



Research article

Role of Bacilli in phytohormone production in rice and grain under different doses of vegetative and generative fertilizer

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Abstract

Importance of the work: The use of plant-growth-promoting bacteria inoculant is a sustainable option to promote rice plant growth and improve the yield. Integrating these bacteria resulting with chemical fertilization may be another relevant option.

Objectives: To assess the physiological responses of Pathum Thani 1 rice under the co-application of different fertilizers and plant growth-promoting bacteria.

Materials & Methods: A pot experiment was conducted to assess plant growth-promoting ability under different vegetative fertilizers, generative fertilizers and bacteria inocula. The pot experiment was a 2×2×5 factorial experiment in a completely randomized design. Plants were measured for endogenous phytohormones—indole-3-acetic acid (IAA), gibberellic acids (GAs) and salicylic acid (SA)—, macronutrients—nitrogen (N), phosphorous (P) and potassium (K)—and their yield parameters were evaluated.

Results: Significant ($p < 0.05$) increases were observed in the inoculated Pathum Thani 1 rice grain yield (18.16–32.77 g/pot), panicle number (19.50–31.25 panicles/pot), tiller number (28.50–45.88 tillers/pot) and plant height (103.34–120.52 cm). Significant ($p < 0.05$) endogenous IAA and SA level changes were observed compared to the uninoculated control. GAs were only significantly ($p < 0.05$) increased in the vegetative phase and not in the generative phase ($p > 0.05$) compared to the uninoculated control. A significant ($p < 0.05$) effect in P and K accumulation was observed in plant tissue, but not in N accumulation.

Main finding: Additions of plant growth-promoting bacteria *Lysinibacillus* sp. N5 or *Bacillus* sp. N7 were the best options to enhance rice growth and yield the most. In addition, adding bacteria decreased the dose of fertilizer required to obtain a higher rice yield. Furthermore, adding bacteria enhanced P and K but not N in the soil under various fertilizer doses, implying that N was in excess for plant growth. Finally, adding bacteria enhanced endogenous phytohormones, such as IAA, GAs and SA, in plant tissue.

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Introduction

Various bacterial species can have beneficial traits, such as nitrogen fixation, phosphate and mineral solubilization, phytohormone production and protection against pathogenic organisms (Islam and Hossain, 2012). Among many phytohormones, indole-3-acetic acid (IAA; Susilowati et al., 2018; Cavite et al., 2021; Pham et al., 2022), gibberellic acids (GAs; Yanni et al., 2001; Sudewi et al., 2020; Maghbolí Balasjin et al., 2022) and salicylic acid (SA; Ali and Elmaghraby, 2020) have been reported to be produced by bacteria and to contribute crucially to the improvement of rice grain yield. Some key factors related to grain yield in rice have been known to be regulated by these phytohormones. Root formation development, tillering, panicle architecture, flower development and seed development are controlled by IAA (Ishimaru et al., 2013; Uga et al., 2013; Yoshikawa et al., 2014; Yang et al., 2017; Wang et al., 2018). GAs control plant growth and plant stature improvement by enhancing the plant height in the vegetative phase (Chu et al., 2019), and later in the generative phase, GAs control panicle exertion, panicle development architecture and generative organ development (Pin et al., 2019; Wang et al., 2020; Su et al., 2021; Castro-Camba et al., 2022; Chun et al., 2022). SA is known to regulate photosynthetic efficiency enhancement, floral induction, panicle formation and development, fertility of spikelets and grain filling (Rong et al., 2016; Feng et al., 2018; Kimbembe et al., 2020; Kittipornkul et al., 2020; Zhou et al., 2021; Chen et al., 2022).

Plant growth-promoting bacteria supplementation has proved effective in enhancing crop physiological parameters and yield, while also reducing the amount of synthetic fertilizer used, as reported in various co-application studies (Castanheira et al., 2017; Nguyen et al., 2019; Kobua et al., 2021). However, it is known that the application of fertilizer sometimes does not harmonize with bacteria. Often, excessive nitrogen (N) in fertilizer is mentioned as the cause of changes in the diversity, community structure and activity of soil microorganisms, which lead to reductions in the growth and yield in various plantations, such as tomatoes (Guo et al., 2018), switchgrass (Bahulikar et al., 2019) and wheat (Chen et al., 2019). Excessive phosphorus (P) may lead to a reduction in microbial respiration and the metabolic quotient, as reported in plantations of lodgepole pine (Thirukkumaran and Parkinson, 2002), eucalyptus (Wang et al., 2008) and oak (DeForest, 2019). In rice plantations, excessive P can lead to severe iron (Fe) deficiency and subsequently

a decreasing grain yield (Panda et al., 2012) and increased rice susceptibility to the pathogenic fungus *Magnaporthe oryzae* (Campos-Soriano et al., 2020). Excessive potassium (K) may not have a deleterious effect in the soil over a short time but will lead to a reduction in soil bacteria and fungal diversity in the long term, as reported by Li et al. (2021) in plantation rotations of rice-oilseed rape. These examples identify some of the possible issues that may be encountered in the co-application of plant growth-promoting bacteria and fertilizer. However, the relationship between IAA, GAs and SA in influencing grain yield is currently unknown and how consistently this mode of enhancement exists in rice is unknown.

The current study hypothesized that endogenous IAA, GAs, SA, N, P and K levels might be involved in grain yield improvement, with influence from different fertilization regimens and plant growth-promoting bacteria to the rice endogenous levels of IAA, GAs, SA, N, P and K. In this study, plant growth-promoting bacterial traits of the bacteria used were also explored and related to the endogenous IAA, GAs, SA, N, P and K levels. The effect of the synthetic fertilizers on the endogenous IAA, GAs, SA, N, P and K levels was also explored. This study aimed to detail the mechanisms influencing the dynamics of endogenous IAA, GAs, SA, N, P and K and how these can improve the grain yield and grain-yield-related traits. Therefore, this study aimed to discover the role and interaction between selected microbial isolates and different fertilizers in improving rice grain yield production. The results of this research have revealed the detailed mechanisms by which bacterial strains can improve the grain yield and grain yield-related traits to IAA, GAs, SA, N, P and K.

Materials and Methods

Plant, soil and bacteria preparation

Oryza sativa var. *indica* cv. Pathum Thani 1 was used in this study. Its seeds were immersed in tap water for 2 d, with the tap water changed daily pre-germination. Pre-germinated seeds were seeded into the seedbed and nurtured for 30 d until becoming seedlings. The seedling nursery was using the wet-bed method. Later, the seedlings were transplanted into the experimental pots. Rice field soil from the Bang Pa-in district, Ayutthaya province, Thailand was collected and used as the plant soil medium for this study. The soil physicochemical properties were recorded as: soil texture was classified in the

silty clay category, with 47.51% silt, 47.36% clay and 5.13% sand; pH (soil: H₂O 1:1) 6.08; electrical conductivity 2.07 dS/m; organic matter 31.90 g/kg, total N 1.70 g/kg, available P 99.96 mg/kg; exchangeable K 194.76 mg/kg; exchangeable Ca 2745.12 mg/kg, exchangeable Mg 501.75 mg/kg; and cation exchange capacity (CEC) 33.51 cmol/kg. Soil elemental content was determined using X-ray fluorescence spectrometry. The soil elemental content was recorded as: Si 64.30%, Al 20.90%, Fe 7.75%, K 2.77%, Ti 1.18%, Ca 1.03%, Mg 1.01%, Na 0.40%, P 0.20%, S 0.13%, Mn 0.09%, Ba 0.06%, V 0.03%, Zr 0.03%, Cl 0.03%, Zn 0.03%, Cr 0.02%, Rb 0.02%, Sr 0.02%, Cu 0.01% and Ni 0.01%.

Bacillus sp. N7, *Lysinibacillus* sp. N5, *Bacillus thuringiensis* B20 and *Bacillus safensis* TISTR2163 were used in this study as plant growth-promoting bacteria inoculum strains. Table 1 presents information regarding the plant growth-promoting bacteria used in this study. All bacterial strains were routinely cultured in nutrient broth (Himedia; India) at 30°C and made up into 30% (volume per volume) glycerol culture stocks. Bacterial stocks were maintained at -80°C for future use.

