agronomy

MDPI

Article

Biofertiliser Production for Agronomic Application and Evaluation of Its Symbiotic Effectiveness in Soybeans

Aung Zaw Htwe [1,2,*], Seinn Moh Moh [3], Kyi Moe [1,2] and Takeo Yamakawa [4]

1 Graduate School of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan; kyimoeyau@gmail.com
2 Department of Agronomy, Yezin Agricultural University, Yezin 15013, Myanmar
3 Department of Agriculture, Ministry of Agriculutre, Livestock and Irrigation, Yezin 15011, Myanmar; hanmohmohyau@gmail.com
4 Laboratory of Plant Nutrition, Department of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan; yamakawa@agr.kyushu-u.ac.jp
* Correspondence: aungzawhtwe333@gmail.com; Tel.: +81-80-6423-8187

Received: 12 February 2019; Accepted: 23 March 2019; Published: 27 March 2019

Abstract: This study was conducted to evaluate the effects of *Bradyrhizobium japonicum* SAY3-7, *Bradyrhizobium elkanii* BLY3-8, and *Streptomyces griseoflavus* P4 on the symbiotic effectiveness of soybeans before biofertiliser production, to produce biofertiliser containing the studied three strains (SAY3-7, BLY3-8, and P4), to test the effectiveness of the biofertiliser on soybean varieties, and to assess the varietal effects and interaction effects between variety and biofertiliser on plant growth, nodulation, nitrogen fixation, nutrient absorption, and seed yield. Nitrogen fixation was measured using the acetylene reduction assay and ureide methods. Contents of nutrients (N, P, K, Ca, and Mg) were also measured to calculate their uptakes. In this study, synergistic effects of nitrogen fixation were induced by combined inoculation with SAY3-7, BLY3-8 and P4 in all tested soybean varieties. Therefore, we assumed that an effective biofertiliser could be produced using these effective bacteria (SAY3-7, BLY3-8, and P4). After making biofertiliser using these effective bacteria, packages were stored at 30 °C. The populations of the bacteria in the biofertiliser were maintained at a density of 1×10^8 colony forming units (cfu) g^{-1} for P4 and 7×10^9 cells g^{-1} for *Bradyrhizobium*. Diluting biofertiliser by 10^{-3} proved more effective for nodulation and nitrogen fixation than other dilution treatments. Moreover, this biofertiliser significantly promoted plant growth, nodulation, nitrogen fixation, nutrient uptakes, and seed yield in Yezin-3 and Yezin-6 soybean varieties. Yezin-6 is a more efficient variety than Yezin-3 for improved plant growth, nodulation, nitrogen fixation, nutrient absorption, and seed yield. Taken together, the application of an effective biofertiliser and the use of an efficient soybean variety can play important roles in promoting plant growth, nodulation, nitrogen fixation, and higher seed yield.

Keywords: *Bradyrhizobium*; *Streptomyces griseoflavus*; biofertiliser production; biofertiliser application; nodulation; nitrogen fixation; seed yield

1. Introduction

Rhizobium inoculation is a well-known agronomic practice for ensuring adequate nitrogen (N) for legumes. Inoculating soybeans with a *Bradyrhizobium* strain is essential for supplying crop N demand and substituting for nitrogenous fertiliser. The use of biofertiliser has become a notable alternative method to chemical fertiliser. A biofertiliser is a substance containing living microorganisms that

when applied to seeds, plant surfaces, or soil, colonise the inner part of the plant or the rhizosphere, and promote growth by increasing the supply or availability of primary nutrients to the host plant [1]. Biofertilisers are cost-effective and ecofriendly in nature. The use of biofertiliser improves soil fertility by fixing atmospheric nitrogen, solubilising insoluble phosphates, and producing plant growth-promoting substances in the soil [2]. It also promotes nodulation efficiency and increases yield by around 16–60% [3].

A group of bacteria (rhizobacteria) that lives in the rhizosphere and induces a beneficial effect on plant growth is referred to as plant growth-promoting rhizobacteria (PGPR) [4]. PGPR belong to several genera such as *Azotobacter*, *Bacillus*, *Rhizobium*, *Bradyrhizobium*, *Streptomyces*, and others [5], and various groups of microorganisms, including *Azotobacter*, *Rhizobium*, *Azospirillum*, *Trichoderma*, and *Bacillus*, are used as biofertilisers [6]. *Streptomyces* are known as 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 [7,8]. *Streptomyces* are also known as plant growth-promoting agents [9].

Biofertiliser for soybean is mainly produced using *Bradyrhizobium* species, and has been distributed for domestic use for soybean production in many countries; however, it is currently not widespread in Myanmar [10]. The Plant Pathology Section of the Department of Agricultural Research (DAR), Myanmar, initiated peat-based inoculant production using exotic strains from Nitrogen Fixation for Tropical Agriculture (NifTAL) for seven legume crops [11]. These exotic strains can be replaced by indigenous strains that are adapted to local environmental conditions and have higher efficiency for nitrogen fixation. The use of indigenous rhizobia has been recommended because these bacteria adapt easily to the specific environmental conditions, facilitating their survival and successful nodulation of the host plant [12].

Many commercially available biofertilisers have been used to improve crop production efficiency worldwide [13]. However, more widespread production and utilisation of biofertilisers are required in Myanmar and in other countries. Farmers in Myanmar have a willingness to use rhizobial inoculants when sowing legumes because the cost of rhizobial inoculant application less expensive (around 150 times cheaper) than other fertilisers [14]. However, current inoculant production is lower than 100,000 packets due to limitations throughout the supply chain, from production and quality assurance to distribution to demand [10]. Therefore, much higher production of inoculants is needed in Myanmar. However, there are many factors limiting the use of rhizobia or inoculants by farmers, among which production of quality inoculants is the major concern.

Soybean plants in symbiosis with *Bradyrhizobium* can fix nitrogen at a rate of up to 300 kg N ha^{-1} under favourable conditions [15]. However, the symbiosis is dependent on host specificity. This specificity may also be related to the nodulation regulatory genes of soybean cultivars and the nodulation types of rhizobia. Ishizuka et al. [16,17] tested the compatibility and preference of *Rj*-genotype soybean cultivars with specific strains of *Bradyrhizobium*. The *Bradyrhizobium* strains were classified as nodulation types A, B, and C based on their compatibility with the *Rj* cultivars. Type A strains are preferred by non-*Rj*-genotype soybean cultivars, but nodulate with all *Rj*-genotype soybean cultivars. Type B strains are preferred by Rj_4-genotype soybean cultivars and inhibit nodulation with Rj_2Rj_3-genotype soybean cultivars. Type C strains are preferred by Rj_2Rj_3 cultivars and restrict effective nodule formation with Rj_4-genotype soybean cultivars.

Htwe et al. [18] and Soe et al. [19] reported that most soybean varieties collected from Myanmar harbour Rj_4 genes and non-*Rj* genes. Therefore, inoculation with type A and type B strains, which prefer non-*Rj* and Rj_4-genotype soybean varieties [16,17], is necessary for successful nodulation and nitrogen fixation. Our previous findings highlighted a synergistic effect of *Bradyrhizobium* strain with *Streptomyces griseoflavus* P4 in soybeans [20,21]. In both experiments, significant differences in plant growth, nodulation, N_2 fixation, N uptake, and yield occurred in co-inoculation compared with control, but not with single inoculation of P4 or *Bradyrhizobium* strain, suggesting a synergistic effect of co-inoculation of SAY3-7 (type A) and P4 in the Yezin-6 (non-*Rj*) soybean variety under field

conditions [20] and co-inoculation of BLY3-8 (type B) and P4 in Yezin-3 (Rj_4) soybean variety in the pot condition [21]. Based on the previous experiments, biofertiliser produced from sole inoculation of P4 or *Bradyrhizobium* strain is not suitable for obtaining significant effects on plant growth, nodulation, and N_2 fixation. Biofertiliser production using mixed inoculants of P4 and *Bradyrhizobium* strains may be a promising method to ensure plant growth, nodulation, N_2 fixation, and nutrient uptake, which support better productivity of soybeans. Therefore, this study was conducted to evaluate the effects of *Bradyrhizobium japonicum* SAY3-7, *Bradyrhizobium elkanii* BLY3-8, and *Streptomyces griseoflavus* P4 on the symbiotic effectiveness of soybeans before biofertiliser production, to produce biofertiliser using SAY3-7, BLY3-8, and P4, to evaluate the symbiotic effectiveness of the biofertiliser on soybean varieties during biofertiliser production, and to assess the varietal effects and interaction effects between variety and biofertiliser on plant growth, nodulation, nitrogen fixation, nutrient absorption, and seed yield.

