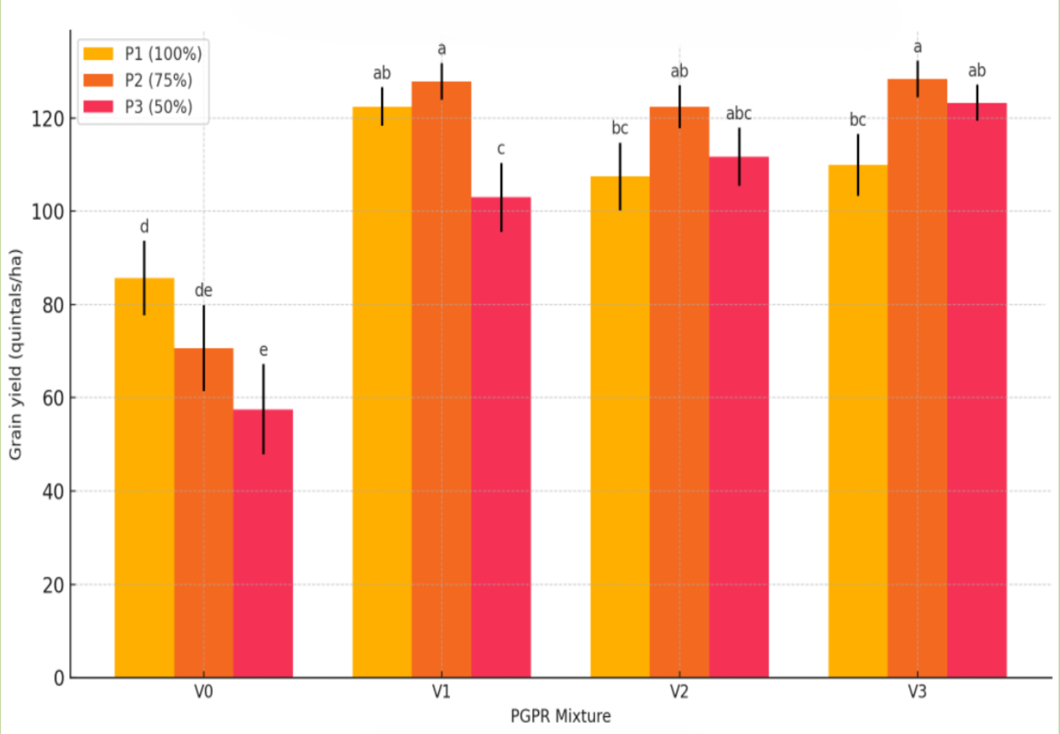

Figure 2: Grain yield of the VH1 purple rice variety under different PGPR mixtures and chemical fertilizer levels V: PGPR mixture, P: Chemical fertilizer level (% of the recommended dose). Error bars represent standard deviations (n = 3). Different superscript letters above the bars indicate statistically significant differences according to Duncan's multiple range test ($p < 0.01$)

As previously reported by Nguyen et al [16], the use of *Stenotrophomonas maltophilia* RDL1B41 in combination with *Enterobacter mori* RDLN105 resulted in improved yield performance of the VH1 purple rice, even under moderate fertilizer inputs-a finding echoed by this current data. The above results are also similar to the study on the role of replacing chemical fertilizers with mixtures of PGPR on rice plants published by Kobua et al. [23], who noted that *Bacillus aryabhattai*, *Burkholderia ambifaria*, *Sphingobium yanoikuyae*) with a 25-50% fertilizer reduction enhanced rice yield by up to 14%.

3.4. Effects of PGPR mixtures and chemical fertilizer levelson anthocyanin contentof the VH1 purple rice variety

Purple rice is considered a medicinal rice type, highly valued for its high level of anthocyanin [24]. Therefore, after harvest, purple rice grains were dried and analyzed for the concentration of anthocyanin to evaluate the effects of rhizobacterial mixture treatments on the pharmaceutical quality of the VH1 variety under field conditions (Table 3).

Table 3: Anthocyanin contentof the VH1 purple rice variety as affected by PGPR mixtures and chemical fertilizer levels

Quality parameter	PGPR mixture (V)	Chemical fertilizer level (P) (% of the recommended dose)			Mean (V)
		P1 (100%)	P2 (75%)	P3 (50%)	
Anthocyanin (mg/100g)	V0	180.16^{c}	176.34^{c}	176.18^{c}	177.56^{B}
	V1	202.26^{ab}	204.94^{ab}	199.53^{b}	202.25^{A}
	V2	200.92^{ab}	205.01^{ab}	201.70^{ab}	202.54^{A}
	V3	201.66^{ab}	206.16^{a}	204.86^{ab}	204.22^{A}
	Mean (P)	196.25^{AB}	198.11^{A}	195.57^{B}	
CV = 1.11%					

Values are the means of three replicates. Different superscript letters within the same column indicate significant differences according to LSD or Duncan’s test. Significance was determined at $p < 0.05$ for PGPR mixture (V), $p < 0.01$ for chemical fertilizer level (P), and $p < 0.01$ for V*P interaction.

Three treatments with PGPR mixtures (V1, V2, and V3) significantly increased the mean anthocyanin content in VH1 rice grains compared to the uninoculated control (V0) ($p < 0.01$). The anthocyanin content of this experiment ranged from 199.53 to 206.16 mg/100 g, depending on the different treatment. The highest values were observed in the V3 treatment (*Stenotrophomonas maltophilia* RDL1B41 + *Enterobacter mori* RDLN105). The mean anthocyanin content in rice grains inoculated with the V3 mixture reached 204.22 mg/100 g, which was 1.97 mg/100 g higher than other PGPR mixtures and 26.66 mg/100 g higher than the uninoculated control (NB medium), with statistically significant differences ($p < 0.05$). Particularly, when the V3 mixture was combined with 50-75% of the recommended fertilizer, the anthocyanin content reached up to 204.86-206.16 mg/100 g, significantly exceeding the values of control treatments receiving 100% fertilizer without PGPR mixtures (V0P1 reached at 180.16 mg/100 g).

Compared to previously published data, Chung et al. [25] reported anthocyanin levels of only 5.73-152.90 mg/100 g under full chemical fertilizer application, which were markedly lower than those observed in this study. Meanwhile, Nguyen et al. [16] documented similar results about anthocyanin content in this experiment, ranging from 194.96 to 211.24 mg/100 g when PGPR mixtures were combined with 50-75% of the recommended fertilizer.

4. Conclusion

Based on the findings, this study confirms the strong potential of PGPR mixtures as biofertilizers for sustainable rice cultivation. Among the tested combinations, the V3 treatment-comprising *Stenotrophomonas maltophilia* RDL1B41 and *Enterobacter mori* RDLN105-combined with a 25-50% reduction in chemical fertilizer, significantly enhanced chlorophyll accumulation, growth performance, grain yield, and anthocyanin content in the VH1 purple rice under field conditions. These results highlight V3 as a promising biofertilizer candidate for reducing fertilizer input while maintaining high productivity and grain quality in purple rice production systems.

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