Plant growth-promoting trait assays and virulence gene profiling of the plant growth-promoting bacteria

The ammonia production assay was determined by colorimetry using the modified Nessler's method, as described by Suksabye et al. (2016). The phosphate solubilization ability

of the bacteria was performed using Fiske and Subbarow's colorimetric method, described by Oteino et al. (2015). The potassium solubilization assay was performed using the method described previously in Sritongon et al. (2017). Phytohormone production by bacteria was also investigated. The bacterial IAA production assay was measured by culturing bacteria in a tryptophan-supplemented Dworkin-Foster minimal medium at 30°C and 150 revolutions per minute (rpm) for 3 d (Setsungnern et al., 2018), followed by colorimetric determination using the method described by Gordon and Weber (1951). Bacterial GAs production was assayed by culturing bacteria in Luria-Bertani medium at 28°C and 200 rpm for 7 d (Ullah et al., 2014) followed by colorimetric determination based on the method described by Holbrook et al. (1961). In addition, bacterial SA production was analyzed by culturing bacteria in Casamino Acid Broth medium at 28°C and 150 rpm for 36 hr (Islam et al., 2020), followed by colorimetric determination based on the method described by Warriar et al. (2013).

To ensure the safety of the plant growth-promoting bacteria, the presence of the bacilli virulence genes *hblA*, *nheC* and *cytK* (Lindbäck and Granum, 2015) was checked using PCR. The three PCR primer sets used in this study were previously described by Kim et al. (2015) and are listed in Table 2. Before the PCR, each plant growth-promoting bacterium was cultivated on a Luria-Bertani agar plate. A sterilized toothpick was used to pick up individual colonies that were dipped into each reaction tube containing 100 µL Luria-Bertani

Table 1 Bacterial strains used in this study

Bacterial strain	Source of isolation	NCBI accession	References
<i>Bacillus</i> sp. N7	Rice rhizosphere	ON860759	(Autarmat et al., 2022)
<i>Lysinibacillus</i> sp. N5	Rice rhizosphere	ON860760	(Autarmat et al., 2022)
<i>Bacillus safensis</i> TISTR2163	Marine coastal area	-	(Tanadchangsang and Pattanasupong, 2022)
<i>Bacillus thuringiensis</i> B20	Fish merchant waste	ON860685	(Siswanto and Thiravetyan, 2016)

Uninoculated condition was used as a control.

Table 2 Primer sets used in this study to detect Bacilli-virulent genes

Primer set	Target gene	Sequence	Amplicon size (bp)
1	<i>hblA</i>	Forward (HBLA1): 5'- GTGCAGATGTTGATGCCGAT -3' Reverse (HBLA2): 5'- ATGCCACTGCGTGACATAT -3'	301
2	<i>nheC</i>	Forward (nheC2820S): 5'- CGGTAGTGATTGCTGGG -3' Reverse (nheC3401A): 5'- CAGCATTCGTACTTGCCAA -3'	564
3	<i>cytK</i>	Forward (CK-F-1859): 5'- ACAGATATCGGKCAAAATGC -3' Reverse (CK-R-2668): 5'- TCCAACCCAGTTWSCAGTTC -3'	809

medium. Later, a 1 μ L sample was combined with a PCR mix consisting of Taq PCR Master Mix (Qiagen, Germany), forward and reverse primers and nuclease-free water, following the manufacturer's protocol. The PCR reaction was performed in a total volume of 25 μ L. The PCR conditions were: initial denaturation at 95°C for 5 min, followed by 25 PCR cycles of denaturation at 95°C for 30 s, annealing at 55°C for 10 s and extension at 72°C for 40 s to 1 min—up to the PCR product size, for extension time for primer, set 1 and 2 = 40 s; primer set 3 = 1 min—and a final extension at 72°C for 7 min. The size and amount of PCR products were visualized and estimated based on 1.5% agarose gel electrophoresis and ethidium bromide staining. Aside from a blank lane that served as a negative blank, for toxin positive and negative control, locally isolated *Bacillus cereus* and *Bacillus megaterium* strains, *Bacillus cereus* ERBP (NCBI Accession: KF475814) (Khaksar et al., 2016) and *Bacillus megaterium* BM (NCBI Accession: KX350034) (Dolphen et al., 2020) were used for comparisons.

Pot experimental preparation, plant growth-promoting bacteria inoculation and agricultural practices

The pot experiment was performed during the dry season of 2021 (started on 16 February and finished on June 19, 2021). During that period, the environmental parameters were recorded (Table 3). Plants were kept in open-air, outdoor pots protected by nets in the Remediation Laboratory, King Mongkut's University Technology Thonburi Bangkhuntien Campus, Bangkok, Thailand, as described in Nashrulloh et al. (2022). Natural soil without sterilization used in this experiment. Each pot (diameter 21 cm, height 21 cm) was filled with 3.5 kg of soil. In total, 200 pots were utilized throughout the pot experiment. A 2×2×5 factorial experiment in a completely randomized design was applied. Factor A was vegetative fertilizer (N-P-K at 16-16-16), consisting of two doses: 2 g/pot, and 4 g/pot. Factor B was generative fertilizer (N-P-K at 8-24-24), consisting of two doses: 2 g/pot, and 4 g/pot. Factor C was bacterial inoculum (five levels), consisted of one uninoculated control and four bacterial strains. There were 10 replicates.

Table 3 Atmospheric parameters during experiments

Atmospheric parameter	Dry season 2021
Daily light-dark cycle (light:dark, hr)	13:11
Atmospheric humidity (%)	70–75
Atmospheric temperature (low/high) (°C)	26–35±1
Average monthly temperature (°C)	30–32
Precipitation (mm)	30.9–202.4

Seedlings were transplanted into pots filled with rice field soil after 30 d in the nursery. Five plants were planted into each pot and left to acclimatize for 7 d. The vegetative fertilizer was applied 7 d after the application of the bacteria and the generative fertilizer was applied when plants started to boot and were preparing to flower.

Plant growth-promoting bacteria inocula were prepared according to Anusaraporn et al. (2020). For plant growth-promoting bacteria inoculation, fresh overnight (24 hr) culture of each bacterial strain containing approximately 1×10^8 colony forming units (CFU)/mL were used as the inoculum. Bacterial inoculum application occurred once on the last day after the plant had acclimatized for 7 d after transplantation (seedlings aged 37 d). Then, 20 mL of bacterial suspension was inoculated in each treatment pot containing approximately 1×10^8 CFU/mL, while the uninoculated nutrient broth was added to pots as the control condition. The water level of each pot was maintained at 1,000 mL/pot by watering twice daily. Plants were allowed to grow until harvest. All mature crops were harvested at 123 d.

Evaluation of endogenous levels of indole-3-acetic acid, gibberellic acids and salicylic acid

Plants were assessed for endogenous phytohormones (IAA, GAs and SA). Leaf sampling was performed at the maximum tillering state (representing the maximum point of the vegetative phase) and panicle exertion state (representing the maximum point of the generative phase), according to Lakaew et al. (2021). The maximum tillering state was observed when rice plants were aged 56 d (1 wk before the rice plants started booting). Rice plants started to boot at around aged 63 d. Generative fertilizer was applied during the booting period. The panicle exertion state occurred around age 77 d (2 wk after the rice plants started to boot). Collected leaf tissue samples were crushed in liquid nitrogen and made into powder. The leaf tissue powder samples were stored at -80°C until used for assays. The IAA and GA extraction and measurement protocols, as described by Bayisa (2020), were used for the measurement of endogenous IAA and GA levels. The SA extraction and measurement protocol described by Khaksar et al. (2017) was used for the measurement of endogenous SA levels.

Evaluation of post-harvest plant macronutrients levels

Plant macronutrients (N, P and K) were assessed. Leaves were collected from residues, cut and dried in an oven at

70°C for 1 wk until dry and a constant weight, before being pulverized into powder. Dried powdered samples were used to measure N, P and K. The standard Kjeldahl method, as described by Eviati and Sulaiman (2009), was used to measure N. The P was measured using the Bray 2 method (Bray and Kurtz, 1945). The K was measured using the ICP-OES method, as described in Dolphen and Thiravetyan (2019). N, P, and K were measured in triplicate.

