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Bacillus Pumilus Strain TUAT-1 and Nitrogen Application in Nursery Phase Promote Growth of Rice Plants under Field Conditions

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Abstract: The aims of this study were to boost growth attributes, yield, and nutrient uptake of rice in paddy fields using a combination of *Bacillus pumilus* strain TUAT-1 biofertilizer and different nitrogen (N) application rates in nursery boxes. *Bacillus pumilus* strain TUAT-1 was applied as an inoculant biofertilizer in conjunction with different rates of N fertilizer to rice seedlings in a nursery. Plant growth and yield parameters were evaluated at two stages: in 21-day-old nursery seedlings and in mature rice plants growing in a paddy field. Inoculation with TUAT-1 significantly increased the seedling growth and root morphology of 21-day-old nursery seedlings. There was a marked increase in chlorophyll content, plant height, number of tillers, and tiller biomass of rice plants with the use of TUAT-1 and N fertilizers alone, and their combinations, at the maximum tillering stage in the field. The combination of TUAT-1 and 100% N (farmer recommended rate of N) resulted in the greatest tiller number and biomass at the maximum tillering stage, and positively affected other growth attributes and yield. The growth and yield were similar in the TUAT-1 + 50% N and 100% N (uninoculated) treatments, because TUAT-1 promoted root development, which increased nutrient uptake from the soil. These results suggest that the *B. pumilus* strain TUAT-1 has a potential to enhance the nutritional uptake of rice by promoting the growth and development of roots.

Keywords: biofertilizer; *Bacillus pumilus*; growth promotion; N fertilizer; rice; yield

1. Introduction

Rice (*Oryza sativa* L.) is considered as one of the world's most important staple foods and is the key to food security, especially under the threats of climate change in the coming decades [1]. The global rice cultivation area in 2015/2016 approached 158.8 million hectares, and total global rice production amounted to 711.24 million metric tons [2]. Nitrogen (N) fertilizers are used extensively in rice cultivation to meet the growth demands of the crop. However, excessive use of chemical fertilizers, in recent decades, has led to soil toxicity by contamination with toxic heavy metals, which adversely affect the health of rice plants [3]. Inoculating rice plants with plant growth promoting rhizobacteria (PGPR) can significantly enhance rice production, thus reducing the need for N fertilizers and contributing to sustainable rice production and reduced environmental problems [4]. Therefore, the use of biological fertilizers for reducing chemical fertilizers is one of the most effective steps towards sustainable agriculture [5].

Several root-colonizing *Bacillus* species have been shown to enhance plant growth [6]. It is likely that the growth-promoting effects of various PGPR are due to bacterial production of plant growth regulators, such as auxins, gibberellins, and cytokinins [7,8]. *Bacillus pumilus* strain TUAT-1 (hereafter referred to as TUAT-1) has been shown to increase shoot and root growth in rice, mustard, radish, and komatsuna [9,10], mainly due to its effects to promote nutrient uptake by plant roots. Inoculation of plants with TUAT-1 biofertilizer at sowing and transplanting resulted in significant changes in plant biomass, nutrient uptake, tissue N content, tiller number, root length, and number of roots in the forage rice "leaf star" [10]. Despite the large body of experimental evidence on the growth-promoting effects of TUAT-1, our knowledge of the conditions required for a consistent positive interaction between the bacteria and the plant (i.e., increased grain yield), in field conditions, is limited. Generally, bacterial inoculation improves plant growth and rice yield, but not uniformly. The yield response to inoculation is more pronounced in the presence of moderate levels of N fertilizer [11]. Fertilization management can also affect the community structures of plant-associated bacteria [12]. There is an ongoing debate regarding the impacts of fertilization strategies on the effects of PGPR [13].

Several studies have focused on interactions between PGPR and N fertilizers. However, the optimization of inoculant biofertilizer in conjunction with chemical N fertilizer for rice from the nursery tray to field stage has not been reported. We hypothesized that the combination of TUAT-1 biofertilizer and nitrogen fertilizer (N) applied to rice seedlings in nursery trays will promote plant growth and ensure a consistent positive interaction between the bacterium and the plant in terms of grain yield under field conditions. The specific objectives of this study were as follows: (1) to determine the effect of nursery application of TUAT-1 biofertilizer and different N fertilizer rates on growth and root morphology of rice seedlings; and (2) to evaluate the effect these treatments on the growth, yield, and N content of the mature rice plants after transplanting into field conditions.