2. Materials and Methods

2.1. Expt. I. Effect of Single, Dual and Combined Inoculation of SAY3-7, BLY3-8 and P4 on Symbiotic Effectiveness of Different Soybean Varieties

Yezin-3 (Rj_4) and Yezin-6 (non-*Rj*) soybean varieties were used to assess nitrogen fixation. One-litre pots were filled with vermiculite and 0.6 L of half-strength modified Hoagland nutrient (MHN) solution at pH 6.5 [22]. The pots were autoclaved at 120 °C for 20 min. Seed surfaces were sterilised by soaking seeds in 2.5% sodium hypochlorite solution for 5 min, rinsing five times with 10 mL 99.5% ethanol, and washing five times with sterilised MHN solution. Five surface-sterilised seeds were sown in each pot. Indigenous *B. japonicum* SAY3-7 (type A) and *B. elkanii* BLY3-8 (type B) strains were obtained from a previous experiment [23]. *Bradyrhizobium* strains were cultured in A1E liquid medium [24] on a rotary shaker (100 rpm) at 30 °C for 7 days. P4 obtained from the Plant Nutrition Laboratory, Kyushu University, Japan, was incubated on IMA-2 medium [25] at 30 °C for 5 days. For inoculation, the liquid bacterial cultures were diluted with sterilised half-strength MHN solution to 10^5 cells mL^{-1}. Each seed was inoculated with 5 mL of the bacterial suspension. The plants were cultivated in an environmentally controlled room (25 °C and 75% relative humidity) for one month. A completely randomised design was used with three replications. This experiment was conducted from January to March 2017.

At harvest time, N_2 fixation was analysed using the acetylene reduction assay (ARA) method, as described by Haider et al. [26]. To measure N_2 fixation, the plants were uprooted and washed carefully with water so as to not detach the nodules. The soybean plants were cut at the cotyledonary nodes. Then, soybean roots with intact nodules were placed in a 100-mL conical flask and sealed with a serum stopper. A 25-mL aliquot of acetylene (C_2H_2) gas was injected into the flask to replace the air with acetylene. The flasks containing roots with intact nodules were incubated at room temperature and 1.0-mL subsamples were analysed at 5 and 65 min. The ARA value, in terms of C_2H_4 production per plant, was measured using a flame ionisation gas chromatograph (GC-14A, Shimadzu, Kyoto, Japan).

2.2. Experiment II: Physicochemical Properties of Peat Soil and Biofertiliser Production Using the Peat Soil

Peat soils were collected from Heho, Shan State, Myanmar. The collected peat soils were spread and air-dried at room temperature for 24 h, then crushed by hand and sieved using a 2-mm mesh sieve. They were sterilised by autoclave at 120 °C for 20 min before importing to Japan. They were stored at 4 °C until soil analysis. The soil pH_{H2O} (1:2.5, soil:H_2O) was measured using a pH meter. To obtain the nutrient contents of the peat soil, samples were digested using the salicylic acid–H_2SO_4–hydrogen peroxide (H_2O_2) digestion method [27]. Total N was determined using the indophenol method [28], and total phosphorus (P) was analysed using the ascorbic acid method [29]. Mineralisable N was assessed using the soil incubation method [30]. The mineralisable N content was measured as described by Cataldo et al. [28]. Available P was measured using Truog's method [31]. Cation exchange capacity (CEC) and exchangeable cations were determined using the ammonium acetate shaking extraction

method [32] followed by analysis using an atomic absorption spectrophotometer (Z-5300, Hitachi, Tokyo, Japan).

Inoculant preparation was conducted as described above. In this study, we used sterilised peat soil as the carrier. Peat soil was sterilised by autoclaving at 120 °C for 20 min to kill all bacteria in the peat soil. First, 100 g of sterilised peat powder was placed into a polyethylene bag. Second, 20 mL of each *Bradyrhizobium* strain (*B. japonicum* SAY3-7 and *B. elkanii* BLY3-8) was added. Third, *S. griseoflavus* P4 spores from IMA-2 plates were added into the polyethylene bag. Then, the polyethylene bag was placed into a black polyethylene bag to protect the bacteria from the effects of light. The population densities of both bacteria were prepared to obtain a density of 10^{10} cells g^{-1}. The water holding capacity of the biofertiliser was maintained at around 45%. The packets were stored in an incubator at 30 °C in the dark. Aside from the biofertiliser packages, we prepared packages without added bacteria to check for contamination. Moreover, all materials used in biofertiliser production processes were sterilised, and all processes were performed on a clean bench to avoid contamination.

The survival of *S. griseoflavus* P4 was assessed using the plate counting method [33]. The survival of rhizobia in the sterilised peat soils was checked monthly using the most probable number (MPN) method [33] with Yezin-6 (non-*Rj*) as the host plant. For the MPN count, the soybean seeds were surface-sterilised. Then, 1 mL of each dilution from 10^{-1} to 10^{-10} was inoculated per seed. Plants were harvested at 4 weeks after sowing. Four replicate plants were used for each dilution. The quality of the biofertiliser was assessed every four months. The Yezin-6 (non-*Rj*) soybean variety was used for quality assessment. Biofertiliser was diluted with autoclaved deionised water to 10^{-1} to 10^{-10}. Then, each sterilised seed was inoculated with 5 mL of the biofertiliser suspension. Cultivation and data collection were as described above. This experiment was conducted from June 2017 to May 2018.

2.3. Experiment III: Evaluation of Symbiotic Effectiveness of Biofertiliser on Yezin-3 (Rj_4) and Yezin-6 (non-Rj) Soybean Varieties at Different Growth Stages

For pot preparation, an a/5000 Wagner pot was filled with 3.7 kg (oven dry basis) of Futsukaichi soil. Then, compound fertiliser containing 3% nitrogen (N), 10% diphosphorus pentoxide (P_2O_5), and 10% potassium oxide (K_2O) was applied at the rate of 1.6 g pot^{-1} at the time of pot preparation. The maximum water holding capacity was maintained at 60% at the time of sowing. The seed inoculation method used in this experiment was performed by mixing 100 soybean seeds with 10 g biofertiliser and 7 mL 20% liquid solution of gum arabic. Four inoculated seeds were planted per pot and covered with soil just after seed sowing. At 20 days after sowing (DAS), thinning was performed to maintain one plant per pot. In this study, factorial design was used with three replications. This experiment was conducted from July 2017 to October 2017.

Plant samples were collected at three growth stages: V6 (six unfolded trifoliate leaves), R3.5 (early pod-fill stage), and R8 (maturity stage). At the V6 stage, ARA was measured as described above. At the R3.5 stage, relative ureide index (RUI) measurement was conducted on xylem sap. Plants were cut just beneath the cotyledonary nodes and inserted into a silicon tube. The xylem sap was collected within 1 h after cutting and stored at −30 °C for long-term use. Amino N [34], NO_3-N [35], and ureide-N [36] were analysed from the xylem sap. The RUI was calculated as follows [37]: RUI (%) = 4 × ureide-N/(4 × ureide-N + amino-N + nitrate-N) × 100. The percentage of N derived from N fixation was calculated as follows [38]: y = 21.3 + 0.67x, where y is the RUI (%) and x is the percentage of nitrogen derived from nitrogen fixation (%Ndf), respectively.

At the V6, R3.5, and R8 stages, the shoots were divided into leaves, stems and petioles, shells, unfilled seeds, and filled seeds to measure total N, P, K, Ca, and Mg contents. Each plant part was dried at 70 °C for 72 h and separately ground into a powder using a mill (100–120 mesh, Tecator AB, Hoedanaes, Sweden). After digestion of the nutrients using the H_2SO_4–H_2O_2 digestion method, total N content was measured with the indophenol method and total P was analysed using the ascorbic acid method. Total K, Ca, and Mg were analysed by atomic absorption spectrophotometer (Z-5300, Hitachi).

2.4. Statistical Analysis

Data were analysed using the STATISTIX 8 software package (Analytical Software, Tallahassee, FL, USA), and means were compared using Tukey's HSD test with a *P*-value < 0.05 indicating statistical significance.

3. Results

3.1. Experiment I. Effect of Single, Dual and Combined Inoculation of SAY3-7, BLY3-8 and P4 on Ssymbiotic Effectiveness of Different Soybean Varieties

The results of plant growth, nodulation, and nitrogen fixation are shown in Table 1. Nodule numbers were not significantly different among treatments in Yezin-3 and Yezin-6 soybean varieties. However, nodule dry weight was significantly different among treatments in Yezin-3 and Yezin-6 soybeans. Single, dual, or combined inoculation showed no significant differences in root dry weight among the tested soybean varieties, but there were significant differences in shoot dry weight. Shoot dry weights of plants inoculated with SAY3-7 and BLY3-8 with P4 were higher than those of controls in Yezin-3 and Yezin-6. Nitrogen fixation in terms of C_2H_4 production was significantly different in all tested varieties. The nitrogen fixation values of the dual inoculation of SAY3-7 + BLY3-8 and the combined inoculation of SAY3-7 + BLY3-8 + P4 were significantly higher in Yezin-3 and Yezin-6 soybean varieties.

