



# Effects of Biofertilizer Produced from *Bradyrhizobium* and *Streptomyces griseoflavus* on Plant Growth, Nodulation, Nitrogen Fixation, Nutrient Uptake, and Seed Yield of Mung Bean, Cowpea, and Soybean

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**Abstract:** The use of biofertilizers is important for sustainable agriculture, and the use of nodule bacteria and endophytic actinomycetes is an attractive way to enhance plant growth and yield. This study tested the effects of a biofertilizer produced from *Bradyrhizobium* strains and *Streptomyces griseoflavus* on leguminous, cereal, and vegetable crops. Nitrogen fixation was measured using the acetylene reduction assay. Under N-limited or N-supplemented conditions, the biofertilizer significantly promoted the shoot and root growth of mung bean, cowpea, and soybean compared with the control. Therefore, the biofertilizer used in this study was effective in mung bean, cowpea, and soybean regardless of N application. In this study, significant increments in plant growth, nodulation, nitrogen fixation, nitrogen, phosphorus, and potassium (NPK) uptake, and seed yield were found in mung beans and soybeans. Therefore, *Bradyrhizobium japonicum* SAY3-7 plus *Bradyrhizobium elkanii* BLY3-8 and *Streptomyces griseoflavus* are effective bacteria that can be used together as biofertilizer for the production of economically important leguminous crops, especially soybean and mung bean. The biofertilizer produced from *Bradyrhizobium* and *S. griseoflavus* P4 will be useful for both soybean and mung bean production.

**Keywords:** biofertilizer; nitrogen fixation; nodulation; plant growth; seed yield

## 1. Introduction

Leguminous crops are important cash crops, and demand for them for domestic consumption and export is increasing in Myanmar. Currently, around 4.6 billion hectares of pulses are sown annually in all states and regions, and Myanmar leads ASEAN (Association of South East Asian Nations) member countries in pulses production [1]. About one-third of human dietary protein is derived from grain legumes [2]. In addition to their being a rich source of protein, legumes are important because they have the unique ability to produce substantial amounts of organic nitrogen

through symbiotic biological nitrogen fixation [3]. Therefore, legume–rhizobia symbiosis can provide an easy, inexpensive way to maintain soil fertility and improve crop production [4].

Leguminous plants can establish symbiosis with bacteria belonging to the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Allorhizobium*, collectively known as rhizobia [5,6]. Inoculation of the soil with *Rhizobium* strains increased nodulation, nitrogen acquisition, and legume yield [7]. The process responsible for reducing molecular nitrogen into ammonia is referred as nitrogen fixation [8], and rhizobia play important roles in agriculture, performing biological nitrogen fixation (BNF). BNF is important agronomically because it reduces the need for chemical nitrogen fertilizers [9]. However, the efficiency of the biological process depends on several factors related to the host plant and bacteria, and edaphic factors such as soil acidity, low soil fertility, high temperatures, and drought often limit the contribution of nitrogen fixation [10]. *Rhizobium* inoculation is very effective at enhancing BFN and crop yields in most legumes, and this practice is adopted by most producers [11].

Nitrogen is important for maintaining and improving crop growth and yield. However, the long-term excessive use of chemical fertilizers in agriculture has unanticipated environmental impacts [12], including soil fertility degradation, soil organic matter deterioration, and decreased water and nutrient holding capacities and nutrient use efficiency [13,14]. An alternative to N fertilizer is effective, efficient rhizobial N-fixing bacteria alone or together. *Rhizobium* inoculants are relatively inexpensive for leguminous crop production [15]. Therefore, the use of efficient inoculants can be considered an important strategy for sustainable management and reduction of environmental problems by decreasing the use of chemical fertilizers [16].

Plant growth-promoting bacteria (PGPB) enhance plant growth by affecting the availability of nutrients originating from processes such as BNF and phosphate solubilization, alleviating stress through the modulation of 1-aminocyclopropane-1-carboxylate deaminase expression, and producing phytohormones and siderophores [17]. Several commercial PGPB inoculants seem to promote growth through one of three mechanisms: plant disease suppression (bioprotectants), nutrient acquisition improvement (biofertilizers), or phytohormone production (biostimulants) [18]. A biofertilizer is a substance containing living microorganisms that, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or interior of the plant and promote growth by increasing the supply or availability of nutrients to the host plant [19]. Biofertilizers are important components of integrated nutrient management. Biological fertilizers could potentially play key roles in the productivity and sustainability of soil. Biofertilizers are cost-effective and ecofriendly. The use of biofertilizers improves soil fertility by fixing atmospheric nitrogen, solubilizing insoluble phosphates, producing plant growth-promoting substances in the soil [20], and promoting nodulation ability, which increases yield by 16–60% [21].

In Myanmar, the Plant Pathology Section of the Department of Agricultural Research (DAR) initiated peat-based inoculant production using exotic strains from NifTAL (Nitrogen Fixation by Tropical Agricultural Legumes) for seven legume crops: groundnut, chickpea, black gram, green gram, soybean, pigeon pea, and cowpea [22]. The use of indigenous rhizobia has been recommended because they adapt easily to environmental conditions, facilitating their survival and successful nodulation by the host plant [23]. However, inoculation is not currently practiced widely in Myanmar [24]. Therefore, this study tested the effects of biofertilizer produced from indigenous *Bradyrhizobium* strains and *Streptomyces griseoflavus* on the symbiotic effectiveness in some leguminous, cereal, and vegetable crops.