2. Materials and Methods

The TUAT-1 and N fertilizer treatments were applied to rice seedlings growing in nursery trays in a greenhouse. The treated rice plants were transplanted into a paddy field and grown to maturity.

2.1. Soil Preparation and Chemical Analysis

The experimental field site was the Fuchu Honmachi paddy field (35°41′ N, 139°29′ E, 59 MSL) of Tokyo University of Agriculture and Technology, Tokyo, Japan. Soil samples were collected from four points in each plot at a depth of 0–15 cm, before the seedlings were transplanted. The samples were air dried, ground, and passed through a 2-mm sieve. The soil physicochemical properties were measured using conventional methods [14,15], and are summarized in Table 1.

Table 1. Physiochemical properties of paddy soil (0–15 cm) in experimental plots.

Soil Physiochemical Property	Value
pH (H_2O)	6.25
Total carbon content (%)	5.25
Total nitrogen content (%)	0.33
Cation-exchange capacity (cmolc/kg)	20.55
Sand (%)	40.12
Silt (%)	32.27
Clay (%)	27.61

2.2. Nursery Preparation

One of the main cultivars of paddy rice (*Oryza sativa* L.) in Japan, Koshihikari, was used in this study. Seeds (100 g nursery tray^{-1}) were surface-sterilized by immersion in 1% sodium hypochlorite solution for 2–3 min, and in 80% ethanol for 3–4 min, before being washed thoroughly with distilled water. The seeds were then allowed to imbibe in tap water for 72 h, and incubated for 12 h to hasten germination. Pre-germinated seeds were uniformly broadcast in each nursery tray (30 cm × 60 cm) on 22 April 2015. Each nursery tray contained 3.2 kg commercial rice nursery soil (Shinano Soil, Shinano Baiyoudo Co., Ltd., Nagano, Japan). Before sowing the pre-germinated seeds, biofertilizer with or without TUAT-1 was mixed thoroughly into the soil at a rate of 5 g granular biofertilizer per 100 g soil (160 g in 3.2 kg soil). The density of TUAT-1 *Bacillus* cells in biofertilizer was approximately 1.2×10^7 colony forming units (cfu) g^{-1}. The experiment included eight different treatments combining N fertilizer at various application rates with or without TUAT1; (1) 0% N, (2) TUAT-1 + 0% N, (3) 50% N, (4) TUAT-1 + 50% N, (5) 100% N, (6) TUAT-1 + 100% N, (7) 150% N, and (8) TUAT-1 + 150% N. The N fertilizer was solid form of ammonium sulfate, $(NH_4)_2SO_4$. The 100% N rate consisted of 2.64 g $(NH_4)_2SO_4$ per nursery tray, which is the application rate recommended for rice farmers in Japan.

The TUAT-1 isolate was grown as a liquid culture in trypticase soy broth (TSB) (Becton Dickinson, Sparks, MD, USA). When the culture density reached 10^7 cfu mL^{-1}, overnight cultures were then centrifuged at 10,000 rpm for 10 min at 6 °C, and were then washed twice in sterile Milli-Q water and diluted to an optical density of 0.4 at 600 nm, corresponding to approximately 10^7 cells mL^{-1}. Five hundred milliliters were applied to each nursery seedling, once per week for 3 successive weeks. The treatments were arranged in a completely randomized design (CRD) and were replicated three times. At 21 days after sowing (DAS), 30 seedlings were randomly selected from three positions in each nursery tray, and the following data were recorded: chlorophyll content (*SPAD* value), shoot length, shoot biomass, total root length, root number, root surface area, root biomass, and total biomass.

Upon harvest, root systems were removed gently from the nursery tray and stored in 70% ethanol until root parameters were measured. At the time of measurement, the roots were washed gently with deionized water and the root surface area and total root length were measured with an image analyzer (Win-Rhizo REG V 2004 b; Regent Inc., Quebec, Canada).

2.3. Transplanting of Rice Plants into the Paddy Field

The field experiment was conducted at the experimental paddy field of the Tokyo University of Agriculture and Technology in Fuchu Honmachi, Tokyo, Japan. The experiment was conducted in a split-plot design with four replications. The N, phosphorus, and potassium fertilizers were applied as basal dressings at the rate of 80 ($(NH_4)_2SO_4$), 100 (P_2O_5), and 30 (K_2O) kg ha^{-1}, respectively. The roots of 21-day-old seedlings were reinoculated in a bacterial suspension (10^7 cfu mL^{-1}) or tap water (control) for 1 night before transplanting. The main plot treatment factor was presence/absence of TUAT-1, and the subplot treatment factor was N fertilization rate (0%, 50%, 100%, and 150% N). Each plot was about 52 m^2 (7.2 m × 7.2 m).