Evaluation of growth and yield traits

Plant height and tiller number were evaluated before the harvest. After the harvest, the panicle number and total grain yield per pot were evaluated.

Statistical analysis

A completely randomized design was chosen as the experimental design for plant growth-promoting bacteria traits evaluation. All data for plant growth-promoting bacteria traits analysis were analyzed based on one-way analysis of variance, followed by Duncan's multiple range tests using the 95% confidence level for comparison between means of bacterial strains. Data were presented as the mean $\pm$ SD of three replications.

The 2 $\times$ 2 $\times$ 5 factorial experiment in a completely randomized design was chosen for plant cultivation and subsequent evaluation of endogenous phytohormones levels, growth and yield factors. All data were analyzed using the same 2 $\times$ 2 $\times$ 5 factorial experiment design, followed by Duncan's multiple range tests at a 95% confidence level for comparing the means among the groups. The data were presented as the mean $\pm$ SD of 10 replications, except for the macronutrient level, which were presented as the mean $\pm$ SD of three replications.

All statistical analyses used statistical procedures described in Aqil and Effendi (2016). Statistical analyses were performed using the SPSS version 26.0 software (IBM; USA).

Results

Plant growth-promoting traits of various bacterial strains

The result of the assays for plant growth-promoting traits of the various bacterial strains used are shown in Table 4. Individual bacteria possessed different plant growth-promoting bacteria traits, indicating that the bacteria might have different ways of interacting positively with plants. Some traits were common to all isolates used in this study, such as NH₄⁺ production, IAA production and SA production. The NH₄⁺ production levels of plant growth-promoting bacterial strains were in the range 24.89–46.83 μ g NH₄⁺/mL ($p < 0.05$). The IAA production levels were in the range 2.47–7.42 μ g IAA/mL ($p < 0.05$). All bacteria used in this study produced SA, with levels in the range 9.69–18.87 μ g SA/mL ($p < 0.05$). However, some traits were apparent only for certain bacterial strains used in this study. For example, only *Bacillus safensis* TISTR2163 and *Bacillus thuringiensis* B20 produced PO₄³⁻, while *Bacillus thuringiensis* B20 did not produce any GAs. None of the bacterial strains could solubilize potassium. Virulence genes—*hblA*, *nheC* and *cytK*—profiled using PCR presented in the fragmented form. The PCR analysis showed that none of the bacterial strains contained any amplicons of virulence genes (Table 4 and Fig. 1), suggesting that the bacterial strains used in this study were not pathogenic and safe to use. No amplicon of *nheC* or *cytK* was detected in any strain compared to the control (Fig. 1), while *hblA* was not detected on the positive control *Bacillus cereus* ERBP and the Bacilli strains used in this study (Fig. 1).

Table 4 Plant growth-promoting bacteria traits of bacterial strains used in this study

Traits	Bacterial strain			
	N7	N5	BST	B20
NH ₄ ⁺ production (μ g/mL)	28.70 $\pm$ 3.90 ^a	24.89 $\pm$ 3.04 ^a	26.89 $\pm$ 2.24 ^a	46.83 $\pm$ 0.17 ^b
PO ₄ ³⁻ production (μ g/mL)	-	-	22.05 $\pm$ 2.24 ^b	8.87 $\pm$ 0.32 ^a
K solubilization	-	-	-	-
IAA production (μ g/mL)	4.47 $\pm$ 0.20 ^b	7.42 $\pm$ 0.5 ^c	2.47 $\pm$ 0.01 ^a	4.11 $\pm$ 0.59 ^b
GAs production (μ g/mL)	187.13 $\pm$ 4.55 ^b	232.28 $\pm$ 5.50 ^c	145.63 $\pm$ 17.66 ^a	-
SA production (μ g/mL)	9.69 $\pm$ 2.82 ^a	15.28 $\pm$ 2.72 ^{ab}	18.87 $\pm$ 4.74 ^b	16.23 $\pm$ 4.64 ^{ab}
<i>hblA</i>	-	-	-	-
<i>nheC</i>	-	-	-	-
<i>cytK</i>	-	-	-	-

N7 = *Bacillus* sp. N7, N5 = *Lysinibacillus* sp. N5, BST = *Bacillus safensis* TISTR2163,

B20 = *Bacillus thuringiensis* B20. - = Negative or no production.

Data presented as mean $\pm$ SD of three replications. Different lowercase superscripts within the same row denote significant ($p < 0.05$) differences.

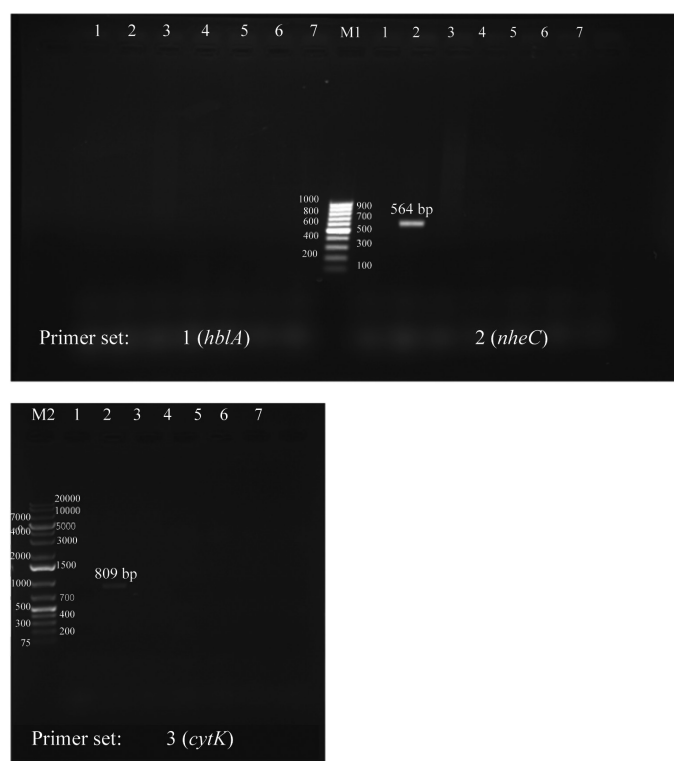


Fig. 1 Representative polymerase chain reaction (PCR) amplicons to detect other putative virulence genes *hblA*, *nheC* and *cytK* among *Bacilli* strains used in this study, where lane codes are M1 = 100 bp ladder (Fermentas, USA), M2 = 1 Kb Plus ladder (Fermentas, USA), 1 = PCR negative control (without DNA sample), 2 = *Bacillus cereus* (virulent strain representative as positive control), 3 = *Bacillus megaterium* (non-virulent strain representative as negative control), 4 = *Bacillus thuringiensis* B20, 5 = *Bacillus safensis* TISTR2163, 6 = *Lysinibacillus* sp. N5, 7 = *Bacillus* sp. N7

Effects of different doses of vegetative and generative fertilizer and plant growth-promoting bacteria inoculation on indole-3-acetic acid, gibberellic acids and salicylic acid phytohormones levels

The application of fertilizer was observed under four different dose conditions (2-2, 2-4, 4-2 and 4-4 in the vegetative and generative phases, respectively). In the vegetative phase, there was a significant interaction effect of vegetative fertilizer × plant growth-promoting bacteria on vegetative IAA. During the generative phase, there were significant interaction effects of generative fertilizer × plant growth-promoting bacteria and vegetative fertilizer × generative fertilizer × plant growth-promoting bacteria influenced generative IAA (Table 5). The interaction effect between study factors for other phytohormones, plant macronutrients, yields and yield components are presented in Table 5. The results showed that the IAA levels of all conditions tended to increase from the vegetative phase to the generative phase. Variations of IAA levels were also observed, IAA values were in the ranges 83.66–151.32 µg IAA/g fresh weight (FW) in the vegetative phase and 104.05–207.76 µg IAA/g FW in the generative phase (Fig. 2A). Inoculation of *Bacillus* sp. N7, *Lysinibacillus* sp. N5, *Bacillus safensis* TISTR2163 and *Bacillus thuringiensis* B20 significantly produced higher vegetative IAA values than for the non-inoculated control under vegetative fertilization of both 2 g/pot and 4 g/pot (Fig. 2A). However, *Bacillus* sp. N7, *Lysinibacillus* sp. N5 and *Bacillus safensis* TISTR2163 produced the highest vegetative IAA values under vegetative