## 2. Materials and Methods

### 2.1. Source of Biofertilizer

A peat-based biofertilizer produced from *B. japonicum* SAY3-7 plus *B. elkanii* BLY3-8 and *S. griseoflavus* P4 was used. This biofertilizer was produced in a previous study [25] using the following processes. First, 100 g of sterilized peat soil imported from Myanmar was put in a polyethylene bag. Then, 20 mL of each of the *Bradyrhizobium* strains (*B. japonicum* SAY3-7 and *B.*

*elkanii* BLY3-8) were added. Next, spores of *S. griseoflavus* P4 from IMA-2 plates were collected and put in the polyethylene bag. Finally, the polyethylene bag was put in a black polyethylene bag to protect the bacteria from light. The population densities of the *Bradyrhizobium* strains and *S. griseoflavus* P4 were  $10^8$  cells  $g^{-1}$  at the time of use.

## 2.2. Evaluation of Biofertilizer on Growth of Cereal and Leguminous Plants

Three cereal crops, rice (*Oryza sativa* L.) cv. Manawthuka and wheat (*Triticum aestivum* L.) (Plant nutrition laboratory, Kyushu university, Japan), and maize (*Zea mays* L.) cv. Kakuteru (Nihon nousan shubyo Co., Ltd., Japan); three horticultural crops, komatsuna (*Brassica rapa* L.) and spinach (*Spinacia oleracea* L.) cv. O-rai hourennsou, Japanese radish (*Raphanus sativus* L.) cv. Tokinashi-daikon (Nihon nousan shubyo Co., Ltd., Japan); and six leguminous plants, common bean (*Phaseolus vulgaris* L.) cv. Suzinashi-saitou (Nihon nousan shubyo Co., Ltd.), sweet pea (*Lathyrus odoratus* L.) cv. spring endo (Nakahara seed product Co., Ltd.), mung bean (*Vigna radiata* L. Wilzeck) cv. Yezin-1, black gram (*Vigna mungo* L. Hepper) cv. Yezin-11, cowpea (*Vigna unguiculata* L. Walp) cv. Yezin-8 and soybean (*Glycine max* (L.) Merr.) cv. Yezin-6 (Department of agriculture, Yezin, Myanmar) were used to evaluate the effects the biofertilizer on plant growth under nitrogen-free and nitrogen-supplemented conditions. The seeds were surface-sterilized by soaking in 2.5% sodium hypochlorite for 5 min, rinsed five times in 10 mL of 99.5% ethanol, and washed five times in sterilized half-strength modified Hoagland nutrient (MHN) solution [26] to remove any traces of the sodium hypochlorite and ethanol. Seven seeds per pot were grown in 1-L plastic pots filled with 1.2 L vermiculite and 0.6 L MHN solution containing 0.5 mM  $NaNO_3$  and autoclaved at 120 °C for 20 min. The vermiculite was sieved through a 4-mm sieve. A liquid suspension of 10-fold diluted biofertilizer was inoculated at 5 mL per seed. Five plants were maintained for 10 days after sowing. The plants were cultivated in a Phytotron (25 °C and 75% relative humidity) for 30 days. When the pot weight decreased by about 400 g, 0.4 L of autoclaved MHN containing 0.5 mM  $NaNO_3$  was added for the first watering, and subsequently, deionized water was used continuously for watering up to the starting weight of each pot. A completely randomized design was used with three replications. Five replicate plants were used from pots of the various crops to measure the growth parameters. This experiment was conducted from March to April 2018.

## 2.3. Evaluation of Biofertilizer on Nitrogen Fixation of Leguminous Plants

Two mung bean varieties; mung bean (*Vigna radiata* L. Wilzeck) cv. greenmappe (Nakahara seed product Co., Ltd., Jpn) and mung bean (*Vigna radiata*) cv. Yezin-1; two cowpea varieties; cowpea (*Vigna unguiculata*) cv. rokushakusage (Nakahara seed product Co., Ltd., Japan) and cowpea (*Vigna unguiculata*) cv. Yezin-8, soybean (*Glycine max*) cv. Akisirome (Plant nutrition laboratory, Kyushu university, Japan) and soybean (*Glycine max*) cv. Yezin-6 were used to evaluate the effects of the biofertilizer on plant growth, nodulation, and nitrogen fixation under the nitrogen-supplemented condition. The growth conditions under N-supplementation were as described above. Three replicate plants of the various crops from each pot were used to measure the acetylene reduction activity (ARA) according to Haider et al. [27]. This experiment was conducted in May 2018.

## 2.4. Evaluation of Biofertilizer on Growth, Nitrogen Fixation and Seed Yield of Three Legume

Before cultivation, the Futsukaichi soil used was mixed thoroughly. Then, a soil sample was collected to analyze its physicochemical properties. The collected soil samples were spread and air-dried at room temperature for 24 h. Then, they were crushed by hand and sieved using a 2-mm sieve. They were stored at 4 °C until soil analysis. The soil  $pH_{H_2O}$  (1:2.5 soil:H<sub>2</sub>O) was measured using a pH meter (Beckman  $\phi$  360 pH/Temp/mV Meter; Beckmann Coulter, Brea, CA, USA). The nutrients in the soil were digested using the salicylic acid–H<sub>2</sub>SO<sub>4</sub>–hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) digestion method [28]; total N was determined using the indophenol method [29], and total phosphorus (P) using the ascorbic acid method [30]. Mineralizable N was assessed using the soil

incubation method [31], and the mineralizable N content was measured as in Cataldo et al. [29]. Available P was measured using the Truog method [32]. The cation exchange capacity (CEC) and exchangeable cations were extracted using the ammonium acetate shaking method [33] and analyzed using an atomic absorption spectrophotometer (Z-5300, Hitachi, Tokyo, Japan).