Table 1. Effect of inoculation of *B. japonicum* SAY3-7, *B. elkanii* BLY3-8 and *S. griseoflavus* P4 on nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW), root dry weight (RDW) and the acetylene reducing activity (ARA) of soybeans at 30 days after sowing.

Variety	Treatment	NN (No. plant^{-1})	NDW (mg plant^{-1})	RDW (g plant^{-1})	SDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1})
Yezin-3	Control	0.0 ± 0.0 b	0.00 ± 0.00 c	0.33 ± 0.04 a	0.61 ± 0.03 b	0.00 ± 0.00 c
	P4	0.0 ± 0.0 b	0.00 ± 0.00 c	0.33 ± 0.01 a	0.64 ± 0.02 b	0.00 ± 0.00 c
	SAY3-7	11.5 ± 3.2 a	54.43 ± 4.42 a	0.30 ± 0.06 a	0.82 ± 0.03 a	1.04 ± 0.09 b
	BLY3-8	14.0 ± 6.1 a	33.50 ± 0.64 b	0.28 ± 0.02 a	0.85 ± 0.05 a	0.90 ± 0.09 b
	SAY3-7 + BLY3-8	10.3 ± 2.6 a	50.10 ± 4.49 a	0.31 ± 0.01 a	0.84 ± 0.02 a	1.32 ± 0.09 a
	SAY3-7 + P4	11.8 ± 4.4 a	51.00 ± 5.51 a	0.33 ± 0.06 a	0.92 ± 0.10 a	1.09 ± 0.04 b
	BLY3-8 + P4	13.8 ± 3.5 a	35.47 ± 3.59 b	0.32 ± 0.05 a	0.83 ± 0.04 a	0.98 ± 0.04 b
	SAY3-7 + BLY3-8 + P4	13.8 ± 2.3 a	50.37 ± 1.77 a	0.26 ± 0.02 a	0.82 ± 0.00 a	1.34 ± 0.06 a
Yezin-6	Control	0.0 ± 0.0 b	0.00 ± 0.00 d	0.41 ± 0.04 a	0.69 ± 0.06 b	0.00 ± 0.00 c
	P4	0.0 ± 0.0 b	0.00 ± 0.00 d	0.43 ± 0.05 a	0.84 ± 0.04 ab	0.00 ± 0.00 c
	SAY3-7	16.0 ± 2.9 a	58.70 ± 2.87 b	0.47 ± 0.04 a	1.04 ± 0.04 a	1.61 ± 0.02 b
	BLY3-8	15.0 ± 1.6 a	47.03 ± 0.71 c	0.39 ± 0.06 a	1.04 ± 0.11 a	1.47 ± 0.15 b
	SAY3-7 + BLY3-8	11.7 ± 1.7 a	65.70 ± 5.19 ab	0.45 ± 0.05 a	0.98 ± 0.01 a	2.22 ± 0.05 a
	SAY3-7 + P4	15.7 ± 5.2 a	57.60 ± 3.05 b	0.37 ± 0.02 a	0.94 ± 0.03 a	1.73 ± 0.23 b
	BLY3-8 + P4	14.3 ± 3.7 a	46.20 ± 3.16 c	0.46 ± 0.11 a	1.05 ± 0.13 a	1.51 ± 0.06 b
	SAY3-7 + BLY3-8 + P4	18.3 ± 3.4 a	71.87 ± 3.03 a	0.47 ± 0.04 a	0.94 ± 0.01 a	2.25 ± 0.10 a

For each cultivar, mean values ± SD with the same letters in each column are not significantly different at $p < 0.05$ (Tukey's test).

3.2. Expteriment II. Physicochemical Properties of Peat Soil and Biofertiliser Production Using the Peat Soil

The physicochemical properties of the peat soil are described in Table 2.

Table 2. Physicochemical properties of peat soil.

Physicochemical Properties	Values
Soil pH (Soil: H_2O; 1:2.5)	7.11
Total N (%)	0.80
Total P_2O_5 (%)	0.09
Available N (mg N/ 100 g soil)	150.26
Available P (mg P_2O_5/ 100 g soil)	4.25
CEC ($cmol_c\ Kg^{-1}$)	20.47
Exc. Ca ($cmol_c\ Kg^{-1}$)	25.61
Exc. Mg ($cmol_c\ Kg^{-1}$)	1.08

The populations of *Bradyrhizobium* strains and P4 on the peat soil were checked monthly. The results are presented in Table 3. The population of P4 decreased gradually to six months after producing the biofertiliser, but the population was maintained at 1×10^8 cfu g^{-1} to 12 months. However, the population of *Bradyrhizobium* did not decrease and was maintained at a density of 7×10^9 cells g^{-1} (MPN) in the peat from the beginning to the end of production. This result indicates that the peat soil collected from Myanmar is a suitable substrate for biofertiliser production.

Table 3. Survival of P4 and *Bradyrhizobium* on peat soil.

Months after Producing	P4	*Bradyrhizobium*
	Plate Count (cfu g^{-1})	**MPN (Cells g^{-1})**
1	1.7×10^{10}	7×10^9
2	2×10^{10}	7×10^9
3	3.2×10^{11}	7×10^9
4	2.8×10^{11}	7×10^9
5	4×10^{10}	7×10^9
6	2×10^{10}	7×10^9
7	1.2×10^9	7×10^9
8	4×10^8	7×10^9
9	4×10^8	7×10^9
10	3×10^8	7×10^9
11	1.8×10^8	7×10^9
12	1×10^8	7×10^9

Bacterial population for *Bradyrhizobium* and P4 was 1×10^{10} at the time of preparation.

Nodulation and nitrogen fixation efficiency of the bacteria from the biofertiliser were measured at 4, 8, and 12 months after producing the biofertiliser. The results are shown in Figure 1. Diluted biofertiliser until 10^{-3} proved more effective for nodulation and nitrogen fixation than the other treatments at 4, 8, and 12 months after producing the biofertiliser.