Table 5 The *p* values for effect of vegetative fertilizers, generative fertilizers, plant growth-promoting bacteria strains inoculated to Pathum Thani 1 rice and their interaction on grain yield (GY), panicle number (PN), tiller number (TN), plant height (PH) and endogenous plant physiological factors—IAA (at vegetative and generative phases), GAs (at vegetative and generative phases), SA (at vegetative and generative phases), N, P and K

Dependent factor	Independent factor			Interaction between independent factors			
	VF	GF	B	VF×GF	VF×B	GF×B	VF×GF×B
GY	<0.001	<0.001	<0.001	0.003	<0.001	0.010	0.146
PN	<0.001	<0.001	0.073	<0.001	0.011	0.022	0.017
TN	0.002	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
PH	0.017	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
IAA _{veg}	<0.001	<0.001	<0.001	0.978	<0.001	<0.001	0.023
IAA _{Gen}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GAS _{veg}	<0.001	0.599	<0.001	0.001	<0.001	0.111	0.082
GAS _{Gen}	<0.001	0.042	<0.001	0.619	<0.001	0.704	0.001
SA _{veg}	<0.001	<0.001	<0.001	0.913	<0.001	0.092	0.072
SA _{Gen}	<0.001	<0.001	<0.001	0.407	0.002	0.402	0.061
N	0.782	<0.001	0.097	0.032	0.048	0.308	0.002
P	<0.001	0.480	<0.001	<0.001	<0.001	<0.001	<0.001
K	0.769	0.833	0.003	<0.001	<0.001	0.005	<0.001

VF = Vegetative fertilizer; GF = Generative fertilizer; B = Plant growth-promoting bacteria strains; GY = grain yield; PN = panicle number; TN = tiller number; PH = plant height; IAA_{veg} = IAA at the vegetative phase; IAA_{Gen} = IAA at the generative phase; GAS_{veg} = GAs at the vegetative phase; GAS_{Gen} = GAs at the generative phase; SA_{veg} = SA at the vegetative phase; SA_{Gen} = SA at the generative phase.

fertilization at 2 g/pot, whereas *Lysinibacillus* sp. N5 had the highest vegetative IAA value under vegetative fertilization at 4 g/pot. During the generative phase, *Bacillus* sp. N7, *Lysinibacillus* sp. N5, *Bacillus safensis* TISTR2163 and *Bacillus thuringiensis* B20 had significantly higher generative IAA values than the non-inoculated control under the two doses of both vegetative and generative fertilizers (Fig. 2A).

The results confirmed that total the GA contents tended to increase from the vegetative phase to the generative phase (Fig. 2B). In the vegetative phase, *Bacillus* sp. N7 and *Bacillus safensis* TISTR2163 induced higher vegetative GA levels than the other strains at 2 g/pot of vegetative fertilizer, whereas *Bacillus* sp. N7 and *Lysinibacillus* sp. N5 induced higher vegetative GA levels than the other strains at 4 g per pot of vegetative fertilizer. In the generative phase with the application of two doses of the vegetative and generative fertilizers, no bacterial strain produced a better GA level than the non-inoculated control. Variations in the GA levels were observed, in the ranges 4.19–5.78 mg GA₃ equivalent/g FW in the vegetative phase and 4.89–6.80 mg GA₃ equivalent/g FW in the generative phases (Fig. 2B). *Bacillus* sp. N7 and *Bacillus safensis* TISTR2163 significantly increased the vegetative GA levels under the half dose of vegetative fertilization (2 g/pot), while *Bacillus* sp. N7 significantly increased the vegetative GA levels under a full dose of vegetative fertilization (4 g/pot), as shown in Fig. 2B. During the generative phase, *Bacillus* sp. N7, *Lysinibacillus* sp. N5, *Bacillus safensis* TISTR2163 and *Bacillus thuringiensis* B20 significantly influenced generative GAs (Fig. 2B); however, the degree of enhancement depended on the amounts of vegetative fertilizer and generative fertilizer applied (Fig. 2B).

The results showed that the generative phase had a higher SA content than the vegetative phase. Variations in the SA levels were observed, in the ranges 1.55–1.93 mg SA/g FW in the vegetative phase and 1.90–2.65 mg SA/g FW in the generative phase (Fig. 2C). In general, the endogenous SA levels of the rice with a full dose of vegetative fertilizer (4 g/pot) were significantly higher than in rice with vegetative fertilizer at 2 g/pot (Fig. 2C). However, during the generative phase, *Bacillus* sp. N7, *Lysinibacillus* sp. N5, *Bacillus safensis* TISTR2163 and *Bacillus thuringiensis* B20 significantly influenced the generative SA levels (Fig. 2C); however, the degree of enhancement depended on the amounts of vegetative fertilizer and generative fertilizers applied.

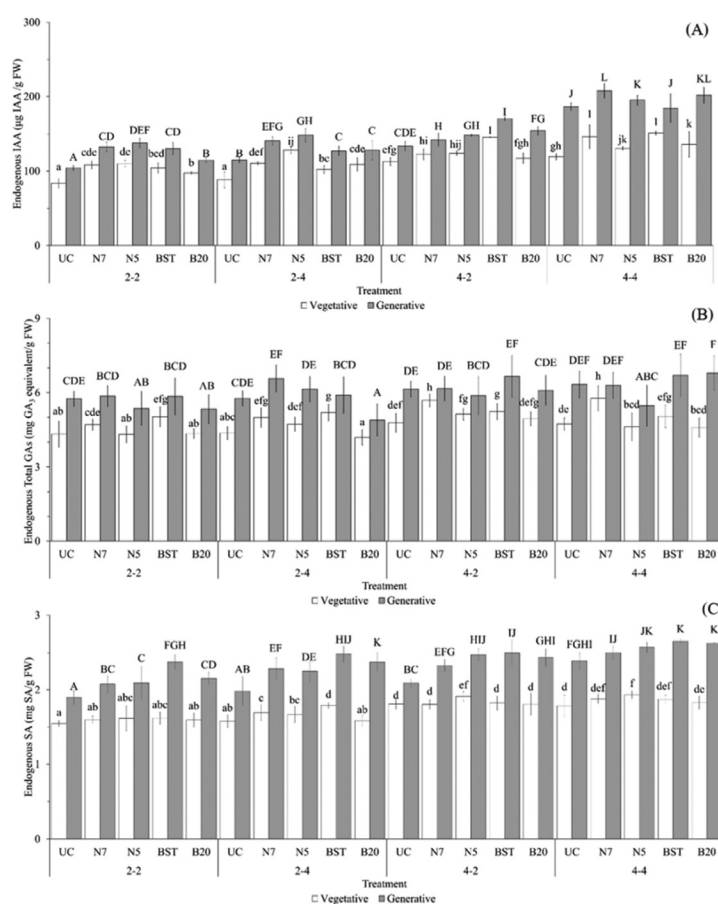


Fig. 2 Effect of adding various bacterial strains to endogenous levels in Pathum Thani 1 rice under different fertilizer regimens for: (A) indole-3-acetic acid (IAA); (B) gibberellic acids (GAs); (C) salicylic acid (SA), where values are mean $\pm$ SD of 10 replications; different letters above bars for each experiment denote significant ($p < 0.05$) differences, with lowercase letters distinguishing between vegetative phases, while capital letters distinguish between generative phases; FW = fresh weight, UC = uninoculated control, N7 = *Bacillus* sp. N7, N5 = *Lysinibacillus* sp. N5, BST = *Bacillus safensis* TISTR2163, B20 = *Bacillus thuringiensis* B20. 2-2 = fertilizer regimens of 2 g vegetative fertilizer N-P-K (16-16-16) and 2 g generative fertilizer N-P-K (8-24-24), 2-4 = fertilizer regimens of 2 g vegetative fertilizer N-P-K (16-16-16) and 4 g generative fertilizer N-P-K (8-24-24), 4-2 = fertilizer regimens of 4 g vegetative fertilizer N-P-K (16-16-16) and 2 g generative fertilizer N-P-K (8-24-24), 4-4 = fertilizer regimens of 4 g vegetative fertilizer N-P-K (16-16-16) and 4 g generative fertilizer N-P-K (8-24-24).