For pot preparation, a 1/5000 Wagner pot was filled with 3.7 kg (oven-dry basis) of Futsukaichi soil. Then, compound fertilizer (Kumiai Mame-kasei 300, Ryoto Fertilizer Co., Ltd., Ooita, Japan) containing 3% N, 10% P<sub>2</sub>O<sub>5</sub>, and 10% K<sub>2</sub>O was applied at a rate of 4 g pot<sup>-1</sup> at the time of pot preparation. The water-holding capacity was kept at 50% at the time of sowing. To prepare the inoculum, a liquid suspension of 10-fold diluted biofertilizer was used at a rate of 5 mL per seed. Five seeds were sown per pot. Fourteen days after sowing, the plants were thinned to two plants per pot. Pesticides were sprayed as necessary. Plant samples were collected from three growing stages: V3 (three unfolded trifoliate leaves), R2 (full bloom stage), and R3.5 (early pod-fill stage). At R8 (maturity stage), the mature plants were harvested to determine the yield.

To measure nitrogen fixation, the ARA was measured at R2 and R3.5. After the ARA assay, the number of nodules was counted, and then shoots, roots, and nodules were collected separately and dried at 70 °C for 72 h to record their dry weights.

At the V3, R2, and R3.5 stages, the dried shoots were ground into a powder using a mill. After digesting the nutrients using the H<sub>2</sub>SO<sub>4</sub>–H<sub>2</sub>O<sub>2</sub> digestion method, the total N in the shoot was measured by the indophenol method; total P was analyzed using the ascorbic acid method, and total K was measured using atomic absorption spectrophotometry. Total N, P and K uptakes were calculated by multiplying nutrient content and shoot dry weight.

At the R8 stage, the plants were cut at the cotyledon nodes to determine the yield parameters, such as the number of pods per plant, number of seeds per pod, and 100-seed weight. This experiment was conducted from June to September 2018.

## 2.5. Statistical Analysis

The data were analyzed using the STATISTIX 8 software package (Analytical Software, Tallahassee, FL, USA), and the means were compared using Tukey's honestly significant difference (HSD) test, with a *p*-value < 0.05 taken to indicate statistical significance.

## 3. Results

### 3.1. Effects of Biofertilizer on Plant Growth of Cereal, Leguminous, and Vegetable Crops

Table 1 summarizes the effects of biofertilizer application on plant growth. Under the N-limited condition, the shoot and root growth of mung bean, cowpea, and soybean were significantly promoted by the biofertilizer compared with the control, but not in the other tested cereals or the leguminous and vegetable crops. With N supplementation, the biofertilizer application significantly increased the shoot and root growth of mung bean, cowpea, and soybean compared with the control. Moreover, the plants to which biofertilizer was applied showed increased shoot growth in komatsuna, and root growth in wheat, maize, sweet pea, and common bean. These results demonstrate that the biofertilizer used in our study was effective in mung bean, cowpea, and soybean regardless of N application.

### 3.2. Effects of Biofertilizer on Plant Growth, Nodulation and Nitrogen Fixation of Leguminous Crops

Table 2 shows the effects of biofertilizer application on plant growth, nodulation, and nitrogen fixation in three leguminous crops. Compared with the control, the biofertilizer significantly promoted these in mung bean, cowpea, and soybean. No nodules formed in the control treatment; consequently, nitrogen fixation did not occur. However, the biofertilizer was compatible with nodule formation and nitrogen fixation in mung beans, cowpeas, and soybeans.

**Table 1.** Effect of biofertilizer on RDW and SDW (g pot<sup>-1</sup>) of various crops with or without nitrogen application.

Crops	Treatment	Without N Application		With N Application	
		RDW (g pot <sup>-1</sup> )	SDW (g pot <sup>-1</sup> )	RDW (g pot <sup>-1</sup> )	SDW (g pot <sup>-1</sup> )
Rice	Control	0.08 ± 0.01 a	0.10 ± 0.00 a	0.20 ± 0.02 a	0.35 ± 0.02 a
	Biofertilizer	0.10 ± 0.00 a	0.11 ± 0.00 a	0.22 ± 0.04 a	0.37 ± 0.03 a
Wheat	Control	0.30 ± 0.02 a	0.17 ± 0.01 a	0.51 ± 0.04 b	0.46 ± 0.03 a
	Biofertilizer	0.32 ± 0.03 a	0.19 ± 0.01 a	0.61 ± 0.01 a	0.49 ± 0.03 a
Maize	Control	0.50 ± 0.09 a	0.42 ± 0.10 a	0.66 ± 0.02 b	0.94 ± 0.03 a
	Biofertilizer	0.56 ± 0.02 a	0.48 ± 0.04 a	0.78 ± 0.05 a	1.04 ± 0.08 a
Komatusna	Control	0.01 ± 0.00 a	0.14 ± 0.01 a	0.08 ± 0.00 a	0.30 ± 0.04 b
	Biofertilizer	0.01 ± 0.00 a	0.16 ± 0.01 a	0.09 ± 0.02 a	0.42 ± 0.04 a
Spinach	Control	0.03 ± 0.00 a	0.08 ± 0.01 a	0.04 ± 0.01 a	0.23 ± 0.02 a
	Biofertilizer	0.03 ± 0.00 a	0.09 ± 0.00 a	0.06 ± 0.01 a	0.31 ± 0.04 a
Raddish	Control	0.03 ± 0.00 a	0.18 ± 0.00 a	0.09 ± 0.02 a	0.66 ± 0.04 a
	Biofertilizer	0.03 ± 0.01 a	0.20 ± 0.02 a	0.09 ± 0.02 a	0.72 ± 0.03 a
Sweet pea	Control	0.44 ± 0.02 a	1.40 ± 0.03 a	0.53 ± 0.07 b	1.58 ± 0.15 a
	Biofertilizer	0.46 ± 0.03 a	1.42 ± 0.04 a	0.73 ± 0.07 a	1.65 ± 0.12 a
Common bean	Control	0.53 ± 0.10 a	1.63 ± 0.20 a	0.96 ± 0.09 b	2.13 ± 0.17 a
	Biofertilizer	0.67 ± 0.02 a	1.75 ± 0.18 a	1.22 ± 0.06 a	2.35 ± 0.17 a
Mung bean	Control	0.35 ± 0.00 b	0.69 ± 0.03 b	0.52 ± 0.03 b	0.95 ± 0.02 b
	Biofertilizer	0.41 ± 0.01 a	0.95 ± 0.06 a	0.63 ± 0.02 a	1.11 ± 0.04 a
Black gram	Control	0.29 ± 0.02 b	0.32 ± 0.02 a	0.39 ± 0.02 a	0.44 ± 0.04 a
	Biofertilizer	0.32 ± 0.01 a	0.35 ± 0.02 a	0.40 ± 0.03 a	0.47 ± 0.02 a
Cowpea	Control	0.60 ± 0.04 b	1.25 ± 0.08 b	1.06 ± 0.04 b	1.68 ± 0.07 b
	Biofertilizer	0.78 ± 0.08 a	1.52 ± 0.01 a	1.29 ± 0.07 a	2.37 ± 0.13 a
Soybean	Control	0.65 ± 0.04 b	1.80 ± 0.07 b	1.21 ± 0.04 b	2.23 ± 0.10 b
	Biofertilizer	0.95 ± 0.14 a	2.25 ± 0.12 a	1.57 ± 0.06 a	2.92 ± 0.10 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). RDW: Root dry weight, SDW: shoot dry weight.