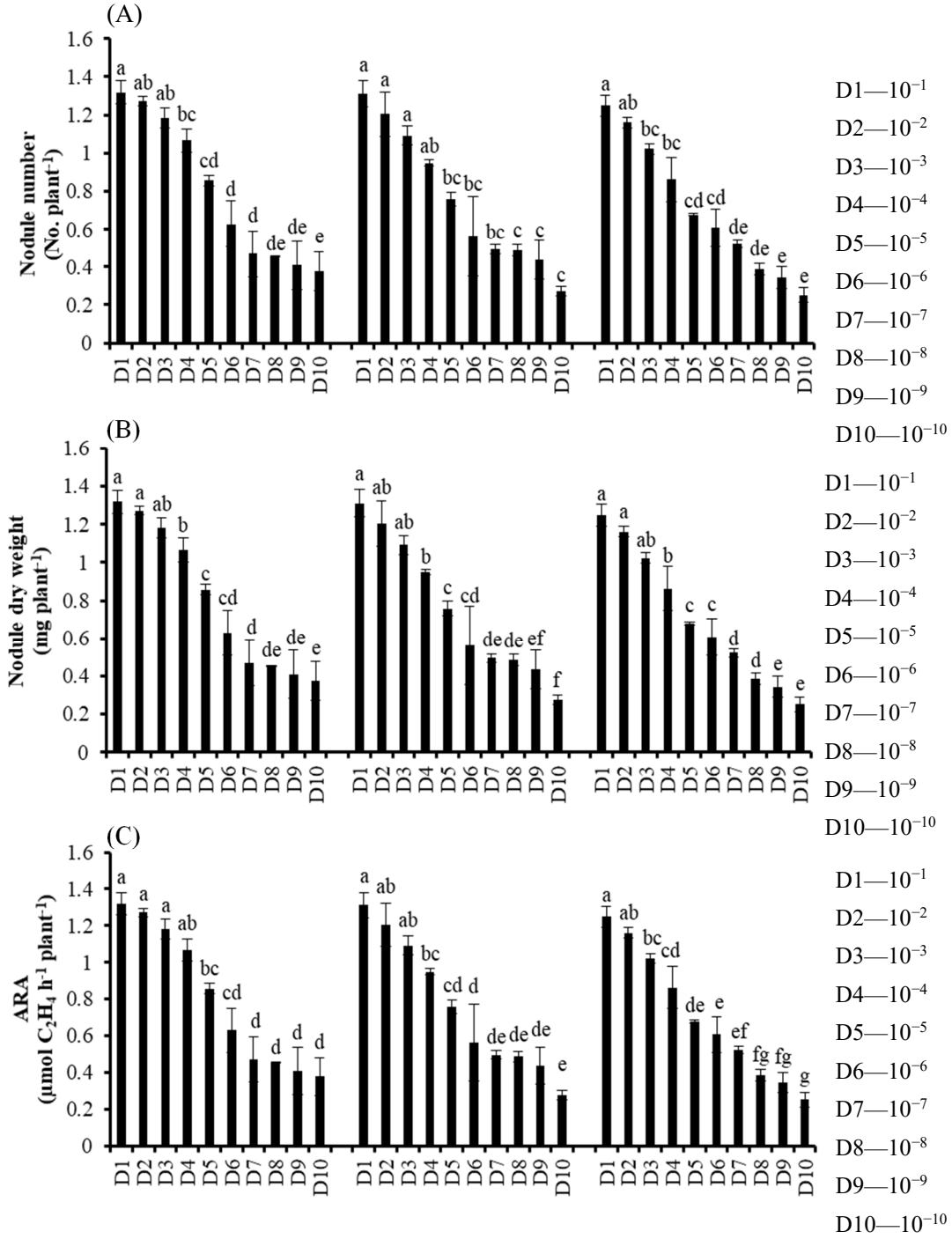

Figure 1. Effect of different dilution times of biofertiliser produced from *B. japonicum* SAY3-7 (type A), *B. elkanii* BLY3-8 (type B) and *S. griseoflavus* P4 on (**A**) nodule number, (**B**) nodule dry weight (**C**) nitrogen fixation in terms of ARA of Yezin-6 soybean varieties at 30 days after sowing. The histograms with the same letter are not significantly different at $p < 0.05$ (Tukey's test). The bar on each histogram indicates SD.

3.3. Experiment III. Evaluation of Ssymbiotic Effectiveness of Biofertiliser on Yezin-3 (Rj_4) and Yezin-6 (non-Rj) Soybean Varieties at Different Growth Stages

The results of plant growth, nodulation, and nitrogen fixation for the Yezin-3 and Yezin-6 soybean varieties at V6 stage are shown in Table 4. Nodule number, nodule dry weight, and root dry weight were not significantly different between the Yezin-3 and Yezin-6 varieties at the V6 stage. However,

shoot dry weight was significantly different between the two varieties. Shoot dry weight of Yezin-3 was significantly higher than that of Yezin-6. Moreover, inoculation with biofertiliser significantly improved nodule, root, and shoot dry weights in both varieties, but did not improve nodule number in either variety. There was no interaction effect between variety and treatment in any parameters at the V6 stage. Nitrogen fixation in terms of ARA was not significantly different between the Yezin-3 and Yezin-6 varieties at the V6 stage. However, inoculation with biofertiliser resulted in significantly higher nitrogen fixation compared with control plants for both varieties. There was no interaction effect between variety and treatment on nitrogen fixation at the V6 stage.

Table 4. Effect of biofertiliser on plant growth, nodulation and nitrogen fixation in terms of ARA of Yezin-3 and Yezin-6 soybean varieties at V6 stage.

Treatment	NN (No. plant^{-1})	NDW (mg plant^{-1})	RDW (g plant^{-1})	SDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1})
Variety					
Yezin-3	10.3 ± 1.7 A	26.98 ± 4.33 A	0.46 ± 0.07 A	1.81 ± 0.11 A	0.62 ± 0.12 A
Yezin-6	12.2 ± 2.1 A	27.57 ± 5.54 A	0.47 ± 0.06 A	1.61 ± 0.11 B	0.60 ± 0.15 A
Treatment of Yezin-3					
Control	11.7 ± 0.5 a	22.97 ± 2.19 b	0.40 ± 0.01 b	1.72 ± 0.04 b	0.50 ± 0.07 b
Biofertiliser	12.7 ± 1.9 a	31.00 ± 0.73 a	0.51 ± 0.04 a	1.91 ± 0.05 a	0.73 ± 0.01 a
Treatment of Yezin-6					
Control	9.3 ± 2.4 a	22.40 ± 0.96 b	0.41 ± 0.01 b	1.51 ± 0.05 c	0.46 ± 0.04 b
Biofertiliser	11.3 ± 1.7 a	32.73 ± 2.67 a	0.52 ± 0.03 a	1.71 ± 0.03 b	0.73 ± 0.07 a
Source of variance ($Pr > F$)					
Variety	ns	ns	ns	**	ns
Treatment	ns	**	**	**	**
Variety × Treatment	ns	ns	ns	ns	ns
CV%	8.64	9.34	4.67	2.79	8.64

NN; nodule number, NDW; nodule dry weight, SDW; shoot dry weight, RDW; root dry weight, ARA; acetylene reducing activity. Mean values ± SD with the same upper-case or lower-case letter in each column are not significantly different at $p < 0.05$ (Tukey's test). ns: nonsignificant, **: significant at $p < 0.01$.

The results of plant growth, nodulation, and nitrogen fixation of the Yezin-3 and Yezin-6 soybean varieties at the R3.5 stage are shown in Table 5. Nodule number and root dry weight were significantly different between the Yezin-3 and Yezin-6 varieties at the R3.5 stage, whereas nodule dry weight and shoot dry weight were not significantly different between the two varieties. Nodule number and root dry weight of Yezin-6 were significantly higher than those of Yezin-3. Inoculation of peat-based biofertiliser significantly improved nodule number in the Yezin-6 variety, but not in the Yezin-3 variety. Moreover, biofertiliser had significant effects on shoot dry weight in both varieties. There were no interaction effects between variety and treatment for nodule number, nodule dry weight, root dry weight, or shoot dry weight at the R3.5 stage. At R3.5, nitrogen fixation in terms of RUI(%) and %Ndfa differed significantly between varieties and between treatments. Yezin-6 had more potential for nitrogen fixation than Yezin-3. Compared with the control, utilisation of biofertiliser had significant effects on RUI(%) and %Ndfa in both soybean varieties. There was an interaction effect between variety and treatment in RUI(%) and %Ndfa at the R3.5 stage.

Table 5. Effect of biofertiliser on plant growth, nodulation and nitrogen fixation in terms of RUI (%) and % Ndfa of Yezin-3 and Yezin-6 soybean varieties at R3.5 stage.

Treatment	NN (No. plant^{-1})	NDW (mg plant^{-1})	RDW (g plant^{-1})	SDW (mg plant^{-1})	RUI (%)	% Ndfa
Variety						
Yezin-3	58.0 ± 5.7 B	370.00 ± 76.16 A	1.50 ± 0.26 B	7.39 ± 0.35 A	80.64 ± 7.10 B	88.56 ± 10.60 B
Yezin-6	83.2 ± 11.6 A	408.33 ± 41.40 A	1.96 ± 0.12 A	7.51 ± 0.31 A	86.42 ± 1.75 A	97.20 ± 2.62 A
Treatment of Yezin-3						
Control	52.7 ± 1.7 c	313.33 ± 49.22 b	1.31 ± 0.05 b	7.05 ± 0.04 b	73.57 ± 0.90 c	78.01 ± 1.34 c
Biofertiliser	63.3 ± 2.4 bc	426.67 ± 52.49 ab	1.69 ± 0.24 ab	7.72 ± 0.10 a	87.71 ± 0.22 a	99.12 ± 0.33 a
Treatment of Yezin-6						
Control	72.7 ± 5.3 b	376.67 ± 4.71 ab	1.85 ± 0.04 a	7.22 ± 0.14 b	84.81 ± 0.16 b	94.80 ± 0.23 b
Biofertiliser	93.7 ± 4.5 a	440.00 ± 37.42 a	2.07 ± 0.06 a	7.80 ± 0.03 a	88.03 ± 0.98 a	99.60 ± 1.47 a
Source of Variance (*Pr* > *F*)						
Variety	**	ns	**	ns	**	**
Treatment	**	**	*	**	**	**
Variety × Treatment	ns	ns	ns	ns	**	**
CV%	7.26	10.32	9.79	1.38	1.06	1.42

NN; nodule number, NDW; nodule dry weight, SDW; shoot dry weight, RDW; root dry weight, RUI (%); relative ureide index (%), %Ndfa; percentage of nitrogen derived from nitrogen fixation. Mean values ± SD with the same upper-case or lower-case letter in each column are not significantly different at $p < 0.05$ (Tukey's test). ns: nonsignificant, *: significant at $p < 0.05$, **: significant at $p < 0.01$.