Effects of different doses of vegetative and generative fertilizer and plant growth-promoting bacteria inoculation on endogenous N, P and K

Variations in the N levels were observed in the ranged 5.30–8.67 mg TKN/g dry weight (DW); however, the N levels, in general, were not significantly different (Fig. 3A), though the significant differences were observed in P and K. Variations in the P levels were observed in the range 0.60–1.53 mg available PO_4^{3-} /g DW (Fig. 3B). The effects of the individual factors of vegetative fertilizer and bacteria were also significant in influencing P levels (Table 5). Variations in the K levels were also observed in the range 0.80–2.55 mg available K/g DW (Fig. 3C). In general, rice plants with lower vegetative fertilization (2 g/pot) had lower P and K accumulation than the rice plants with higher vegetative fertilization (Fig. 3B–3C).

Bacillus sp. N7, *Lysinibacillus* sp. N5, *Bacillus safensis* TISTR2163 and *Bacillus thuringiensis* B20 significantly increased P accumulation in plant tissue in all fertilization regimens. However, K accumulation assisted by plant-growth-promoting bacteria was only significant in the fertilization regimens with full vegetative fertilization (4 g/pot).

Effects of different doses of vegetative and generative fertilizer and plant growth-promoting bacteria inoculation on growth and yield traits

The results confirmed that all bacteria had a role in increasing rice productivity and reduced the amount of fertilizer used. There were significant increases in grain yield (10.16–32.77 g/pot), panicle number (16.00–31.25 panicles/pot), tiller number (27.00–45.88 tillers/pot) and plant height (103.34–120.52 cm) in Pathum Thani 1 rice (Figs. 4A–4D). *Bacillus* sp. N7, *Lysinibacillus* sp. N5, *Bacillus safensis* TISTR2163 and *Bacillus thuringiensis* B20 significantly improved the grain yield in the fertilization regimens with a one-half dose of vegetative fertilizer (2 g/pot); however, in the fertilization regimens with the full dose of vegetative fertilizer (4 g/pot), bacterial inoculation had no significant effect (Fig. 4A). Rice inoculated with *Lysinibacillus* sp. N5 and *Bacillus* sp. N7 with vegetative fertilization at 2 g/pot had higher grain yields than for the other treatments.

Rice with *Lysinibacillus* sp. N5 inoculation had significant panicle number enhancement under the one-half dose of vegetative fertilizer (2 g/pot) and the one-half dose of generative fertilizer (2 g/pot) fertilization regimen. However, with the one-half dose of vegetative fertilizer (2 g/pot) and a full dose

of generative fertilizer (4 g/pot), there was no no significant panicle number enhancement. Similarly, no significant panicle number enhancement was observed among the rice crops with fertilizer regimens including full dose fertilization regimens (Fig. 4B).

The interaction effect of vegetative fertilization $\times$ generative fertilization $\times$ plant-growth-promoting bacteria significantly promoted tillering (Fig. 4C) and plant height (Fig. 4D).

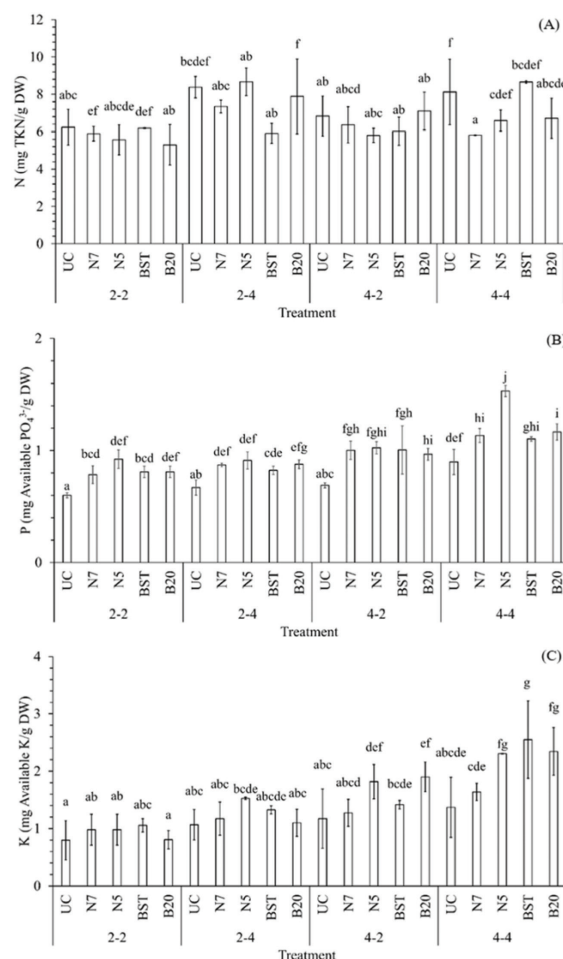


Fig. 3 Effect of adding various bacterial strains in Pathum Thani 1 rice under different doses of fertilizer on post-harvest levels of: (A) N, (B) P; (C) K, where values are mean $\pm$ SD of three replications; different lowercase letters above bars for each experiment denote significant ($p < 0.05$) differences; DW = dry weight, UC = uninoculated control, N7 = *Bacillus* sp. N7, N5 = *Lysinibacillus* sp. N5, BST = *Bacillus safensis* TISTR2163, B20 = *Bacillus thuringiensis* B20. 2-2 = fertilizer regimens of 2 g vegetative fertilizer N-P-K (16-16-16) and 2 g generative fertilizer N-P-K (8-24-24), 2-4 = fertilizer regimens of 2 g vegetative fertilizer N-P-K (16-16-16) and 4 g generative fertilizer N-P-K (8-24-24), 4-2 = fertilizer regimens of 4 g vegetative fertilizer N-P-K (16-16-16) and 2 g generative fertilizer N-P-K (8-24-24), 4-4 = fertilizer regimens of 4 g vegetative fertilizer N-P-K (16-16-16) and 4 g generative fertilizer N-P-K (8-24-24)