**Table 2.** Effects of biofertilizer on NN, NDW, RDW, SDW, and ARA of mung beans, cowpeas and soybeans at 30 DAS.

Crop	Treatment	NN (no. pot <sup>-1</sup> )	NDW (g pot <sup>-1</sup> )	RDW (g pot <sup>-1</sup> )	SDW (g pot <sup>-1</sup> )	ARA ( $\mu\text{mol C}_2\text{H}_4$ h <sup>-1</sup> plant <sup>-1</sup> )
<b>Mung bean (JP)</b>	Control	0.0 $\pm$ 0.0 b	0.00 $\pm$ 0.00 b	0.50 $\pm$ 0.04 b	0.91 $\pm$ 0.01 b	0.00 $\pm$ 0.00 b
	Biofertilizer	17.3 $\pm$ 2.1 a	0.02 $\pm$ 0.00 a	0.61 $\pm$ 0.03 a	1.06 $\pm$ 0.08 a	0.43 $\pm$ 0.04 a
<b>Mung bean (MM)</b>	Control	0.0 $\pm$ 0.0 b	0.00 $\pm$ 0.00 b	0.64 $\pm$ 0.03 b	1.06 $\pm$ 0.06 b	0.00 $\pm$ 0.00 b
	Biofertilizer	16.7 $\pm$ 0.5 a	0.02 $\pm$ 0.00 a	0.75 $\pm$ 0.05 a	1.27 $\pm$ 0.07 a	0.51 $\pm$ 0.13 a
<b>Cowpea (JP)</b>	Control	0.0 $\pm$ 0.0 b	0.00 $\pm$ 0.00 b	0.94 $\pm$ 0.03 b	1.45 $\pm$ 0.05 b	0.00 $\pm$ 0.00 b
	Biofertilizer	23.0 $\pm$ 1.4 a	0.02 $\pm$ 0.00 a	1.08 $\pm$ 0.02 a	1.73 $\pm$ 0.08 a	0.53 $\pm$ 0.07 a
<b>Cowpea (MM)</b>	Control	0.0 $\pm$ 0.0 b	0.00 $\pm$ 0.00 b	1.11 $\pm$ 0.03 b	2.15 $\pm$ 0.04 b	0.00 $\pm$ 0.00 b
	Biofertilizer	21.0 $\pm$ 2.2 a	0.02 $\pm$ 0.00 a	1.28 $\pm$ 0.06 a	2.40 $\pm$ 0.09 a	0.49 $\pm$ 0.07 a
<b>Soybean (JP)</b>	Control	0.0 $\pm$ 0.0 b	0.00 $\pm$ 0.00 b	1.10 $\pm$ 0.02 b	1.97 $\pm$ 0.03 b	0.00 $\pm$ 0.00 b
	Biofertilizer	12.3 $\pm$ 1.7 a	0.04 $\pm$ 0.00 a	1.21 $\pm$ 0.04 a	2.23 $\pm$ 0.10 a	1.01 $\pm$ 0.04 a
<b>Soybean (MM)</b>	Control	0.0 $\pm$ 0.0 b	0.00 $\pm$ 0.00 b	1.32 $\pm$ 0.09 b	2.69 $\pm$ 0.07 b	0.00 $\pm$ 0.00 b
	Biofertilizer	15.0 $\pm$ 0.8 a	0.04 $\pm$ 0.00 a	1.57 $\pm$ 0.06 a	2.92 $\pm$ 0.10 a	1.05 $\pm$ 0.07 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, NN: nodule number, NDW: nodule dry weight, RDW: root dry weight, SDW: shoot dry weight, ARA: acetylene reduction activity, DAS: days after sowing.

### 3.3. Effects of Biofertilizer on Symbiotic Nitrogen Fixation and Nutrient Uptake of Mung Bean, Cowpea, and Soybean Cultivated in Futsukaichi Soil

Table 3 describes the physicochemical properties of the Futsukaichi soil. The soil pH was 6.61 (soil:water, 1:2.5). The soil contained 80 mg of total nitrogen, 0.39 mg of available nitrogen, 120 mg total phosphorus (P), and 33.89 mg available nitrogen (N) per 100 g soil. The cation exchange capacity (CEC) of the soil was 9.96 cmolc kg<sup>-1</sup>. There were 0.56, 3.12, and 0.26 c molc kg<sup>-1</sup> of exchangeable potassium (K), calcium (Ca), and magnesium (Mg), respectively.