The 21-day-old seedlings were manually transplanted on 13 May 2015 into the paddy field at a planting density of 22.2 hills m^2. Each hill contained three seedlings with 30 cm row spacing and 15 cm intra-row spacing. The experiment was conducted in irrigated paddy conditions. At 45 days after transplanting (DAT) in the field, chlorophyll content (*SPAD* value), plant height, tiller number, and tiller biomass were measured at the maximum tillering stage.

Upon harvest, 120 DAT, the following data were recorded: number of panicles, panicle length, panicle weight, straw yield, aboveground biomass, and grain yield. Straw yield and grain yield were recorded from 44 hills (2 m^2) of each plot, which is reasonable for sampling in some area of Japan [16,17]. Grain yield was adjusted to 14% moisture content while straw yield was recorded on an oven-dry basis (80 °C). Nitrogen contents in the grain and straw samples were estimated colorimetrically after H_2SO_4–H_2O_2 wet digestion, as described by Mizuno and Minami [18].

2.4. Data Analysis

Analysis of variance was performed for all measurements with the CROP-STAT version 7.2 software (International Rice Research Institute, IRRI, Philippines). The statistical model included replication, TUAT-1, N rate, and the interaction between TUAT-1 treatment and N rate. The results were subjected to a two-way analysis of variance, and mean values were then compared by 5% level by Fisher (LSD) test ($p < 0.05$) when the F probability value was significant, using XLSTAT Version 2017 (Addinsoft, Paris, France). Data were reported as means ± the standard deviation (SD).

3. Results

3.1. Seedling Growth at Nursery Stage

Root morphology was significantly changed with TUAT-1 inoculation in each N treatment when compared with its uninoculated treatment (Table 2). Rice seedlings receiving TUAT-1 + 150% N showed the greatest root surface area and total length than that of these root parameters of uninoculated plants and other inoculated plants receiving 100% N, 50% N, and 0% N (Table 2). Treatments of TUAT-1 + 150% N and TUAT-1 + 100% N were on par with each other with respect to root surface area and it did not reach significant level between these two treatments. Root parameters increased by either of inoculation or N fertilizer application alone. The TUAT-1 inoculation and N fertilizer interactions were significant with respect to total root length.

Table 2. Effects of nitrogen (N) levels and TUAT-1 on chlorophyll content (*SPAD* value), shoot length plant-1 (SL), shoot biomass plant-1 (SB), root biomass plant-1 (RB), Total root length plant-1 (TRL) and root surface area plant-1 (RSA) (g) of 21 days old seedlings. Means in columns followed by the different letters are significantly different according to Least Significant difference (LSD) test ($p < 0.05$). p values indicate that the differences are statistically significant (2-way ANOVA, $p < 0.05$), ns = non-significant.

Treatments	*SPAD*	SL (cm)	SB (g)	RB (g)	TRL (cm)	RSA (cm^2)
0% N	18.6 ^{c}	12.1 ^{cd}	16.0 ^{b}	15.3 ^{d}	12.7 ^{f}	240.3 ^{d}
0% N + TUAT-1	19.6 ^{c}	13.6 ^{c}	21.6 ^{b}	20.6 ^{c}	19.7 ^{de}	335.7 ^{c}
50% N	18.1 ^{bc}	15.2 ^{ab}	27.0 ^{a}	18.3 ^{c}	17.1 ^{ef}	268.7 ^{cd}
50% N + TUAT-1	24.8 ^{cd}	15.0 ^{bc}	29.1 ^{a}	25.0 ^{b}	29.2 ^{bc}	466.9 ^{b}
100% N	21.4 ^{bc}	15.0 ^{ab}	25.3 ^{a}	19.6 ^{c}	18.7 ^{de}	315.7 ^{c}
100% N + TUAT-1	26.8 ^{bc}	15.2 ^{ab}	25.9 ^{a}	25.9 ^{b}	31.9 ^{b}	520.9 ^{ab}
150% N	25.4 ^{ab}	16.3 ^{a}	26.9 ^{a}	23.8 ^{b}	26.6 ^{cd}	405.3 ^{b}
150% N + TUAT-1	32.1 ^{a}	16.2 ^{a}	28.1 ^{a}	29.2 ^{a}	39.2 ^{a}	682.0 ^{a}
Analysis of variance			*p* value			
Nitrogen (N)	0.001	0.0001	0.0001	0.0001	0.0001	0.0001
TUAT-1 (T)	0.006	0.957	0.127	0.0001	0.0001	0.0001
N × T	0.439	0.512	0.387	0.76	0.074	0.127