The results of N, P, K, Ca, and Mg uptakes at the V6 stage are shown in Table 6. There were no significant differences in uptakes between the Yezin-3 and Yezin-6 varieties at the V6 stage. Application of biofertiliser did not significantly improve N, P, Ca, and Mg uptake in either variety, but biofertiliser increased K uptake in the Yezin-6 variety (but not in the Yezin-3 variety) compared with the control. There was no interaction effect between variety and treatment in nutrient uptakes at the V6 stage. The N, P, K, Ca, and Mg uptake results at the R3.5 stage are shown in Table 7. N, K, Ca, and Mg uptakes were significantly different between the Yezin-3 and Yezin-6 varieties at the R3.5 stage. The N uptake of Yezin-3 was significantly higher than that of Yezin-6. In contrast to the N uptake, the K, Ca, and Mg uptakes of Yezin-3 were significantly lower than those of Yezin-6. Application of biofertiliser did not significantly improve N, K, and Ca uptakes in either variety, but application of biofertiliser increased P and Mg uptakes in both varieties compared with the control. There was no interaction effect between variety and treatment in nutrient uptakes at the R3.5 stage. The N, P, K, Ca, and Mg uptake results at the R8 stage are shown in Table 8. N and K uptakes were significantly different between the Yezin-3 and Yezin-6 varieties at the R8 stage, but those of other nutrients were not different. The N and K uptakes of Yezin-6 were significantly higher than those of Yezin-3. Application of biofertiliser significantly increased N and K uptakes in both varieties, but biofertiliser application did not increase P, Ca, and Mg uptakes at the R8 stage in either variety compared with control. There was no interaction effect between variety and treatment on the nutrient uptakes at the R8 stage.

Table 6. Effect of biofertiliser on N, P, K, Ca and Mg uptakes of Yezin-3 and Yezin-6 soybean varieties at V6 stage.

Treatment	N (mg plant^{-1})	P (mg plant^{-1})	K (mg plant^{-1})	Ca (mg plant^{-1})	Mg (mg plant^{-1})
Varieties					
Yezin-3	33.88 ± 7.16 A	1.13 ± 0.07 A	0.38 ± 0.02 A	0.37 ± 0.06 A	0.18 ± 0.02 A
Yezin-6	38.10 ± 5.82 A	1.12 ± 0.11 A	0.39 ± 0.04 A	0.37 ± 0.04 A	0.16 ± 0.01 A
Treatment of Yezin-3					
Control	27.27 ± 2.92 b	1.15 ± 0.04 a	0.36 ± 0.03 ab	0.33 ± 0.05 a	0.18 ± 0.02 a
Biofertiliser	40.50 ± 2.51 ab	1.11 ± 0.09 a	0.41 ± 0.02 ab	0.41 ± 0.04 a	0.19 ± 0.01 a
Treatment of Yezin-6					
Control	32.82 ± 2.41 ab	1.06 ± 0.08 a	0.35 ± 0.01 b	0.38 ± 0.03 a	0.16 ± 0.01 a
Biofertiliser	43.37 ± 2.50 a	1.17 ± 0.11 a	0.43 ± 0.01 a	0.36 ± 0.04 a	0.16 ± 0.01 a
Source of Variance (*Pr* > *F*)					
Variety	ns	ns	ns	ns	ns
Treatment	**	ns	**	ns	ns
Variety × Treatment	ns	ns	ns	ns	ns
CV%	13.2	9.05	5.7	15.7	8.64

Mean values ± SD with the same upper-case or lower-case letter in each column are not significantly different at $p < 0.05$ (Tukey's test). ns: nonsignificant, **: significant at $p < 0.01$.

Table 7. Effect of biofertiliser on N, P, K, Ca and Mg uptakes of Yezin-3 and Yezin-6 soybean varieties at R3.5 stage.

Treatment	N (mg plant^{-1})	P (mg plant^{-1})	K (mg plant^{-1})	Ca (mg plant^{-1})	Mg (mg plant^{-1})
Varieties					
Yezin-3	158.59 ± 6.63 A	26.31 ± 0.68 A	2.09 ± 0.23 B	0.95 ± 0.11 B	1.20 ± 0.07 B
Yezin-6	142.16 ± 9.69 B	27.40 ± 0.99 A	2.72 ± 0.10 A	1.31 ± 0.15 A	1.38 ± 0.06 A
Treatment of Yezin-3					
Control	153.15 ± 0.87 ab	23.03 ± 0.46 b	1.76 ± 0.01 b	0.72 ± 0.02 b	1.05 ± 0.06 c
Biofertiliser	164.02 ± 5.29 a	29.60 ± 0.78 a	2.42 ± 0.13 ab	1.19 ± 0.09 ab	1.35 ± 0.08 ab
Treatment of Yezin-6					
Control	141.11 ± 8.58 b	23.70 ± 1.28 b	2.38 ± 0.06 ab	1.07 ± 0.18 ab	1.22 ± 0.07 bc
Biofertiliser	143.21 ± 10.58 b	31.09 ± 0.34 a	3.06 ± 0.05 a	1.55 ± 0.04 a	1.54 ± 0.01 a
Source of Variance (*Pr* > *F*)					
Variety	**	ns	**	*	**
Treatment	**	**	**	**	**
Variety × Treatment	ns	ns	ns	ns	ns
CV%	5.89	5.61	10.18	15.05	6.67

Mean values ± SD with the same upper-case or lower-case letter in each column are not significantly different at $p < 0.05$ (Tukey's test). ns: nonsignificant, *: significant at $p < 0.05$, **: significant at $p < 0.01$.

Table 8. Effect of biofertiliser on N, P, K, Ca and Mg uptakes of Yezin-3 and Yezin-6 soybean varieties at R8 stage.

Treatment	N (mg plant^{-1})	P (mg plant^{-1})	K (mg plant^{-1})	Ca (mg plant^{-1})	Mg (mg plant^{-1})
Varieties					
Yezin-3	192.24 ± 29.23 B	9.87 ± 3.43 A	1.49 ± 0.36 B	1.29 ± 0.25 A	0.89 ± 0.16 A
Yezin-6	238.10 ± 28.44 A	9.87 ± 3.92 A	1.73 ± 0.41 A	1.41 ± 0.30 A	0.82 ± 0.18 A
Treatment of Yezin-3					
Control	163.53 ± 3.14 c	9.66 ± 1.34 a	1.28 ± 0.12 b	1.21 ± 0.11 a	0.87 ± 0.07 a
Biofertiliser	220.94 ± 7.16 b	10.08 ± 0.44 a	1.70 ± 0.14 a	1.38 ± 0.03 a	0.90 ± 0.2 a
Treatment of Yezin-6					
Control	212.24 ± 11.44 b	9.54 ± 1.78 a	1.64 ± 0.29 a	1.34 ± 0.15 a	0.78 ± 0.11 a
Biofertiliser	263.97 ± 12.23 a	10.20 ± 0.60 a	1.81 ± 0.14 a	1.47 ± 0.22 a	0.86 ± 0.08 a
Source of Variance ($Pr > F$)					
Variety	**	ns	**	ns	ns
Treatment	**	ns	**	ns	ns
Variety × Treatment	ns	ns	ns	ns	ns
CV%	4.26	8.21	5.84	10.03	8.34

Mean values ± SD with the same upper-case or lower-case letter in each column are not significantly different at $p < 0.05$ (Tukey's test). ns: nonsignificant, **: significant at $p < 0.01$.

The results of yield and yield components at the R8 stage are shown in Table 9. Inoculation of soybeans with biofertiliser produced significantly higher seed yield in Yezin-6 than in the control, but not in Yezin-3. Moreover, the seed yield of Yezin-6 was significantly higher than that of Yezin-3. There were no interaction effects between variety and treatment on yield or yield-related parameters at the R8 stage.

Table 9. Effect of biofertiliser on yield and yield related parameters of Yezin-3 and Yezin-6 soybean varieties at R8 stage.

Treatment	Pods (No. plant^{-1})	Seed Per Pod (No. pod^{-1})	100 Seed Weight (g)	Seed Yield (g plant^{-1})
Variety				
Yezin-3	20.8 ± 3.0 A	1.8 ± 0.1 A	13.48 ± 0.95 A	5.07 ± 0.41 B
Yezin-6	20.3 ± 2.4 A	1.9 ± 0.1 A	14.51 ± 0.89 A	5.84 ± 0.59 A
Treatment of Yezin-3				
Control	19.3 ± 1.7 a	1.8 ± 0.1 a	13.75 ± 0.71 a	4.66 ± 0.06 b
Biofertiliser	22.3 ± 3.3 a	1.9 ± 0.1 a	13.22 ± 1.07 a	5.31 ± 0.11 b
Treatment of Yezin-6				
Control	18.7 ± 1.9 a	2.0 ± 0.1 a	14.31 ± 0.93 a	5.47 ± 0.25 b
Biofertiliser	22.0 ± 1.4 a	2.0 ± 0.1 a	14.71 ± 0.80 a	6.36 ± 0.30 a
Source of Variance ($Pr > F$)				
Variety	ns	ns	ns	**
Treatment	ns	ns	ns	**
Variety × Treatment	ns	ns	ns	ns
CV%	13.57	7.95	7.86	5.22

Mean values ± SD with the same upper-case or lower-case letter in each column are not significantly different at $p < 0.05$ (Tukey's test). ns: nonsignificant, **: significant at $p < 0.01$.