**Table 3.** Physicochemical properties of Futsukaichi soil.

Physicochemical Property	Values
Soil pH (Soil:H <sub>2</sub> O; 1:2.5)	6.61
Total N (mg N/100 g soil)	80 mg
Total P <sub>2</sub> O <sub>5</sub> (mg P <sub>2</sub> O <sub>5</sub> /100 g soil)	120 mg
Available N (mg N/100 g soil)	0.34 mg
Available P (mg P <sub>2</sub> O <sub>5</sub> /100 g soil)	33.89 mg
CEC (c mol <sub>c</sub> Kg <sup>-1</sup> )	9.94 c mol <sub>c</sub> Kg <sup>-1</sup>
Exc. K (c mol <sub>c</sub> Kg <sup>-1</sup> )	0.56 c mol <sub>c</sub> Kg <sup>-1</sup>
Exc. Ca (c mol <sub>c</sub> Kg <sup>-1</sup> )	3.12 c mol <sub>c</sub> Kg <sup>-1</sup>
Exc. Mg (c mol <sub>c</sub> Kg <sup>-1</sup> )	0.26 c mol <sub>c</sub> Kg <sup>-1</sup>

CEC: Cation exchange capacity, Exc: Exchangeable

Table 4 presents the effects of the biofertilizer on the growth of mung beans, cowpeas, and soybeans. The application of biofertilizer significantly increased the shoot biomass of mung beans and soybeans at all sampling stages compared with the control. However, the growth-promoting effect in cowpeas occurred at the early growth stages (V3 and R2), but not at the later growth stages (R3.5 and R8 (maturity stage)).

**Table 4.** Effect of biofertilizer on SDW (g pot<sup>-1</sup>) of mung beans, cowpeas and soybeans at different growth stages.

Crop	Treatment	V3	R2	R3.5	R8
Mung bean (JP)	Control	0.31 ± 0.02 b	16.27 ± 0.62 b	24.88 ± 0.41 b	21.89 ± 1.33 b
	Biofertilizer	0.36 ± 0.01 a	17.97 ± 0.07 a	27.49 ± 1.09 a	26.26 ± 1.16 a
Mung bean (MM)	Control	0.29 ± 0.02 b	14.38 ± 0.58 b	27.76 ± 1.42 b	39.74 ± 0.35 b
	Biofertilizer	0.35 ± 0.01 a	17.32 ± 1.07 a	32.05 ± 0.53 a	42.23 ± 0.76 a
Cowpea (JP)	Control	0.38 ± 0.04 b	15.67 ± 0.92 b	25.89 ± 1.77 a	19.51 ± 5.06 a
	Biofertilizer	0.47 ± 0.01 a	18.49 ± 0.66 a	27.39 ± 0.83 a	21.93 ± 2.64 a
Cowpea (MM)	Control	0.45 ± 0.03 b	15.23 ± 0.82 b	29.11 ± 0.59 a	26.66 ± 3.11 a
	Biofertilizer	0.56 ± 0.03 a	17.64 ± 0.38 a	30.62 ± 0.49 a	28.38 ± 1.59 a
Soybean (JP)	Control	0.35 ± 0.03 b	16.72 ± 0.32 b	32.00 ± 1.04 b	32.13 ± 1.29 b
	Biofertilizer	0.45 ± 0.02 a	17.77 ± 0.22 a	35.64 ± 1.00 a	35.50 ± 0.45 a
Soybean (MM)	Control	0.35 ± 0.03 b	16.55 ± 0.63 b	28.96 ± 1.44 b	39.45 ± 1.52 b
	Biofertilizer	0.48 ± 0.02 a	19.03 ± 0.83 a	33.20 ± 1.07 a	43.58 ± 2.03 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, SDW: shoot dry weight, V3: three unfolded trifoliate leaves, R2: full bloom stage, R3.5: early pod-fill stage, R8: maturity stage.

Tables 5 and 6 describe the effects of the biofertilizer on symbiotic nitrogen fixation in the mung bean, cowpea, and soybean. The biofertilizer significantly improved nodule formation and

nitrogen fixation by mung beans and soybeans at R2 and R3.5 compared with the control, but not the nodulation and nitrogen-fixation ability of cowpea.

**Table 5.** Effect of biofertilizer on NN, NDW and ARA of mung beans, cowpeas and Soybeans at R2 (full bloom stage).

Crop	Treatment	NN (no. pot <sup>-1</sup> )	NDW (g pot <sup>-1</sup> )	ARA ( $\mu\text{mol C}_2\text{H}_4 \text{ h}^{-1} \text{ pot}^{-1}$ )
Mung bean (JP)	Control	7.3 $\pm$ 1.2 b	0.06 $\pm$ 0.01 b	0.76 $\pm$ 0.06 b
	Biofertilizer	13.0 $\pm$ 1.6 a	0.10 $\pm$ 0.01 a	1.32 $\pm$ 0.17 a
Mung bean (MM)	Control	22.7 $\pm$ 1.9 b	0.14 $\pm$ 0.03 b	2.66 $\pm$ 0.28 b
	Biofertilizer	29.3 $\pm$ 3.3 a	0.27 $\pm$ 0.04 a	4.23 $\pm$ 0.41 a
Cowpea (JP)	Control	17.7 $\pm$ 2.1 a	0.20 $\pm$ 0.00 a	3.03 $\pm$ 0.64 a
	Biofertilizer	2.0 $\pm$ 5.0 a	0.22 $\pm$ 0.01 a	3.58 $\pm$ 0.71 a
Cowpea (MM)	Control	25.7 $\pm$ 4.2 a	0.23 $\pm$ 0.02 a	7.53 $\pm$ 2.31 a
	Biofertilizer	39.0 $\pm$ 7.0 a	0.25 $\pm$ 0.03 a	9.37 $\pm$ 2.14 a
Soybean (JP)	Control	58.33 $\pm$ 6.2 b	0.32 $\pm$ 0.25 b	10.11 $\pm$ 0.09 b
	Biofertilizer	113.33 $\pm$ 26.2 a	0.54 $\pm$ 0.14 a	11.68 $\pm$ 0.41 a
Soybean (MM)	Control	36.33 $\pm$ 3.9 b	0.19 $\pm$ 0.07 b	7.73 $\pm$ 0.93 b
	Biofertilizer	66.00 $\pm$ 18.5 a	0.37 $\pm$ 0.09 a	10.76 $\pm$ 1.04 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, NN: nodule number, NDW: nodule dry weight, ARA: acetylene reduction activity.