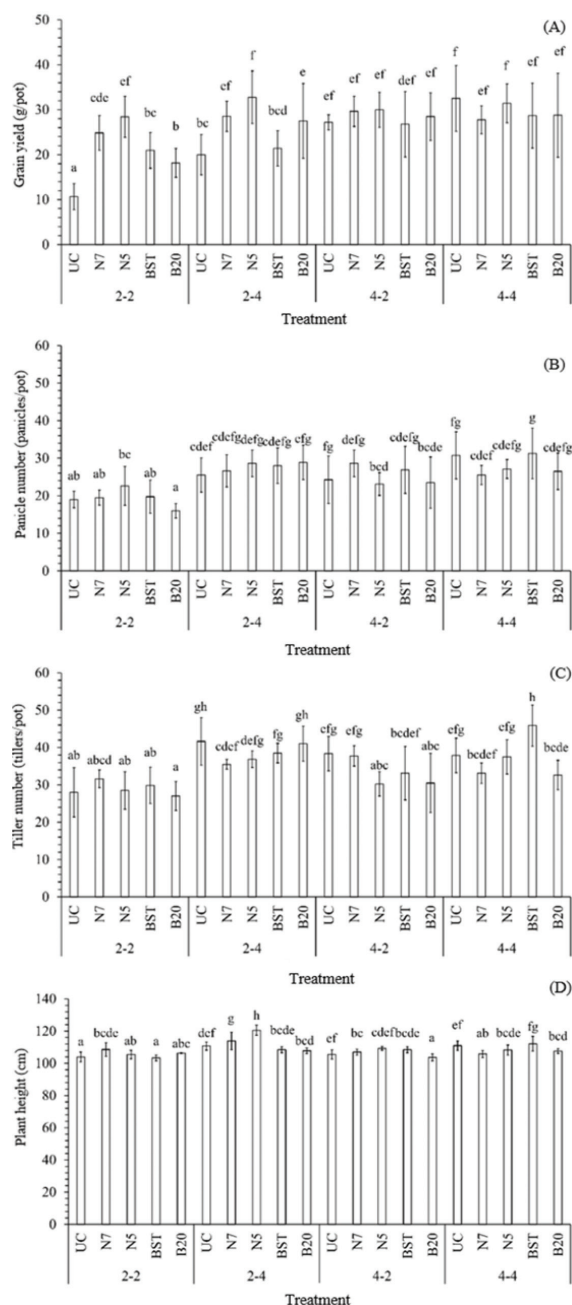


Fig. 4 Effect of adding bacterial strains to Pathum Thani 1 rice after harvesting under different doses of fertilizer on (A) grain yield; (B) panicle number; (C) tiller number; (D) plant height, where values are mean $\pm$ SD of 10 replications; different lowercase letters above bars for each experiment denote significant ($p < 0.05$) differences; UC = uninoculated control, N7 = *Bacillus* sp. N7, N5 = *Lysinibacillus* sp. N5, BST = *Bacillus safensis* TISTR2163, B20 = *Bacillus thuringiensis* B20. 2-2 = fertilizer regimens of 2 g vegetative fertilizer N-P-K (16-16-16) and 2 g generative fertilizer N-P-K (8-24-24), 2-4 = fertilizer regimens of 2 g vegetative fertilizer N-P-K (16-16-16) and 4 g generative fertilizer N-P-K (8-24-24), 4-2 = fertilizer regimens of 4 g vegetative fertilizer N-P-K (16-16-16) and 2 g generative fertilizer N-P-K (8-24-24), 4-4 = fertilizer regimens of 4 g vegetative fertilizer N-P-K (16-16-16) and 4 g generative fertilizer N-P-K (8-24-24)

Discussion

Roles of indole-3-acetic acid, gibberellic acids and salicylic acid levels in rice yield determination under co-application of fertilizer and plant growth-promoting bacteria

Hormonal control is one of the aspects determining rice grain yield. Plants possess various phytohormones and some phytohormones have been identified as key phytohormones that play a role in the determination of grain yield. Due to the very broad mechanisms controlled by phytohormones, some phytohormones have overlapping functions with each other or there may be some crosstalk mechanisms between them. In tillering, IAA, cytokinins (CKs), GAs, brassinosteroids (BRs) and strigolactones (SLs) together control tillering activity (Liu et al., 2011; Yu et al., 2020; Wu et al., 2021). In panicle development, spikelet and flowering organs development, grain formation and grain filling, IAA, GAs, CKs, abscisic acid (ABA), BRs and ethylene play roles in these processes (Mohapatra and Sahu, 2022); thus, these are very important to obtain considerable and calculable rice grain yield. To maintain the spikelet until it develops into grain, IAA and SA are important to ensure the maintained status of the spikelet, so the spikelet can mature and reach seed form (Zhang et al., 2017a).

Three endogenous phytohormones (IAA, GAs and SA) were investigated in the current study. Notably, all the studied endogenous phytohormones showed similar trends of increasing over time from the vegetative and generative phases. Autarmat et al. (2022) reported an increase in IAA during the progression from the vegetative to the generative phase in Pathum Thani 1 rice. Similarly, Zhang et al. (2016) with *Oryza sativa* var. *japonica* cv. Azucena and *Oryza sativa* var. *indica* cv. Zhefu802 reported that IAA tended to reach its maximum rate of increase in the first 2 wk after anthesis and subsequently declined after the period of maximum increase. The increase in IAA during this period was likely related to the initiation of the formation of panicle and spikelet primordia (Yang et al., 2017). Plant growth-promoting bacteria appeared to increase endogenous IAA levels. Hsu (2010) reported on *Streptomyces scabies* 87-22 and *Raphanus sativus* cv. Burpee White plant-microbe interactions and found that the IAA produced by the bacteria enabled the interaction between the bacteria and the plant to gain advantage from the plant physiological processes for bacterial benefit. A similar result in *Xanthomonas oryzae* pv. *oryzae* PXO61 and *Oryza sativa* var. *indica* cv. Minghui 63 and Zhenshan 97 plant-microbe interactions was reported

by Liu et al. (2012). Ambreetha et al. (2018) reported that in the *Bacillus altitudinis* FD48 and *Oryza sativa* var *indica* cv. Co51 plant-microbe interaction, endogenous IAA levels were elevated by beneficial bacteria. Thus, IAA was one of the molecules used by bacteria to interact with the plant. The results of this study confirmed the co-effect between the fertilizers and plant growth-promoting bacteria in influencing endogenous IAA levels.

GAs showed a different way of modulation compared to IAA, which could be divided into two parts: during the vegetative and during the generative phases. During the vegetative phase, GAs levels were influenced by bacteria, fertilizer and their interaction. Nagai et al. (2020) working with *Oryza sativa* var. *japonica* cv. Taichung 65 and C9285, reported that GAs facilitated rice growth in the vegetative phase. This growth-promoting bacteria influence on vegetative GAs was similar Joo et al. (2005), who reported increased endogenous GAs levels in the vegetative phase of *Capsicum annuum* inoculated with *Bacillus cereus* MJ-1, *Bacillus macroides* CJ-29 and *Bacillus pumilus* CJ-69. Furthermore, a similar result was observed in the vegetative phase of *Oryza sativa* cv. Waito-C inoculated with *Azospirillum lipoferum* USA 5b and *Azospirillum brasilense* Cd (Cassán et al., 2001). In the current study, over time, the GAs showed a tendency to elevate from the vegetative to the generative phase. This result was similar to López-Cristoffanini et al. (2019), who reported that in *Oryza sativa* var. *japonica* cv. NRVC980385, Bomba and dwarf-Bomba the GAs levels were elevated during the formation of the flag leaf and the 50% heading state. The increase in GAs during the generative phase could be attributed to the GAs being needed in spikelet formation (Kwon and Paek, 2016). However, in the current study, no different generative GAs levels were observed during the generative phases for all fertilization regimens. To date, there has been no explanation for why generative fertilization does not affect GA levels. In literature, however, the reverse has been the subject of considerable discussion. For example, with *Brassica juncea* cv. T-59 (Khan, 1996), *Brassica juncea* cv. Varuna (Khan et al., 2002) and *Solanum lycopersicum* cv. Yingfen No. 8, (Zhang et al., 2017b) reported that GAs increased plant nitrate reductase activity, thus affecting the nitrogen metabolism and nitrogen translocation in plants, resulting in improved utilization of N by plants. Not much is known about the extent and dynamics of GAs in determining grain yield (Mohapatra and Sahu, 2022). The current study may shed some light on the co-effect between the fertilizers and plant growth-promoting bacteria in influencing endogenous GAs levels.