TUAT-1 inoculation significantly enhanced seedling growth. Rice nursery seedlings receiving TUAT-1 + 150% N showed the highest chlorophyll content, shoot length, and shoot biomass (Table 2). The lowest growth parameters were recorded in uninoculated plants receiving 0% N. Chlorophyll content was not significantly different among the treatments with and without TUAT-1 inoculation (Figure 1A). At each N treatment, TUAT-1 inoculation showed a significant greater root biomass, as compared to those of their respective uninoculated plants. TUAT-1 inoculation did not enhance the shoot length and shoot biomass significantly over its respective uninoculated plants.

3.2. Growth at Maximum Tillering Stage

The effect of TUAT-1 on growth at maximum tillering stage was significant at an F probability level $p < 0.002$. Increase of growth parameters measured at maximum tillering stage were also significant, due to N fertilization, with an F probability level of $p < 0.004$. The interaction effect between TUAT-1 and N fertilizer was also significant for chlorophyll content, plant height, and tiller biomass (Table 3).

Table 3. Effects of N levels and TUAT-1 on chlorophyll content (*SPAD* value), plant height, tiller number (TN), and tiller biomass plant^{-1} (TB) at 45 days after transplanting (DAT). The significance (*) derived from two ways analysis of p value *, **, and ***, significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. Means in columns, followed by the different letters, are significantly different according to LSD test ($p < 0.05$). p values indicate that the differences are statistically significant (2-way ANOVA, $p < 0.05$), ns = non-significant.

Treatments	*SPAD*	Plant Height (cm)	TN	TB (g)
0% N	39.6 ^{d}	82.2 ^{c}	8.7 ^{c}	9.5 ^{d}
0% N + TUAT-1	40.2 ^{d}	86.7 ^{ab}	13.6 ^{ab}	17.0 ^{c}
50% N	40.0 ^{d}	82.8 ^{c}	11.2 ^{bc}	10.7 ^{d}
50% N + TUAT-1	41.4 ^{c}	86.8 ^{ab}	13.4 ^{ab}	18.4 ^{bc}
100% N	40.5 ^{c}	84.4 ^{bc}	12.5 ^{ab}	16.2 ^{bc}
100% N + TUAT-1	41.7 ^{b}	88.4 ^{a}	15.7 ^{a}	20.9 ^{a}
150% N	42.1 ^{a}	87.6 ^{a}	13.7 ^{ab}	18.6 ^{ab}
150% N + TUAT-1	42.3 ^{ab}	89.1 ^{a}	16.4 ^{a}	21.8 ^{a}
Analysis of variance		p value		
Nitrogen (N)	0.002	0.0001	0.004	0.0001
TUAT-1 (T)	0.001	0.001	0.002	0.0001
N × T	0.232	0.023	0.536	0.029

Under the field condition, chlorophyll content at maximum tillering stage was significant difference between the treatments with and without TUAT-1 inoculation at 50% N and 100% N. TUAT-1 inoculation also promoted plant height at 0%, 50%, and 100% N (Table 3). Besides plant height, tiller number and tiller biomass are also important parameters in terms of the vegetative growth of the plants, and these were also found to be increased in response to nursery application of TUAT-1 inoculation. The best combination of nursery seedling treatment with TUAT-1 and different N rates on rice tiller number was observed in TUAT-1 + 100% N. The combined application of TUAT-1 + 100% N also gave the best effect on dry matter accumulation (tiller biomass) by plants at maximum tillering stage followed by TUAT-1 + 50% N. The nursery application of TUAT-1 + 150% N did not further enhance the above plant growth parameters significantly over application of TUAT-1 + 100% N.