**Table 6.** Effect of biofertilizer on NN, NDW and ARA of mung beans, cowpeas and soybeans at R3.5 (early pod-fill stage).

Crop	Treatment	NN (No. pot <sup>-1</sup> )	NDW (g pot <sup>-1</sup> )	ARA ( $\mu\text{mol C}_2\text{H}_4 \text{ h}^{-1} \text{ pot}^{-1}$ )
Mung bean (JP)	Control	60.0 $\pm$ 4.9 b	0.54 $\pm$ 0.04 b	47.01 $\pm$ 2.69 b
	Biofertilizer	82.3 $\pm$ 2.1 a	0.76 $\pm$ 0.04 a	52.53 $\pm$ 1.07 a
Mung bean (MM)	Control	87.7 $\pm$ 8.8 b	0.93 $\pm$ 0.05 b	18.59 $\pm$ 3.07 b
	Biofertilizer	111.0 $\pm$ 3.7 a	1.21 $\pm$ 0.06 a	31.01 $\pm$ 3.02 a
Cowpea (JP)	Control	158.3 $\pm$ 8.6 a	1.11 $\pm$ 0.16 a	26.19 $\pm$ 4.32 a
	Biofertilizer	172.0 $\pm$ 12.0 a	1.32 $\pm$ 0.11 a	32.23 $\pm$ 2.05 a
Cowpea (MM)	Control	157.0 $\pm$ 12.0 a	0.87 $\pm$ 0.03 a	46.02 $\pm$ 2.68 a
	Biofertilizer	162.3 $\pm$ 8.2 a	0.97 $\pm$ 0.11 a	50.91 $\pm$ 5.44 a
Soybean (JP)	Control	140.7 $\pm$ 7.4 b	1.47 $\pm$ 0.05 b	63.16 $\pm$ 5.57 b
	Biofertilizer	181.3 $\pm$ 21.7 a	1.62 $\pm$ 0.06 a	75.42 $\pm$ 3.67 a
Soybean (MM)	Control	113.0 $\pm$ 8.8 b	1.10 $\pm$ 0.13 b	46.62 $\pm$ 3.46 b
	Biofertilizer	146.7 $\pm$ 7.8 a	1.51 $\pm$ 0.09 a	84.85 $\pm$ 10.04 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, NN: nodule number, NDW: nodule dry weight, ARA: acetylene reduction activity.



Table 7 shows the effects of the biofertilizer on N uptake of mung bean, cowpea, and soybean. The biofertilizer significantly increased the N uptake of mung bean and soybean at V3, R2, and R3.5 compared with the control. By contrast, a significant increase in the N uptake of cowpea occurred at V3 stage, but not at R2 and R3.5. Table 8 shows the effects of the biofertilizer on P uptake by mung bean, cowpea, and soybean. The biofertilizer application significantly increased the P uptake of the Myanmar mung bean variety at all stages compared with the control. In comparison, in the Japanese mung bean variety, the significant increase in P uptake occurred at V3. In cowpea, a significant increase in P uptake occurred at V3, but not at R2 and R3.5. The biofertilizer significantly increased the P uptake of soybean at all stages compared with the control. Table 9 shows the effects of the biofertilizer on the K uptake of mung bean, cowpea, and soybean. The biofertilizer significantly increased the K uptake of the Myanmar mung bean variety at the V3, R2, and R3.5 stages compared with the control, whereas the significant increase in K uptake occurred at V3 in the Japanese mung bean variety. Similarly, the application of biofertilizer significantly increased the K content of the Myanmar cowpea variety at V3, R2, and R3.5 compared with the control, whereas the significant increase in K uptake occurred at V3 and R2 in the Japanese cowpea variety. In soybean, the use of the biofertilizer significantly increased the K uptake at V3 in Japan soybean variety and at V3 and R2 in Myanmar soybean variety compared with the control, but not at the R8 growth stage [in both Japan and Myanmar soybean].

**Table 7.** Effect of biofertilizer on N uptake (mg pot<sup>-1</sup>) of mung beans, cowpeas and soybeans at different growth stage.

Crop	Treatment	V3	R2	R3.5
Mung bean (JP)	Control	16.02 ± 0.84 b	203.92 ± 22.96 b	452.17 ± 46.87 b
	Biofertilizer	19.22 ± 0.76 a	271.66 ± 9.75 a	551.48 ± 23.54 a
Mung bean (MM)	Control	15.02 ± 1.31 b	242.85 ± 41.76 b	547.06 ± 60.47 b
	Biofertilizer	18.40 ± 0.65 a	321.88 ± 21.22 a	683.20 ± 63.57 a
Cowpea (JP)	Control	18.35 ± 2.01 b	315.69 ± 19.63 a	687.56 ± 51.53 a
	Biofertilizer	23.38 ± 1.04 a	354.51 ± 11.68 a	666.05 ± 37.64 a
Cowpea (MM)	Control	23.25 ± 1.51 b	449.94 ± 10.94 a	790.34 ± 96.43 a
	Biofertilizer	28.16 ± 2.25 a	477.13 ± 34.69 a	877.11 ± 40.68 a
Soybean (JP)	Control	21.14 ± 1.78 b	281.08 ± 28.72 b	664.16 ± 24.46 b
	Biofertilizer	28.24 ± 1.34 a	346.09 ± 50.50 a	777.03 ± 41.12 a
Soybean (MM)	Control	20.80 ± 1.88 b	406.71 ± 12.20 b	740.31 ± 45.07 b
	Biofertilizer	29.26 ± 1.87 a	466.06 ± 9.35 a	866.08 ± 54.32 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, V3: three unfolded trifoliate leaves, R2: full bloom stage, R3.5: early pod-fill stage.