SA was maintained at low levels in the rice vegetative phase, becoming elevated in the generative phase. This result was supported by Malaga et al. (2020) who reported on *Hordeum vulgare* DH lines, in which the SA levels were also maintained at low levels during the vegetative phase and were elevated in the generative phase. SA increases during the progression to the flowering stage (Abreu and Munné-Bosch, 2009; Shah et al., 2021), which is consistent with its role as a flowering phytohormone. The possible reason why fertilizer resulted in increased SA levels was likely due to the precursors of SA (phenylalanine and tyrosine) both being amino acids (Widhalm et al., 2015; Qian et al., 2019), which contain N as constituent. Thus, increasing the levels of SA may be seen as an indirect effect from N fertilization. There are reports supporting such a mechanism in the literature. Phenylalanine was found to be increased by N fertilization in *Populus x canescens* (Jiao et al., 2018). An increased tyrosine content resulted from fertilization of *Solanum tuberosum* cv. Eshu 3 (Zhang et al., 2020). On the other hand, the contents of phenylalanine and tyrosine can be increased by plant growth-promoting bacteria, as reported by Hutapea et al. (2018) in *Indigofera zollingeriana* inoculated with *Bradyrhizobium* sp. and *Roseomonas* sp. strains. A similar result was reported by İpek (2019) in *Rubus idaeus* cv. Heritage inoculated with *Alcaligenes* sp. 637Ca, *Staphylococcus* sp. MFDCa1, *Staphylococcus* sp. MFDCa2, *Agrobacterium* sp. A18, *Pantoea* sp. FF1 and *Bacillus* sp. M3. Phenylalanine and tyrosine are precursors for the phenylalanine ammonia-lyase and tyrosine ammonia-lyase pathways, which produce aromatic phenolic compounds, including SA (Tashackori et al., 2018). Since the production of both precursors is determined by N, this may be the reason why SA was elevated under higher fertilizer regimens. SA has been reported to increase productivity and yield as reported for *Capsicum annum* cv. Yolo wonder (Ahmed et al., 2020), *Triticum aestivum* cv. PAK 2013 (Azmat et al., 2020), *Zea mays* cv. Xinzhongyou 801 (Shemi et al., 2021) and *Oryza sativa* var. *indica* cv. Jinliangyouhuazhan (Yang et al., 2022). SA-producing plant growth-promoting bacteria also assist yield improvement as reported in the inoculation of *Pseudomonas aeruginosa* PaRsG18, *Pseudomonas aeruginosa* PaRsG27 and *Pseudomonas aeruginosa* PaRsG28 in *Oryza sativa* var. *indica* cv. IR 50 (Saikia et al., 2006), of *Achromobacter xylosoxidans* SF2, *Bacillus pumilus* SF3 and *Bacillus pumilus* SF4 in *Helianthus annuus* cv. B59 and B71 (Forchetti et al., 2010), of *Serratia marcescens* NBR11213 in *Zea mays* cv. Arkil (Lavania and Nautiyal, 2013) and of *Pseudomonas vancouverensis* OB155 in *Solanum lycopersicum* (Subramanian

et al., 2014). These reports have established that SA is one of the essential molecules for determining rice yield. The current study identified a co-effect between the fertilizers and plant growth-promoting bacteria in influencing endogenous SA levels.

All measured phytohormones in the current study tended to increase with the increase in chemical fertilizers. In a study of the addition of NPK 20-20-20 at various doses performed by Mohamed (2018) with *Dypsis cabadae*, increasing doses of NPK 20-20-20 increased the levels of endogenous IAA and total GAs. A similar result was reported for the addition of NPK 19-19-19 by Ashour et al. (2023) with *Ervatamia coronaria*. There are no available reports on the effect of a variety of chemical fertilization regimens in enhancing endogenous SA. Thus, the current study has provided some insights into how fertilization affects endogenous SA levels in plants, especially rice. While many studies emphasized only a single type of chemical fertilizer applied in a plant, the current study demonstrated the impacts of different types and combinations of fertilizers and their amounts when applied at certain life states of plants encountered in real-life situations.

Roles of N, P, K levels in rice yield determination under co-application of fertilizer and plant growth-promoting bacteria

N, P and K are important elements for supporting plant life and vital for plant productivity, including rice (Shrestha et al., 2020). Chemical fertilizers containing N, P and K are still required by farmers for application to their rice crops to provide ready-to-absorb N, P and K for plants, because in nature, such elements are not readily available for plants, despite being abundant in the environment (Chandini et al., 2019; Sinha and Tandon, 2020). The elemental N in NPK fertilizer has the function of providing building blocks of vital nitrogenous biochemicals, such as amino acids, nucleotides and chlorophylls (Uchida, 2000). The elemental P in NPK fertilizer has a function in storing energy and facilitating its transfer (Wahid et al., 2019; Mussarat et al., 2021). The elemental K in NPK fertilizer serves as an enzyme activator and assists in the transport of assimilated results from the leaf to the plant tissue (Ragel et al., 2019; Bhardwaj et al., 2022). If properly used in rice in adequate amounts, NPK fertilization can lead to improvements in crop height, tiller number, panicle number, total grains per panicle and grain filling, thus increasing the potential yield of grain produced (Barita et al., 2018; Cahyono et al., 2019; Moe et al., 2019; Paiman et al., 2021).

N is an important element that promotes crop growth and improves grain yield and quality by increasing photosynthesis activity, metabolism and the development of both leaf area and root system, thus promoting the number of yield-associated components such as tillers, panicles and grain (Wang et al., 2022). In the current study, fertilizer and bacteria did not significantly affect the endogenous N content. This result was contrary to Duarah et al. (2011), who observed *Oryza sativa* and *Vigna unguiculata* treated with fertilizer and various bacteria inocula, such as *Staphylococcus epidermidis* TP06, *Erwinia tasmaniensis* TP08, *Pseudomonas aeruginosa* TP13, *Pseudomonas aeruginosa* TP14, *Pseudomonas aeruginosa* TP17, *Pseudomonas aeruginosa* TP373 and *Bacillus subtilis* TP27(7). According to Fitriatin et al. (2019), this was possibly caused by innate physiological factors of the rice plant in responding to both fertilizer and bacteria and to environmental factors, such as climate, soil and to biotic factors during planting that may affect N accumulation.

P is the second-most important element required by rice that is responsible for better plant vigor and a good root system, for promoting increased plant storage capacity to store assimilates and for promoting flower and grain production (Choudhury et al., 2007; Dunn and Stevens, 2008; Irfan et al., 2020; Bhatta et al., 2021). In the current study, fertilizer and bacteria were proven to affect the endogenous P content. This result was similar to the report by Fitriatin et al. (2021) in their observations of *Oryza sativa* treated with fertilizer and a consortium consisting of *Aspergillus* sp., *Penicillium* sp., *Pseudomonas mallei*, *Pseudomonas cepaceae*, *Azotobacter* sp. and *Azospirillum* sp. Notably, in the current study, *Bacillus* sp. N7 and *Lysinibacillus* sp. N5 (despite lacking P solubilization activity *in vitro*), showed enhanced P levels in their plant tissue possibly because some bacteria needed certain environmental conditions to exhibit activity that was not apparent *in vitro*. The review by Alori et al. (2017) noted that microbes required certain environmental conditions to exhibit P solubilization activity due to the soil P availability in soil greatly differing from one to another place and soil P behavior being different between acidic and alkaline soils, mainly due to the influence of soil organic matter, pH and exchangeable and soluble aluminum (Al), iron (Fe) and calcium (Ca). The phosphate ion (PO_4^{3-}) is soluble; however, in certain conditions, it can form a complex with other ions in the soil. In acidic soils, the formation of insoluble Al-PO_4 , Fe-PO_4 and Mn-PO_4 compounds limits the availability of P, while in alkaline soils, insoluble Ca-PO_4 and Mg-PO_4 compounds limit the availability of P (Gyaneshwar et al., 2002). A soil pH range of 6.0–7.0 was

considered the best pH to ensure the availability of P (Elliott et al., 2014). Relatively high background available P and earth alkali metals Ca and Mg of the soil were also considered to be other factors influencing P accumulation in plant tissue (El-Nagar et al., 2019; Johan et al., 2021; Weih et al., 2021).

K is important for plant N absorption and translocation of assimilates to the grain, thus making K important in the quality improvement of rice grain yield (Atapattu et al., 2018). K is biologically important as it plays a role in maintaining the homeostasis of cells, regulation of protein synthesis and enzyme activation in all living systems, including plants and bacteria (Kumar et al., 2020; Stautz et al., 2021). In the current study, fertilizer and bacteria were proven to affect the endogenous K content. A similar result was reported by Hindersah et al. (2023) in their observations regarding *Lactuca sativa* co-treated with fertilizer and a proprietary *Bacillus* inoculum. Notably, no K solubilization activity was exhibited *in vitro* in any of the bacteria used in the current study. Etesami et al. (2017) noted the ability of bacteria to solubilize K depended on: the bacteria's own innate K solubilization capability, the types of minerals that bound K, the presence of pre-existing soluble K, water, environmental pH, the presence of oxygen (O), the soil clay content and the presence of organic matter; thus, the performance of K solubilizing bacteria may differ between *in vitro* and real applications. This was supported by Sun et al. (2020) in their observations regarding *Leclercia* sp. GZ18 isolated from a *Mikania micrantha* rhizosphere. Compared to the high *in vitro* K solubilizers of *Burkholderia* sp. HZ18 and *Burkholderia* sp. SZ5 isolated from the same study, when applied to the *Mikania micrantha* plant, plants inoculated with *Leclercia* sp. GZ18 exhibited more K uptake than plants inoculated with *Burkholderia* sp. HZ18 or *Burkholderia* sp. SZ5. This result suggested that certain conditions are required by the plant-growth-promoting rhizobacteria (PGPR) used in this study to enable K solubilization.