3.3. Yield and Yield Component Parameters

The yield and yield components showed similar trends to those of other growth parameters at the maximum tillering stage. Application of N to nursery seedlings, alone or in combination with TUAT-1, led to significant increases in the number of panicles, panicle length, panicle weight, straw yield, aboveground biomass, and grain yield, compared with uninoculated plants with 0% N (Figure 1). The values of yield and yield components were significantly higher in TUAT-1-inoculated plants than in uninoculated plants, with F probability levels range of p-0.002 to p-0.048. N fertilization also increased yield and yield components with F probability values of $p < 0.001$. Interaction between TUAT-1 and N levels was not observed for yield and yield component characters.

Nursery treatments of TUAT-1 + 100% N gave the best values on panicle length, number, and weight (Figure 1A–C). No further significant increase in the above parameters was found in the treatment of TUAT-1 + 150% N. In the 0% N treatments, panicle length and number were significantly greater in TUAT-1-inoculated plants than in uninoculated plants (Figure 1A,B). In addition, TUAT-1 led to significant increases in the panicle weight compared with uninoculated plants with 50% N treatment (Figure 1C).

The maximum estimated straw yield was in the TUAT-1 + 150% N treatment (11.16 ton ha^{-1}), followed by the 150% N treatment (9.72 ton ha^{-1}), the TUAT-1 + 100% N treatment (9.69 ton ha^{-1}), and then the TUAT-1 + 50% N treatment (9.18 ton ha^{-1}) (Figure 1D). In the 50% and 150% N treatments, the estimated straw yield was significantly higher in TUAT-1-inoculated plants than in the uninoculated plants. The aboveground biomass was also significantly higher in TUAT-1-inoculated plants with the 50% N treatment (Figure 1E).

The highest estimated grain yield was in the TUAT-1 + 100% N treatment (4.89 ton ha^{-1}) and the TUAT-1 + 150% N treatment (5.05 ton ha^{-1}) (Figure 1F). The grain yield of plants in the TUAT-1 + 50% N treatment (4.4 ton ha^{-1}) was statistically insignificant to that of plants in the 100% N treatment (4.6 ton ha^{-1}) or TUAT-1 + 100 % N.