**Table 8.** Effect of biofertilizer on P uptake (mg pot<sup>-1</sup>) of mung beans, cowpeas and soybeans at different growth stage.

Crop	Treatment	V3	R2	R3.5
Mung bean (JP)	Control	1.58 ± 0.07 b	53.34 ± 4.29 a	83.20 ± 4.60 a
	Biofertilizer	2.05 ± 0.07 a	51.24 ± 3.98 a	81.75 ± 4.32 a
Mung bean (MM)	Control	1.31 ± 0.10 b	37.51 ± 4.05 b	83.49 ± 1.60 b
	Biofertilizer	1.71 ± 0.07 a	48.31 ± 2.87 a	96.03 ± 8.34 a
Cowpea (JP)	Control	1.73 ± 0.16 b	42.41 ± 3.67 a	91.45 ± 8.75 a
	Biofertilizer	2.23 ± 0.13 a	50.55 ± 3.51 a	88.34 ± 8.57 a
Cowpea (MM)	Control	2.13 ± 0.15 a	51.50 ± 2.03 a	103.57 ± 3.58 a
	Biofertilizer	2.35 ± 0.01 a	57.93 ± 2.76 a	107.03 ± 2.77 a
Soybean (JP)	Control	2.15 ± 0.21 b	48.47 ± 3.28 b	84.82 ± 3.76 b
	Biofertilizer	2.89 ± 0.14 a	57.64 ± 1.98 a	107.31 ± 8.25 a
Soybean (MM)	Control	2.08 ± 0.17 b	49.94 ± 0.97 b	87.13 ± 3.06 b
	Biofertilizer	2.89 ± 0.18 a	60.10 ± 4.43 a	101.09 ± 5.09 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, V3: three unfolded trifoliolate leaves, R2: full bloom stage, R3.5: early pod-fill stage.

**Table 9.** Effect of biofertilizer on K uptake (mg pot<sup>-1</sup>) of mung beans, cowpeas and soybeans at different growth stage.

Crop	Treatment	V3	R2	R3.5
Mung bean (JP)	Control	0.26 ± 0.01 b	7.58 ± 0.68 a	9.07 ± 0.91 a
	Biofertilizer	0.32 ± 0.01 a	6.91 ± 0.56 a	9.81 ± 0.51 a
Mung bean (MM)	Control	0.25 ± 0.02 b	6.07 ± 0.62 b	10.19 ± 0.69 b
	Biofertilizer	0.33 ± 0.01 a	8.10 ± 0.22 a	12.87 ± 0.52 a
Cowpea (JP)	Control	0.30 ± 0.03 a	5.05 ± 0.32 b	9.55 ± 0.56 a
	Biofertilizer	0.34 ± 0.00 a	6.15 ± 0.31 a	9.26 ± 0.67 a
Cowpea (MM)	Control	0.33 ± 0.02 b	8.38 ± 0.21 b	11.96 ± 0.54 b
	Biofertilizer	0.47 ± 0.03 a	9.62 ± 1.10 a	14.33 ± 0.66 a
Soybean (JP)	Control	0.24 ± 0.02 b	5.84 ± 0.14 a	10.15 ± 0.91 a
	Biofertilizer	0.32 ± 0.01 a	6.33 ± 0.45 a	12.07 ± 0.92 a
Soybean (MM)	Control	0.24 ± 0.02 b	6.72 ± 0.33 b	10.86 ± 0.95 a
	Biofertilizer	0.32 ± 0.02 a	8.24 ± 0.24 a	12.28 ± 0.91 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, V3: three unfolded trifoliolate leaves, R2: full bloom stage, R3.5: early pod-fill stage.

Table 10 shows the effects of the biofertilizer on the yield and yield components of mung bean, cowpea, and soybean. The biofertilizer significantly increased the number of pods per pot in mung bean and soybean compared with the control, but not the seeds per pod or 100-seed weight. Moreover, the seed yield of mung bean and soybean increased with the biofertilizer application. In cowpea, the application of biofertilizer did not significantly increase the yield and yield components.

**Table 10.** Effect of biofertilizer on yield and yield components of mung beans, cowpeas and soybeans at R8 (maturity stage).

Crop	Treatment	Pod. No (no. pot <sup>-1</sup> )	Seeds Per Pod (no. pod <sup>-1</sup> )	100 Seed Weight (g)	Seed Yield (g pot <sup>-1</sup> )
Mung bean (JP)	Control	22.0 ± 2.2 b	4.9 ± 0.1 a	4.90 ± 0.14 a	5.22 ± 0.28 b
	Biofertilizer	26.7 ± 0.9 a	4.8 ± 0.1 a	5.11 ± 0.19 a	6.46 ± 0.23 a
Mung bean (MM)	Control	19.3 ± 1.2 b	5.4 ± 0.1 a	4.44 ± 0.31 a	4.67 ± 0.49 b
	Biofertilizer	25.3 ± 2.1 a	5.0 ± 0.4 a	4.75 ± 0.18 a	5.94 ± 0.27 a
Cowpea (JP)	Control	11.7 ± 1.2 a	6.4 ± 0.4 a	7.48 ± 0.62 a	5.58 ± 0.55 a
	Biofertilizer	13.3 ± 1.2 a	6.4 ± 0.3 a	7.49 ± 0.42 a	6.37 ± 0.24 a
Cowpea (MM)	Control	16.0 ± 2.9 a	5.7 ± 0.5 a	7.69 ± 0.39 a	6.90 ± 0.96 a
	Biofertilizer	17.7 ± 1.7 a	5.9 ± 0.5 a	7.64 ± 0.73 a	7.87 ± 0.81 a
Soybean (JP)	Control	42.3 ± 1.9 b	1.9 ± 0.0 a	18.47 ± 1.42 a	14.68 ± 1.55 b
	Biofertilizer	53.0 ± 5.1 a	1.9 ± 0.1 a	18.11 ± 0.33 a	18.03 ± 1.01 a
Soybean (MM)	Control	53.3 ± 5.2 b	1.9 ± 0.1 a	17.10 ± 0.14 a	16.87 ± 0.50 b
	Biofertilizer	67.3 ± 2.1 a	1.9 ± 0.0 a	17.06 ± 0.33 a	21.49 ± 0.68 a