4. Discussion

Biological nitrogen fixation (BNF) is an essential component of sustainable agriculture [38], and inoculant of rhizobia has been applied frequently as a biofertiliser. Biofertiliser is usually produced using *Rhizobium* species. However, in this study, *Bradyrhizobium* and *Streptomyces* species were used to produce biofertiliser because the presence of the biocontrol microorganism *Streptomyces* in the rhizosphere plays an essential role in enhancing the growth and development of useful bacteria, such as nitrogen-fixing bacteria [39]. Plant growth promotion can be induced by the function of a single

bacterial strain and the synergistic functions of different bacterial species (*Streptomyces* sp. R170 with *Sphingomonas* sp. T168 or *Methylibium* sp. R182) [40]. The endophytic actinomycete strain, P4 used in this experiment was isolated from sweet pea root at Kurima, Tsu-City, Japan and its 16S rRNA sequence has 99.7% sequence identity with that of *Streptomyces griseoflavus* (Accession number: EU741217) [41]. In this study, nitrogen fixation was significantly increased by dual inoculation with *B. japonicum* SAY3-7 + *B. elkanii* BLY3-8 and also combined inoculation with *B. japonicum* SAY3-7 + *B. elkanii* BLY3-8 + *S. griseoflavus* P4 in all tested soybean varieties. The result was interesting in that different *Bradyrhizobium* species (*B. japonicum* SAY3-7 + *B. elkanii* BLY3-8) induced synergistic effects on nitrogen fixation. In contrast to the previous finding in which synergistic effects occurred due to co-inoculation of *S. griseoflavus* P4 with *B. japonicum* SAY3-7 or *B. elkanii* AHY3-1 [42], more synergistic effects on nitrogen fixation occurred with co-inoculation of different *Bradyrhizobium* species (*B. japonicum* SAY3-7 + *B. elkanii* BLY3-8). Based on the previous findings and those of the present study, we consider that the combination of *B. japonicum* SAY3-7 + *B. elkanii* BLY3-8 + *S. griseoflavus* P4 has potential for inoculant production.

Peat soil collected from Heho was used as a suitable carrier to produce the biofertiliser. Thein and Hein [43] reported that peat soil collected from Myanmar can be used as a suitable carrier, because the rhizobial population was maintained until 6 months after making the biofertiliser at local room temperatures (30–32 °C). Our results were in agreement with their finding. Diluted biofertiliser until 10^{-3} improved nodulation and nitrogen fixation until 12 months after producing the biofertiliser. The inoculation density of biofertiliser diluted by factors of 10^{-1}, 10^{-2} and 10^{-3} contained 10^{8}, 10^{7}, and 10^{6} cells, respectively. The incensement in nodulation and nitrogen fixation in the plants applied with diluted biofertiliser until 10^{-3} is might be due to the proper inoculation density. Many studies highlighted that nodulation of soybean was inhibited when inoculated with high density cell inoculum (10^{9} cell seed^{-1}) [44]. Yamakawa and Fukushima [45] reported that the application of a higher inoculum density decreased acetylene reduction activity (10^{9} cell seed^{-1}). There was also a significant increment in yield and nitrogen fixation observed at inoculation rates of 10^{5} and 10^{7} cells seed^{-1}. Albareda et al. [46] stated that nodule dry weight, seed yield, and seed N content increased when the number of rhizobia applied to seeds was increased from 10^{4} to 10^{7} cell seed^{-1}.

In this study, the plants with applied biofertiliser showed significant increases in shoot and root weight at the V6 and/or R3.5 stages. These findings are similar to those of our previous study, in which the combined use of P4 with *Bradyrhizobium* increased shoot and root biomass of soybeans [47]. This plant growth-promoting effect of P4 may be due to the secretion of growth-promoting hormones. The P4 used in biofertiliser manufacture can secrete the plant growth hormone indole acetic acid [47]. IAA controls many processes in plant growth and development and plays an important role in shaping plant root architecture, such as regulation of lateral root initiation, root vascular tissue differentiation, polar root hair positioning, root meristem maintenance and root gravitrophism [48]. In another experiment, Soe et al. [49] concluded that the Myanmar *Bradyrhizobium* strain and *S. griseoflavus* P4 will be useful as biofertiliser because of their synergy in plant growth, nodulation, and productivity of soybean. Our results confirmed that *Bradyrhizobium* strain and *S. griseoflavus* P4 could be produced as biofertiliser and we could see its effectiveness on soybean.

Our results showed that nodule number and/or nodule dry weight in plants with applied biofertiliser were significantly higher than those of the control plants at the V6 and/or R3.5 stages in the Yezin-3 and/or Yezin-6 soybean varieties. The soybean resulted in high nodule number and dry weight by *Bradyrhizobium* inoculant [50,51] and single or *mixed inoculants* of *Bradyrhizobium* with *Azotobacter chroococcum* [52]. The use of biofertiliser produced from *Bradyrhizobium* strains and P4 increased nitrogen fixation in terms of ARA at the V6 stage and increased RUI(%) and %Ndfa at the R3.5 stage compared with uninoculated controls in both soybean varieties. It has been reported that the combined use of *Bradyrhizobium* with P4 induces higher nitrogenase activity in terms of ARA [53] and increased nitrogen fixation in terms of RUI(%) and %Ndfa [54] compared with uninoculated control. Symbiotic N fixation in soybeans can provide 40–70% of the total N requirement [55]. Soe et al. [56]

found that co-inoculation with *Streptomyces* spp. P4 and indigenous Myanmar *Bradyrhizobium* was more effective for N uptake than single inoculation with *Bradyrhizobium*. In our previous study, N, P, K, Ca, and Mg uptakes were significantly improved by the combined use of SAY3-7 and P4 [20]. Moreover, N uptake was increased by the combined application of BLY3-8 and P4 [21]. Similarly, in the present study, the use of biofertiliser produced from SAY3-7, BLY3-8, and P4 significantly improved N, P, K, Ca, and Mg uptakes at some growth stages in Yezin-3 and Yezin-6 soybean varieties. The use of biofertiliser produced from *Bradyrhizobium* and P4 significantly increased seed yield in the Yezin-6 soybean variety compared with the control. These results support other findings from our group showing that the use of *Bradyrhizobium* strains with P4 increased seed yield of soybeans [57]. Combined inoculation of *Rhizobium leguminosarum*-PR1 with *Pseudomonas* sp. strain, PGERs17 also increases N, P and K uptakes of field pea (*Pisum sativum* L. variety VL Matar 42) compared with uninoculated control [58].

The legume-rhizobia symbiosis exhibits widely differing degrees of specificity. In some instances, the symbiosis is highly specific in that a particular species or strain of root nodule bacteria can induce an effective symbiosis association with only a particular legume specie or variety [59]. Senaratne et al. [60] reported that plant dry weight, nitrogen yield, percent nitrogen derived from the atmosphere and amount of nitrogen fixed were significantly influenced by specific combinations of host genotype and *Bradyrhizobium* strain. In our study, soybean variety also had influential effects on plant growth, nodulation, nitrogen fixation, and nutrient uptakes at some growth stages. This was in agreement with the findings of Soe et al. [54,56] and Soe and Yamakawa [57]. However, an interaction effect between variety and treatment occurred only on nitrogen fixation at the R3.5 stage, and not at the V6 stage or for any other parameters. This result supports the findings of others showing that biological nitrogen fixation in soybean can be increased by using effective *Bradyrhizobium* strains and efficient soybean cultivars [61].

5. Conclusions

According to the results of the tests prior to biofertiliser production, the combination of SAY3-7 + BLY3-8 + P4 has potential for inoculant production. During biofertiliser production, peat soil collected from Myanmar served as a suitable substrate for production, with the assurance of nodulation and nitrogen fixation in the Yezin-6 soybean variety. Moreover, the use of the biofertiliser produced from SAY3-7 + BLY3-8 + P4 was very effective in improving plant growth, nodulation, nitrogen fixation, nutrient uptakes, and productivity of the soybeans. Yezin-6 is the more efficient variety for improved plant growth, nodulation, nitrogen fixation, nutrient absorption, and higher seed yield. Moreover, there was an interaction effect between variety and treatment only on nitrogen fixation in terms of RUI (%) and %Ndfa at the R3.5 stage, suggesting that nitrogen fixation can be increased by using an effective biofertiliser and an efficient soybean cultivar. It can be concluded that application of an effective biofertiliser and use of an efficient soybean variety are promising methods for promoting plant growth, nodulation, nitrogen fixation, and seed yield.