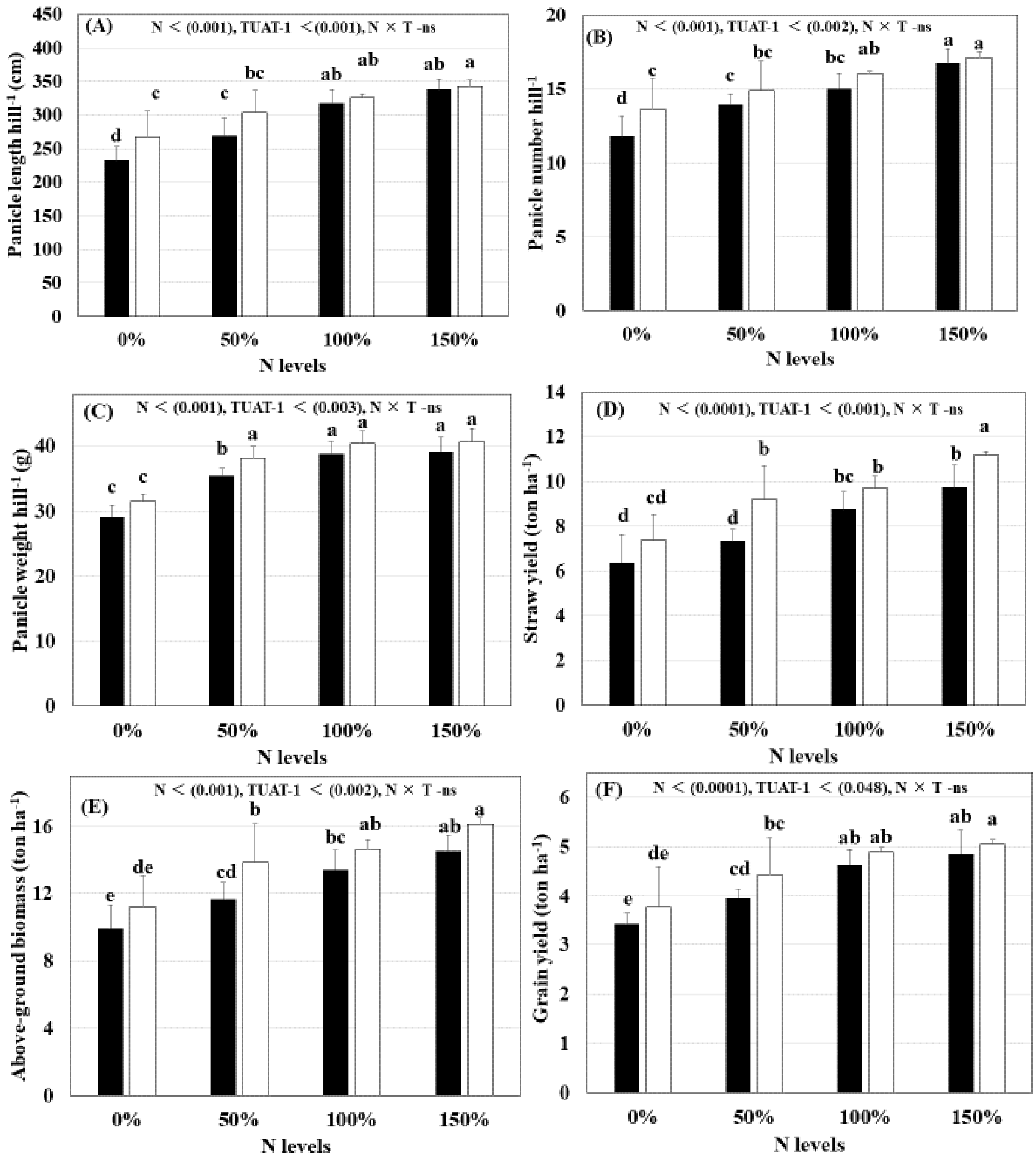

Figure 1. Effect of TUAT-1 biofertilizer and nitrogen fertilizer treatments at the seedling stage on yield and yield components of mature rice plants. (**A**) Panicle length $hill^{-1}$, (**B**) panicle number $hill^{-1}$, (**C**) panicle weight $hill^{-1}$, (**D**) straw yield, (**E**) aboveground biomass, and (**F**) grain yield. Black and white bars represent uninoculated and inoculated with TUAT-1. Error bars indicate standard deviation. Different letters indicate significant difference at 5% level by Fisher (LSD) test ($p < 0.05$). p values are shown in brackets. p values indicate that the differences are statistically significant (2-way ANOVA, $p < 0.05$), ns = non-significant.

3.4. Nitrogen Content (%) in Grain and Straw

The patterns of N content in rice grain and straw were affected by TUAT-1 inoculation and N application levels in the nursery (Figure 2A,B). In the 50% N treatment, grain N content was significantly higher in TUAT-1-inoculated plants than in uninoculated plants. However, TUAT-1 inoculation did not significantly increase grain N content in the other treatments. Nitrogen fertilization alone did not significantly promote N content in rice grain. TUAT-1 significantly increased the N content in straw in the 0% N treatments (Figure 2B).

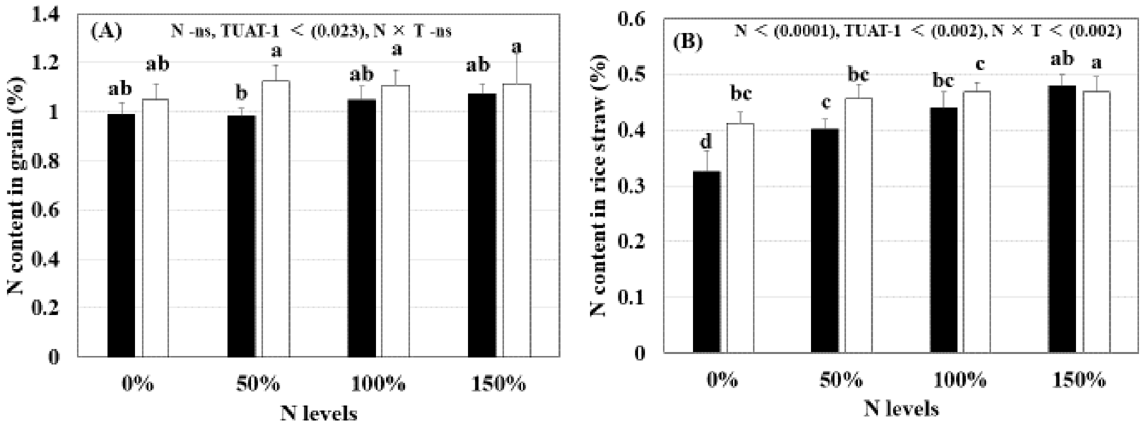

Figure 2. Effect of TUAT-1 biofertilizer and nitrogen fertilizer treatments at the seedling stage on N content (%) (**A**) in grain and (**B**) in straw of mature rice plants. Black and white bars represent uninoculated and inoculated with TUAT-1, respectively. Error bars indicate standard deviation. Different letters indicate significant difference at 5% level by Fisher (LSD) test ($p < 0.05$). p values are shown in brackets. p values indicate that the differences are statistically significant (2-way ANOVA, $p < 0.05$), ns = non-significant.