For each cultivar, mean values with the same letters in each column are not significantly different at  $p < 0.05$  (Tukey's test). JP: Japanese variety, MM: Myanmar variety, V3: three unfolded trifoliate leaves, R2: full bloom stage, R3.5: early pod-fill stage.

#### 4. Discussion

The bacteria that live in the rhizosphere and induce beneficial effects on plant growth are referred to as plant growth-promoting rhizobacteria (PGPR) [34]. PGPR belong to several genera, including *Azotobacter*, *Bacillus*, *Rhizobium*, *Bradyrhizobium*, and *Streptomyces* [35]. Some PGPR are used as biofertilizers [36]. *Bradyrhizobium* are nitrogen fixers due to their potential to fix nitrogen in soybean. *Streptomyces* are promising biocontrol agents because of their potential to produce a wide range of secondary substances such as vitamins, alkaloids, plant growth factors, enzymes, and enzyme inhibitors [37,38]. *Streptomyces* are plant growth-promoting bacteria because of their potential to promote the growth of crop plants [39]. Therefore, there is interest in evaluating the symbiotic effectiveness of biofertilizers produced from *Bradyrhizobium* strains and *Streptomyces* on leguminous crops.

In our study, biofertilizer application increased the shoot growth of komatsuna, root growth of wheat, maize, sweet pea, and common bean, and shoot and root growth of mung bean, cowpea, and soybean grown in vermiculite (Tables 1 and 2). Moreover, biofertilizer application increased the shoot growth of mung bean and soybean from early (V3 and R2) to later (R3.5 and R8) growth stages and that of cowpea cultivated in soil at early growth stages (V3 and R2) (Table 4). Meguro et al. [40] reported that the endophytic strain *Streptomyces* spp. MBR-52 enhanced the emergence and

elongation of plant adventitious roots. Soe and Yamakawa [41] found that inoculation of *S. griseoflavus* P4 enhanced the growth of soybean, sweet pea, rice, spinach, maize, and wheat, perhaps via the production of IAA. Soe and Yamakawa [41] revealed that with longer incubation times, *S. griseoflavus* P4 secreted more IAA, ranging from 0.8 to 2.8  $\mu\text{g mL}^{-1}$ . In our study, the *S. griseoflavus* P4 in the biofertilizer promoted growth because IAA-producing microorganisms stimulate root elongation and enhance plant growth [42].

The plants with biofertilizer produced from *Bradyrhizobium* strains plus *S. griseoflavus* P4 promoted nodulation ability (Tables 5 and 6). Soe et al. [43] found that the co-inoculation of P4 with *B. yuanmingense* MAS34 significantly improved the nodule dry weight of soybean. The combined use of bradyrhizobial strains and *S. griseoflavus* P4 increased nodulation [44].

In this study, the application of biofertilizer produced from *Bradyrhizobium* and *S. griseoflavus* P4 increased N fixation by soybean and mung bean compared with the control (Tables 5 and 6). Our study supports previous findings that co-inoculation of bradyrhizobial strains and *S. griseoflavus* P4 increased nitrogen fixation by soybean [45–47].

The application of the biofertilizer produced from *Bradyrhizobium* and *S. griseoflavus* P4 increased the N, P, and K uptakes of soybean and mung bean compared with the control (Tables 7–9). These results support our previous finding that inoculation of *Bradyrhizobium* with P4 significantly increased the N, P, and K uptakes of soybean compared with an un-inoculated control [48,49]. Beneficial microorganisms can keep the soil environment rich in all kinds of micro- and macronutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, the release of plant growth regulators, the production of antibiotics, and the biodegradation of organic matter in the soil [18].

Soe et al. [50] reported that the combined use of *Bradyrhizobium* strains and *S. griseoflavus* P4 had a significant effect on the number of pods per plant in Yezin-12 and CM-6 compared with the uninoculated control. This was in line with our findings that the use of a biofertilizer produced from effective *Bradyrhizobium* strains and *S. griseoflavus* P4 significantly increased the number of pods per pot in both soybean and mung bean (Table 10). Consequently, biofertilizer application increased the seed yield of mung bean and soybean. Our study supports the findings of Soe and Yamakawa [51] showing that co-inoculation of bradyrhizobial strains and P4 increased the seed yield of soybean. Moreover, the seed yield of mung bean significantly improved with the application of biofertilizer produced from *Bradyrhizobium* and *S. griseoflavus* P4.

In this study, we observed significant increases in plant growth, nodulation, and nitrogen fixation in mung bean and soybean. Our experimental results confirmed the findings of Akarapisan et al. [52] that *S. griseoflavus* P4 is an effective endophytic actinomycete that can be used together with selected root nodule bacterial strains for the production of economically important leguminous crops by enhancing symbiotic nitrogen fixation, increasing nodulation, and promoting plant growth.

## 5. Conclusions

In conclusion, the biofertilizer was effective in mung bean, cowpea, and soybean regardless of N application. Significant increases in plant growth, nodulation, nitrogen fixation, NPK uptake, and seed yield were seen in mung bean and soybean. Therefore, the Myanmar biofertilizer produced from *Bradyrhizobium* strains with *S. griseoflavus* P4 should be useful not only for soybean production but also for mung bean production.

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