Author Contributions: Conceptualization, A.Z.H. and T.Y.; methodology and investigation, A.Z.H., S.M.M., K.M., data organization and formal analysis, A.Z.H.; writing—original draft preparation, A.Z.H.; writing—review and editing, S.M.M., K.M.; supervision, T.Y.; and all authors read and approved the manuscript.

Funding: This research and APC were granted by the Ministry of Education, Culture, Sports, Science, and Technology Grant Number: [130287].

Acknowledgments: We are thankful to Ministry of Education, Culture, Sports, Sciences and Technology (MEXT) of Japan for their financial support of the present study.

Conflicts of Interest: There is no financial support or relationships that may pose conflict of interest.

References

1. Vessey, J.K. Plant growth promoting rhizobacteria as bio-fertilizers. *Plant Soil* **2003**, *255*, 571–586. [CrossRef]
2. Mazid, M.; Khan, T.A. Future of bio-fertilizers in Indian agriculture: An overview. *Int. J. Agric. Food Res.* **2015**, *3*, 10–23. [CrossRef]
3. DAR. Rhizobium biofertilizer. In *The Result of Research of Agricultural Research, Golden Jubilee*; Department of Agricultural Research: Yezin, Myanmar, 2004; pp. 114–118.
4. Schroth, M.N.; Hancock, J.G. Selected topics in biological control. *Ann. Rev. Microbiol.* **1981**, *35*, 453–476. [CrossRef] [PubMed]
5. Weller, D.M. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopathol.* **1988**, *26*, 379–407. [CrossRef]
6. Bharathi, V.; Sudhakar, R.; Parimala, K.; Reddy, V.A. Evaluation of bioagents and biofertilizers for the management of seed and seedling diseases of *Sesamum indicum* (Sesame). *eSci. J. Plant Pathol.* **2013**, *2*, 179–186.
7. Lehr, N.A.; Schrey, S.D.; Hampp, R.; Tarkka, M.T. Root inoculation with a forest soil. *Streptomycete* leads to locally and systemically increased resistance against phytopathogens in Norway Spruce. *New Phytol.* **2008**, *177*, 965–976. [PubMed]
8. Rothrock, C.S.; Gottlieb, D. Role of antibiosis in antagonism of *Streptomyces hygroscopicus* var. *Geldans to Rhizoctonia solani in Soil. Can. J. Microbiol.* **1984**, *30*, 1440–1447. [CrossRef]
9. Compant, S.; Duffy, B.; Nowak, J.; Clement, C.; Barka, E.A. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* **2005**, *71*, 4951–4959. [CrossRef]
10. Herridge, D.; Maw, J.B.; Thein, M.M.; Rupela, O.P.; Boonkerd, N.; Thao, T.Y.; Deaker, R.; Hartley, E.; Gemell, G. Expanding production and use of legume inoculants in Myanmar and Vietnam. In Proceedings of the 14th Australian Agronomy Conference, Adelaide, Australia, 21–25 September 2008.
11. Than, M.M.; San, K.K.; Thein, M.M. Evaluation of effective rhizobial strains for commercial legume inoculants. *J. Agric. For. Livest. Fish. Sci.* **2006**, *6*, 264–280.
12. Topre, S.D.; Panikar, S.S.; Mahajani, S.U. Biofertilizer: A novel approach for agriculture. *J. Agric. Biotechnol. Sustain. Dev.* **2011**, *3*, 205–208.
13. Mahanty, T.; Bhattacharjee, S.; Goswami, M.; Bhattacharyya, P.; Das, B.; Ghosh, A.; Tribedi, P. Biofertilizers: A potential approach for sustainable agriculture development. *Environ. Sci. Pollut. Res.* **2107**, *24*, 3315–3335. [CrossRef]
14. Than, H.; Aung, N.N.; Kyi, P.P. Response of rhizobial peat inoculants on five important legumes. In Proceedings of the 18th Congress Myanmar Agricultural Science Research Division, Yezin, Myanmar, 1987; pp. 1–12.
15. Smith, D.L.; Hume, D.J. Comparison of assay methods for N_2 fixation utilizing white bean and soybean. *Can. J. Plant Sci.* **1987**, *67*, 11–19. [CrossRef]
16. Ishizuka, J.; Suemasu, Y.; Mizogami, K. Preference of *Rj*-soybean cultivars for *Bradyrhizobium japonicum* for nodulation. *Soil Sci Plant Nutr.* **1991**, *37*, 15–21. [CrossRef]
17. Ishizuka, J.; Yokoyama, A.; Suemasu, Y. Relationship between serotypes of *Bradyrhizobium japonicum* and their compatibility with *Rj*-cultivars for nodulation. *Soil Sci Plant Nutr.* **1991**, *37*, 23–30. [CrossRef]
18. Htwe, A.Z.; Saeki, Y.; Moe, K.; Yamakawa, T. Determining nodulation regulatory (*Rj*) genes Myanmar soybean cultivars and their symbiotic effectiveness with *Bradyrhizobium japonicum* USDA110. *Am. J. Plant Sci.* **2015**, *6*, 2799–2810. [CrossRef]
19. Soe, K.M.; Yamakawa, T.; Hashimoto, S.; Sarr, P. Phylogenetic diversity of indigenous soybean bradyrhizobia from different agro-climatic regions in Myanmar. *Sci. Asia* **2013**, *39*, 574–583.
20. Htwe, A.Z.; Moh, S.M.; Moe, K.; Yamakawa, T. Effects of co-inoculation of *Bradyrhizobium japonicum* SAY3-7 and *Streptomyces griseoflavus* P4 on plant growth, nodulation, nitrogen fixation, nutrient uptake, and yield of soybean in a field condition. *Soil Sci. Plant Nutr.* **2018**, *64*, 222–229. [CrossRef]
21. Htwe, A.Z.; Moh, S.M.; Moe, K.; Yamakawa, T. Effects of co-inoculation of *Bradyrhizobium elkanii* BLY3-8 and *Streptomyces griseoflavus* P4 on Rj_4 soybean varieties. *Soil Sci. Plant Nutr.* **2018**, *64*, 449–454. [CrossRef]
22. Nakano, Y.; Yamakawa, T.; Ikeda, M.; Ishizuka, J. Nodulation of *Rj*-soybean varieties with *Rhizobium fredii* USDA193 under limited supply of nutrients. *Soil Sci. Plant Nutr.* **1997**, *43*, 929–932. [CrossRef]