4. Discussion

Seedling growth is the most important growth stage of any crop, as it determines the amount of biomass generated. In rice, it is also important for tiller development [19]. In our study, nursery seedling growth was increased by TUAT-1 inoculation (Table 2). The values of root parameters (total root length and root surface area) were significantly higher for TUAT-1-inoculated plants than for uninoculated plants. A larger and stronger root system can increase seedling vigor, plant growth, and micronutrient status [20]. An increase in rice root biomass in response to PGPR inoculation was also reported by Souza et al. [4]. The root system plays an important role in plant productivity because roots take up essential nutrients from the soil [21]. Various PGPR have been shown to enhance root hair proliferation and deformation, increase root branching, promote seedling emergence, and increase leaf surface area, vigor, biomass, endogenous plant hormone levels, and uptake of minerals and water [22,23].

After 21 days in the nursery, in each N treatment, rice seedlings inoculated with TUAT-1 produced approximately 17% greater total biomass than did uninoculated seedlings (Supplementary Figure S1). Like other PGPR, TUAT-1 produces growth-promoting and growth-regulating substances to support root growth, allowing inoculated rice plants to absorb more nutrients to enhance their growth. A wide range of nutrients and signaling molecules are exuded from roots, directing plant–microbe interactions [24]. The associated microbes may affect root morphogenesis [25], and many studies suggest that the acceleration of plant growth by PGPR involves phytohormone modulation. Numerous studies have reported on Plant growth-promoting bacteria (PGBs), particularly on *Bacillus* spp. exerting a number of characteristics enabling mobilization of soil nutrients and synthesis of phytohormones, leading to plant growth promotion [26–28]. Further research is required to determine the exact mechanism by which TUAT-1 promotes rice growth.

The combination of TUAT-1 and different rates of N application in the nursery also have a significant effect on chlorophyll content, plant height, and tiller biomass at 45 DAT (Figure 2A). Promoting crop growth in the early stage is important for early and rapid tiller production. Herein, we suggested that TUAT-1 inoculation had the higher tiller biomass production as a result of vigorous early growth and early leaf expansion at tillering stage. Similar responses to fertilizer addition at the nursery stage were reported by Ros et al. [29] and Panda et al. [30]. Increased tiller number and tiller biomass have been reported for rice plants treated with PGPR [31].

Furthermore, in the other section of this study, the results of the present study also showed that the seedling growth parameters of root, such as total root surface area, total root length, and root biomass, increased significantly due to TUAT-1 inoculation, which can improve uptake of water and nutrition from soil. Wang et al. [32] also recently reported vigorous root growth and long and dense root hairs ensured efficient acquisition of macro- and micronutrients during early growth, and a high root length to shoot dry matter ratio favored high macronutrient concentrations in the shoots, which is assumed to be important for later plant development.

Herein, inoculating rice with TUAT-1 was also reported to improve the water and nutrition absorption capacity of the root system, and promote the absorption of N from soil during the early growth period [10]. Matsumura et al. [33] suggested that increased N absorption may promote tillering, increase the number of ears, and maintain photosynthetic activity during the growth period of the plant, so that crop yield is boosted. The increase in tiller number and tiller biomass by TUAT-1 may be due to the effects of *Bacillus* species to improve tolerance of plants to the drought and salinity stresses [34,35] and their water and nutrient uptake ability [10]. Furthermore, compared with untreated seedlings, the seedlings treated with TUAT-1 and N fertilizer with vigorous dense root systems and strong stems may have coped better with transplanting shock by rapidly developing new roots. It is likely that the vigorous dense root systems and strong stems contributed to the higher tiller production (biomass) in TUAT-1-inoculated plants than in uninoculated plants.