Plant growth-promoting bacteria safety

Three bacilli virulence molecular markers—*hblA*, *nheC* and *cytK*—were targeted in the current study to investigate toxicity potential, where *hblA* is a gene encoding hemolysin A, a hemolytic toxin protein, *nheC* is a gene encoding the non-hemolytic enterotoxin C toxin protein and *cytK* is a gene encoding cytotoxin K, a necrotic cytotoxin (Böhm, 2017; Montone et al., 2020)(Reference). All these toxin genes are well-known as a result of being studied and characterized and have been used to identify the toxin potential of *Bacillus*

species (Lindbäck and Granum, 2015). Despite being mainly reported in widely recognized as 'not-safe' species of *Bacillus cereus*, several species of *Bacillus* have also been reported to have these genes (From et al., 2005; Yu et al., 2023). Owing to the diversity of genomic structure between *Bacillus* species and strains, the presence of one gene may exist in one species and be non-existent in another or even between strains within the same species (Rossi et al., 2018; de Magalhães, 2019; Zhang et al., 2022) with the possible presence of multiple toxin-encoding genes in a *Bacillus* strain (Jeżewska-Frąckowiak et al., 2017). Thus, multi toxin gene loci detection based on PCR has been chosen as an effective way to ensure the toxicity potential of a *Bacillus* strain, and so one cannot simply rely on a single gene (Hwang and Park, 2015; Jeżewska-Frąckowiak et al., 2017; Bianco et al., 2023). In the current study, no amplicons of *nheC* and *cytK* were detected in any of the PGPR bacteria used in this study. No amplicon of *hblA* was detected in the tested bacterial strains or the positive control. This result was similar to the results reported by López and Alippi (2010), who did not detect an amplicon of *hblA* in *Bacillus cereus* ATCC 1778 that was used in their study as a control; however, the *hblA* amplicon possessed by other *Bacillus cereus* and *Bacillus megaterium* strains were discovered in their study.

Importance of fertilization in plant growth-promoting bacteria affecting the grain yield improvement and relation to the bacteria phytohormones production capability

Bacillus sp. N7, *Lysinibacillus* sp. N5, *B. safensis* TISTR2163 and *B. thuringiensis* B20 increased the yield by different amounts under given fertilization regimens. In the fertilization regimens with lower vegetative fertilizer amounts (2-2 and 2-4), *Bacillus* sp. N7, *Lysinibacillus* sp. N5 and *B. thuringiensis* B20 produced yield increases compared to the uninoculated control. However, *B. safensis* TISTR2163 only produced a yield increase compared to the uninoculated control under the 2-2 fertilizer regimen and not with the in 2-4 fertilizer regimens. In this study, the best result was achieved with inoculation of *Lysinibacillus* sp. N5 and *Bacillus* sp. N7. Rice plants inoculated with *Lysinibacillus* sp. N5 yielded 28.43 ± 4.58 g/pot under the 2-2 and 32.77 ± 5.85 g/pot under the 2-4 fertilizer regimens, whereas rice plants inoculated with *Bacillus* sp. N7 yielded 24.83 ± 3.85 g/pot under the 2-2 and 28.52 ± 3.38 g/pot under the 2-4 fertilizer regimens.

This study reported for the first time the IAA-production, GAs-production, and SA-production capabilities of a bacterial strain. *Bacillus* sp. N7, *Lysinibacillus* sp. N5 and *Bacillus*

safensis TISTR2163 were IAA-producing, GAs-producing and SA-producing bacteria. One strain, *Bacillus thuringiensis* B20, was an IAA-producing and SA-producing bacterium. In the literature, much rice grain yield improvement has been reported by applying certain bacterial strains but with the emphasis only one phytohormone production. For IAA-producing bacteria, Nghia et al. (2017) reported that the IAA-producing *Bacillus megaterium* ST2-1 improved the grain yield in *Oryza sativa* cv. RD6 via improved of overall growth, biomass accumulation and root growth. Purwanto and Suharti (2021) reported that the IAA-producing *Rhizobium* sp. LM-5 and *Rhizobium* sp. R11 increased the grain yield in *Oryza sativa* cv. Inpago Unsoed 1 via improved root growth, nutrient uptake, chlorophyll content and biomass accumulation. Narwal et al. (2022) reported the IAA-producing *Pseudomonas protegens* Ekn03 improved grain yield in *Oryza sativa* cv. PB1509 via improved plant height, biomass accumulation, total chlorophyll content and acetylene reduction activity both in soil and *in planta*. Bacteria with known sole GA-producing or SA-producing ability in rice have been studied only quite rarely. *Leifsonia soli* SE134, a GA-producing bacteria, is known to improve the rice growth of *Oryza sativa* cv. Waito-C by improving the shoot growth, biomass accumulation and chlorophyll content (Kang et al., 2014); however, as it was only observed in the vegetative phase, the grain yield improvement capability of *Leifsonia soli* SE134 is currently unknown. Ali and Elmaghraby (2020) reported *Pseudomonas fluorescens* PsF and *Azotobacter chroococcum* Azoto improved the growth and grain yield by improving plant height, biomass accumulation, water content, chlorophyll content and macronutrient (N, P, and K) accumulation, as well reducing sodium (Na) absorption.

Yanni et al. (2001) reported the *Rhizobium leguminosarum* bv. *trifolii* E11, an IAA-producing and GA-producing bacterium, increased the grain yield in laboratory and field scale experiments by improving biomass accumulation and increasing N efficiency. This bacterium also exhibited consistent grain yield improvement in five Egyptian rice cultivars of *Oryza sativa* cv. Giza 175, Giza 177, Giza 181, Sakha 101 and Sakha 102. Currently, no information has been published in the literature on rice regarding bacteria with dual IAA-producing and SA-producing capability and dual GA-producing and SA-producing capability.

Considering the reduction of fertilizer while maintaining plant growth and grain yield improvement, the current study's results were similar to other reported results. Lai et al. (2019) reported that the application of *Bacillus subtilis* inoculum reduced chemical fertilizer input to 25% and resulted in tiller

and grain yield comparable to the full (100%) chemical fertilizer input without bacterial inoculum. Another study by Kobua et al. (2021) reported that the partial replacement of chemical fertilizer with a bacterial consortium, consisting of *Bacillus aryabhattai*, *Burkholderia ambifaria*, *Stenotrophomonas maltophilia*, *Sphingomonas* sp., *Burkholderia caribensis*, *Sphingobium yanoikuyae* and *Paenibacillus glucanolyticus*, positively reduced by 25–50% the chemical fertilizer input, while improving overall rice growth, grain yield and grain yield components. The current study confirmed the inoculation of rice using bacteria, especially *Lysinibacillus* sp. N5 and *Bacillus* sp. N7, to enhance the grain yield despite inputting less fertilizers, implying that adding bacteria could reduce the use of fertilizer.

In this study, the co-effect of fertilization and plant growth-promoting bacteria inoculation increased the levels of IAA and SA in the vegetative and generative phases compared to the control (without plant growth-promoting bacteria inoculation) for Pathum Thani 1 rice. The results implied that IAA and SA were involved in enhancing grain yield. However, the observable co-effect of fertilization and plant growth-promoting bacterial inoculation to GA levels was observed in the vegetative phase, but not in the generative phase. P and K were significantly affected by the co-effect of fertilization and plant growth-promoting bacterial inoculation, whereas N was not, implying that the available N in the soil was sufficient for the plant to grow under any of the tested fertilizer conditions. Therefore, adding bacteria did not affect N but affected P and K. This study confirmed that the combined effects of bacteria and fertilizers resulted in changes in the phytohormone, P and K levels of the applied plants. For future work, transcriptomic, proteomic and metabolomic studies should be considered to provide hormonomics information regarding the regulation using fertilization and plant growth-promoting bacteria to influence molecular mechanisms related to grain yield *in planta*.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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