23. Htwe, A.Z.; Yamakawa, T.; Sarr, P.S.; Sakata, T. Diversity and distribution of soybean-nodulation bradyrhizobia isolated from major soybean-growing regions in Myanmar. *Afr. J. Microbiol. Res.* **2015**, *9*, 2183–2196.
24. Kuykendall, L.D. Isolation and identification of genetically marked strains of nitrogen-fixing microsymbionts of soybeans. In *Practical Symbiotic Nitrogen Fixation Methodology*; Elkan, G.H., Ed.; Marcel Dekker: New York, NY, USA, 1987; pp. 205–220.
25. Shimizu, M.; Nakagawa, Y.; Sato, Y.; Furumai, T.; Igarashi, Y.; Onaka, H.; Yoshida, R.; Kunoh, H. Studies on endophytic actinomycetes (I) *Streptomyces* sp. isolate from rhododendron and its antifungal activity. *J. Gen. Plant Pathol.* **2000**, *66*, 360–366. [CrossRef]
26. Haider, J.; Hussam, A.K.M.A.; Ikeda, M.; Yamakawa, T.; Ishizuka, J. Effects of nitrate application on growth, nodulation and nitrogen fixation of nitrate-tolerant mutant soybean. *Soil Sci. Plant Nutr.* **1991**, *37*, 521–529. [CrossRef]
27. Ohyama, T.; Ito, M.; Kobayashi, K.; Araki, S.; Yasuyoshi, S.; Sasaki, O.; Yamazaki, T.; Soyama, K.; Tanemura, R.; Mizuno, Y.; et al. Analytical procedures of N, P, K contents in plant and manure materials using H_2SO_4–H_2O_2 Kjeldahl digestion method. *Jpn. Bull. Facul. Agric. Niigata Univ.* **1991**, *43*, 110–120.
28. Cataldo, D.A.; Schrader, L.E.; Youngs, V.L. Analysis by digestion and colrimetric assay of total nitrogen in plant tissues high in nitrate. *Crop Sci.* **1974**, *14*, 854–856. [CrossRef]
29. Murphy, J.; Riley, J.P. A modified single solution for the determination of phosphate in natural waters. *Anal. Chim. Acta* **1962**, *27*, 31–36. [CrossRef]
30. Sahrawat, K.L. Nitrogen availability indexes for submerged rice soils. *Adv. Agron.* **1983**, *36*, 415–451.
31. Truog, E. The determination of the readily available phosphorus in soils. *J. Am. Soc. Agron.* **1930**, *22*, 874–882. [CrossRef]
32. Muramoto, J.; Goto, I.; Ninaki, M. Rapid analysis of exchangeable cation and cation exchange capacity (CEC) of soils by shaking extraction method. *Soil Sci Plant Nutr.* **1992**, *63*, 210–215.
33. Somasegaran, P.; Hoben, H.J. Methods in Legume-Rhizobium Technology. Available online: https://www.ctahr.hawaii.edu/bnf/Downloads/Training/Rhizobium%20technology/Title%20Page.PDF (accessed on 12 February 2019).
34. Moore, S.; Stein, W.H. A Modified ninhydrin reagent for the photometric determination of amino acids and realted compounds. *J. Biol. Chem.* **1954**, *211*, 907–913.
35. Cataldo, D.A.; Haroon, M.; Schrader, L.E.; Youngs, V.L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 71–80. [CrossRef]
36. Young, E.G.; Conway, C.F. On the estimation of Allantoin by the Rimini-Schryver reaction. *J. Biol. Chem.* **1942**, *142*, 839–853.
37. Herridge, D.F.; Peoples, M.B. Ureide assay for measuring nitrogen fixation by nodulated soybean calibrated by 15N methods. *Plant Physiol.* **1990**, *93*, 495–503. [CrossRef] [PubMed]
38. Sessitsch, A.; Howieson, J.G.; Perret, X.; Antoun, H.; Martínez-Romero, E. Advances in rhizobium research. *Crit. Rev. Plant Sci.* **2002**, *21*, 323–378. [CrossRef]
39. Doolotkeldieva, T.; Bobusheva, S.; Konurbaeva, M. Effects of *Streptomyces* biofertilizer to soil fertility and rhizosphere's functional biodiversity of agricultural plants. *Adv. Microbiol.* **2015**, *5*, 555–571. [CrossRef]
40. Santiago, C.D.; Yagi, S.; Ijima, M.; Nashimoto, T.; Sawada, M.; Ikeda, S.; Asano, K.; Orikasa, Y.; Ohwada, T. Bacterial compatibility in combined inoculations enhances the growth of potato seedlings. *Microbes Environ.* **2017**, *32*, 14–23. [CrossRef]
41. Tang-um, J.; Niamsup, H. Chitinase production and antifungal potential of endophytic Streptomyces strain P4. *Maejo Int. J. Sci. Technol.* **2012**, *6*, 95–104.
42. Htwe, A.Z.; Yamakawa, T. Enhanced plant growth and/or nitrogen fixation by leguminous and non-leguminous crops after single or dual inoculation of *Streptomyces griseoflavus* P4 with *Bradyhizobium* strains. *Afr. J. Microbiol. Res.* **2015**, *9*, 2337–2344.
43. Thein, M.M.; Hein, M. Rhizobial inoculants production and their on-farm use in Myanmar. Extending nitrogen fixation research to farmer's fields. In Proceedings of the International Workshop on Managing Legume Nitrogen Fixation in The Cropping Suystems of Asia, Hyderabad, India, 20–24 August 1996.
44. Jitacksorn, S.; Sadowsky, M.J. Nodulation gene regulation and Quorum sensing control density- dependent suppression and restriction of nodulation in the *Bradyrhizobium japonicum*-soybean symbiosis. *Appl. Environ. Microbiol.* **2008**, *74*, 3749–3756. [CrossRef]

45. Yamakawa, T.; Fukushima, Y. Low inoculum densities of *Bradyrhizobium japonicum* USDA110 is effective on production of soybean (Glycine max L. Merr.) cultivar Fukuyutaka.). *J. Faculty Agric. Kyushu Univ.* **2014**, *59*, 45–53.
46. Albareda, M.; Rodrı'guez-Navarro, D.N.; Temprano, F.J. Soybean inoculation: Dose, N fertilizer supplementation and rhizobia persistence in soil. *Field Crops Res.* **2009**, *113*, 352–356. [CrossRef]
47. Soe, K.M.; Yamakawa, T. Evaluating the effects of *Streptomyces griseoflavus* P4 on dry weight of different crops and examine phytohormones activity in term of indole acdic acid (IAA) production. *Myanmar Agric. Res. J.* **2018**, *4*, 165–17145.
48. Fukaki, H.; Okushima, Y.; Tasaka, M. Auxin-mediated lateral root formation in higher plants. *Int. Rev. Cytol.* **2007**, *256*, 111–137.
49. Soe, K.M.; Myint, S.S.; Win, M.M.; Aung, T.T.; San, K.K.; Myint, S.S. Co-inoculation of Myanmar *Bradyrhizobium yuanmingense* MAS34 and *Streptomyces griseoflavus* P4 inoculants to improve plant Growth, seed Yield of soybean cultivars and soil fertility improvement. *Myanmar Agric. Res. J.* **2018**, *4*, 154–164.
50. Ntambo, M.S.; Chilinda, I.S.; Taruvinga, A.; Hafeez, S.; Anwar, T.; Sharif, R.; Chambi, C.; Kies, L. The effect of rhizobium inoculation with nitrogen fertilizer on growth and yield of soybeans (*Glycine max* L.). *Int. J. Biosci.* **2017**, *10*, 163–172.
51. Ulzen, J.; Abaidoo, R.C.; Mensah, N.E.; Masso, C.; AbdelGadir, A.H. *Bradyrhizobium* inoculants enhance grain Yields of soybean and cowpea in Northern Ghana. *Front. Plant Sci.* **2016**, *7*, 1770. [CrossRef]
52. Kozieł, M.; Gebala, B.; Martyniuk, S. Response of soybean to seed inoculation with *Bradyrhizobium japonicum* and with mixed inoculants of *B. japonicum* and *Azotobacter chroococcum*. *Pol. J. Microbiol.* **2013**, *62*, 457–460. [PubMed]
53. Soe, K.M.; Yamakawa, T. Evaluation of effective Myanmar *Bradyrhizobium* strains isolated from Myanmar soybean and effects of coinoculation with *Streptomyces griseoflavus* P4 for nitrogen fixation. *Soil Sci. Plant Nutr.* **2013**, *59*, 361–370. [CrossRef]
54. Soe, K.M.; Bhromsiri, A.; Karladee, D.; Yamakawa, T. Effects of endophytic actinomycetes and *Bradyrhizobium japonicum* strains on growth, nodulation, nitrogen fixation and seed weight of different soybean varieties. *Soil Sci. Plant Nutr.* **2012**, *58*, 319–325. [CrossRef]
55. Klubeck, B.P.; Hendrickson, L.L.; Zablotowicz, R.M.; Skwara, J.E.; Varsa, E.C.; Smith, S.; Isleib, T.G.; Maya, J.; Valdes, M.; Dazzo, F.B.; et al. Competitiveness of selected *Bradyrhizobium japonicum* strains in Midwestern USA soils. *Soil Sci. Soc. Am. J.* **1988**, *52*, 662–666. [CrossRef]
56. Soe, K.M.; Bhromsiri, A.; Karladee, D. Effect of selected endophytic actinomycetes (*Steptomyces* sp.) and bradyrhizobia from Myanmar on growth, nodulation, nitrogen fixation and yield of different soybean varieties. *CMU J. Nat. Sci.* **2010**, *9*, 95–109.
57. Soe, K.M.; Yamakawa, T. Low-density co-inoculation of Myanmar *Bradyrhizobium yuanmingense* MAS34 and *Streptomyces griseoflavus* P4 to enhance symbiosis and seed yield in soybean varieties. *Am. J. Plant Sci.* **2013**, *4*, 1879–1892. [CrossRef]
58. Mishra, P.K.; Bisht, S.C.; Mishra, S.; Selvakumar, G.; Bisht, J.K.; Gupta, H.S. Coinoculation of *Rhizobium Leguminosarum*-PR1 with a cold tolerant *Pseudomonas* sp. improves iron acquisition, nutrient uptake and growth of filed pea (*Pisum Sativum* L.). *J. Plant Nutr.* **2012**, *35*, 243–256. [CrossRef]
59. Somasegaran, P.; Hoben, H.J. *Handbook for Rhizobia: Methods in Legumes-Rhizobium Technology*; Springer: New York, NY, USA, 1994; pp. 1–450.
60. Senaratne, R.; Amornpinol, C.; Hardarson, G. Effect of combined nitrogen on nitrogen fixation of soybean (Glycine max L. Merril) as affected by cultivar and rhizobial strain. *Plant Siol* **1987**, *103*, 45–50. [CrossRef]
61. Htwe, A.Z.; Yamakawa, T.; Moe, K.; Dien, D.C. Symbiotic effectiveness of different indigenous *Bradyrhizobium* strains on selected *Rj*-genes harboring Myanmar soybean cultivars. *Afr. J. Microbiol. Res.* **2015**, *9*, 2345–2353.