Nursery application of TUAT-1 with N fertilizer led to higher tiller number at the maximum tillering stage, which resulted in increased panicle number, panicle length, panicle weight, and yield (Figure 1). The higher panicle number resulted from increased number of effective tillers. Hence, increased panicle number resulted in increased panicle weight, leading to increased grain number per panicle and ultimately increased yield. The beneficial effects of PGPR on rice yield have been observed in both greenhouse and field conditions [36,37]. In our study, the combination of TUAT-1 and N fertilizer applied to rice plants at the nursery stage clearly increased the values of most growth parameters.

The greatest yield was shown in the TUAT-1 + 150% N and the TUAT-1 + 100% N treatments. The grain yield of plants in the TUAT-1 + 50% N treatment (4.4 ton ha^{-1}) was statistically insignificant to that of uninoculated plants in the 100% N treatment (4.6 ton ha^{-1}); that is, inoculation with TUAT-1 has a potential of reducing fertilizer use at the seedling stage by 50% without negatively affecting yield. This result is consistent with earlier studies by Okon and Labandera-Gonzalez [38] and Dobbelaere et al. [39], and beneficial effects of PGPB on crop yield were mainly observed under intermediate levels of fertilizer, rather than maximum or minimum fertilization. The similar rice grain yield in TUAT-1 + 50% N and 100% N may be ascribed to the significantly increased root to shoot growth at the seedling stage (Table 2), which can improve dry matter accumulation (tiller biomass) at maximum tillering stage (Table 3) by supplying a sufficient amount of nutrients, water, and phytohormones to shoots and, subsequently, ensure an increase in rice productivity [10]. We suggested that the nursery applications of N fertilizer at appropriate levels with TUAT-1 biofertilizer in this field study may have masked our ability to observe significant growth impacts on rice grain yields. Previous investigations suggested that strategies combining both reduced rates of agriculture fertilizers and biofertilizers can benefit plant development and nutrient uptake [40,41].

Hence, on the basis of our results, we suggested that the inoculation of TUAT-1 resulted in improving the growth of the rice plants and grain yield, and its inoculation may be applied to spare the use of chemical N fertilization. However, biofertilizer performance may be specific to each situation, as its effectiveness depends on factors like plant species, soil type, and environmental conditions [42]. Since the results reported here for enhancing of growth and yield of rice in the field experiment, as influenced by the application of a combination of TUAT-1 and N fertilizer at the nursery stage, was conducted only for a one year experiment, we suggest that further studies should evaluate the performance of TUAT-1 in different field conditions/locations with different rice cultivars and environmental field conditions of specific issues for a given year. Such studies will determine whether TUAT-1 can substantially decrease the use of chemical N fertilizers in rice cultivation, which is an important economic and environmental goal.

The increases in N contents in grain and straw as a result of N fertilization were consistent with the results of a previous study [43]. Growth promotion by inoculation with the TUAT-1 was accompanied by increased N levels in all plant tissues tested in forage rice [10]. TUAT-1 inoculation significantly promoted straw N content (%) at 0% N and grain N content (%) at 50% N than those of their respective uninoculated plants (Figure 2). It has been suggested that PGPR improves mineral nutrition, especially under low-N input conditions [19]. Hence, it is also postulated that growth promotion effects of TUAT-1 with a moderate amount of N application yielded the result, at least partly, of an enhanced N uptake efficiency. In the other studies, the enhancement of N uptake by plants inoculated with the PGPR strains might be through associative N fixation and phosphorus solubilization [44]. However, such evidence is lacking for TUAT-1. These observations could indicate that growth promotion mechanisms other than nitrogen fixation, such as phytohormone production, improved nutrient uptake balance. In addition, the higher nutrient uptake might be attributed to morphological changes in rice roots, such as increased root number, length, and thickness [45].

5. Conclusions

The increase in grain yield resulting from application of TUAT-1 combined with N fertilizer to rice seedlings at the nursery stage could be related to the increased size of the root system at the early growth stage, which increased nutrient uptake to promote tiller growth (biomass), and yield. As observed the straw and yield were similar in the TUAT-1 + 50% N and 100% N (un-inoculated) treatments, we conclude that TUAT-1 biofertilizer should be used with N fertilizer at appropriate levels to maximize benefits in terms of saving fertilizer and improving yield.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/8/10/216/s1, Figure S1: Effect of TUAT-1 biofertilizer and nitrogen fertilizer on total biomass plant^{-1} at nursery stage.

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