academicJournals

Vol. 9(49), pp. 2345-2353, 14 December, 2015
DOI: 10.5897/AJMR2015.7751
Article Number: 364A9CE56544
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Symbiotic effectiveness of different indigenous *Bradyrhizobium* strains on selected *Rj*-genes harboring Myanmar soybean cultivars

Aung Zaw Htwe[1,3]*, Takeo Yamakawa[2], Kyi Moe[3] and Doan Cong Dien[1]

[1]Laboratory of Plant Nutrition, Graduate School of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.
[2]Laboratory of Plant Nutrition, Division of Molecular Biosciences, Department of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.
[3]Department of Agronomy, Yezin Agricultural University, Yezin, Naypyitaw, Myanmar.

Received 15 September, 2015; Accepted 19 November, 2015

Many scientists are working to identify effective strains of rhizobia to increase nitrogen fixation and reduce nitrogen fertilizer application. Symbiotic nitrogen fixation of leguminous crops has become an alternative to nitrogenous fertilizer, due to its higher efficiency for nitrogen fixation. This experiment was conducted to select strains for improved nitrogen fixation of soybean (*Glycine max* L.). *Bradyrhizobium* strains isolated from Myanmar were evaluated for symbiotic efficacy, using the cultivar Yezin-6 (*non-Rj*). Five *Bradyrhizobium* strains, *B. japonicum* SAY3-7, *B. elkanii* AHY3-1, *B. liaoningense* SMY3-1, *Bradyrhizobium* spp. AHY3-6 and *B. yuanmingense* SMY6-10 were shown to have superior nitrogenase activity. These five strains were evaluated for their effectiveness on different *Rj*-genes in soybean cultivars. The nitrogen fixation of *B. japonicum* SAY3-7, *B. elkanii* AHY3-1 and *B. liaoningense* SMY3-1 were higher than other indigenous strains and the standard strain, *B. japonicum* USDA110. This was particularly the case for the cultivars Yezin-6 (*non-Rj*) and Yezin-11 (Rj_4), but not for the cultivarsYezin-9 (Rj_3) and Yezin-10 (Rj_2Rj_3). *Bradyrhizobium japonicum* SAY3-7, *B. elkanii* AHY3-1 and *B. liaoningense* SMY3-1 were also evaluated on Yezin-8 (*non-Rj*) and Yezin-3 (Rj_4). *Bradyrhizobium japonicum* SAY3-7 had significantly higher nitrogenase activity on Yezin-8 (*non-Rj*), although *B. japonicum* SAY3-7 was not significantly different than *B. elkanii* AHY3-1 and *B. liaoningense* SMY3-1 on Yezin-3 (Rj_4). Therefore, *B. japonicum* SAY3-7, which was the most effective nitrogen fixing strain in all the experiments, was selected for inoculant production. According to this study, it can be concluded that the strains were specific to cultivars and,thus, selection of a strain compatible to a specific cultivar is necessary to increase symbiotic nitrogen fixation.

Key words: *Bradyrhizobium* strains, Myanmar, nitrogenase activity, *Rj*-genes, soybean.

INTRODUCTION

Soybean (*Glycine max* L.) has become an important crop in Myanmar, as it plays a significant role in human, animal and plant nutrition. It can be grown in various parts of the country. The majority of soybean production is located in the Shan State, followed by the Mandalay, Sagaing, Ayeyarwady, and Bago regions (CSO, 2006). Soybean acreage has gradually increased since 1995 to 1996, but the productivity of soybean (1.51 tons ha^{-1}) is still low compared to the world average yield (2.52 tons ha^{-1}) (MOAI, 2013).

In Myanmar, the Department of Agricultural Research (DAR) has been producing improved soybean cultivars, such as Yezin-3 and Yezin-6, to replace local varieties and to increase soybean production. Moreover, *Bradyrhizobium* strains such as TAL 377, TAL 379 and TAL 102, produced by Nitrogen Fixation for Tropical Agricultural Legumes (NifTAL), are being used as inoculants. The Plant Pathology Section of the DAR initiated rhizobial inoculant research and production. However, effective, locally adapted *Rhizobium* strains are not readily available on the Myanmar market. Therefore, exotic strains must be replaced by those that are indigenous, as they have adapted to local environmental conditions and are readily available. The symbiotic effectiveness of native *Bradyrhizobium* strains on different Myanmar soybean cultivars has been reported (Aung, 2007; Soe and Yamakawa, 2013a).

The responses of soybean cultivars vary with the *Rhizobium* strains. While some cultivars are fully compatible with other *Rhizobium*, others cannot form nodules even though the *Rhizobium* belongs to certain serogroups of *Bradyrhizobium* (Van et al., 2007). This might be due to nodulation regulatory genes called *Rj* genes found in cultivars. The *Rj* genotypes (non-*Rj*, rj_1, Rj_2, Rj_3 and Rj_4) have been found to exist in nature (Devine and Kuykendall, 1996). The nitrogen fixation rate varies among both cultivars and inoculant strains. Yamakawa et al. (1999, 2003) reported on the higher nodulation ability of $Rj_2Rj_3Rj_4$. Soe and Yamakawa (2013a, 2013c) reported that indigenous strains are effective on Yezin-6 (*non-Rj*) and Yezin-3 (Rj_4). Therefore, it is necessary to select strains that are compatible with different *Rj*-genotypes to promote soybean production through enhancing symbiotic nitrogen fixation.

Inoculation with symbiotic rhizobia for higher biological nitrogen fixation is a common agronomic practice for agricultural production in other countries. In Myanmar, the majority of farmers rely on nitrogenous fertilizer for crop production, although it is very expensive (Than and Han, 1988). An alternative to nitrogenous fertilizer is the use of effective and efficient strains of N-fixing bacteria. *Rhizobium* inoculant production is relatively inexpensive and affordable to most farmers (Than et al., 1987). Inoculation of soybean with *Rhizobium* is essential to increase productivity.

In Myanmar, researchers have been emphasizing the selection of inoculants to promote soybean production. However, the selection of strains compatible with cultivars grown in Myanmar is still limited. To foster soybean productivity, it is necessary to select strains compatible with cultivars. Therefore, the goal of the present study was to screen strains for nitrogen fixation by using Yezin-6 (*non-Rj*) and to evaluate the symbiotic effectiveness of selected *Bradyrhizobium* strains on different *Rj*-gene harboring Myanmar soybean cultivars.

MATERIALS AND METHODS

Origin of *Bradyrhizobium* strains

The *Bradyrhizobium japonicum* strain USDA110 was obtained from the Laboratory of Plant Nutrition, Kyushu University. Indigenous *Bradyrhizobium* strains were obtained from a previous experiment **(**Htwe et al., 2015a). The strains were selected from different groups identified in a phylogenetic tree. The origins of the isolates are provided in Table 1.

Origin of soybean varieties

Soybean varieties

Yezin-3 (Rj_4), Yezin-6 (*non-Rj*), Yezin-8 (*non-Rj*), Yezin-9 (Rj_3), Yezin-10 (Rj_2Rj_3) and Yezin-11 (Rj_4) were obtained from the Food Legume Section, Department of Agricultural Research, Yezin, Myanmar. Nodulation regulatory genes (*Rj* genes) indicated in parentheses were identified by Soe et al. (2013b) and Htwe et al. (2015b).

Cultivation

The 1 L pots were filled with vermiculite and 0.6 L of half-strength modified Hoagland nutrient (MHN) solution (Nakano et al., 1997). The pots were autoclaved at 120°C for 20 min. Control pots were also prepared to check for contamination. For surface sterilization, the seeds were soaked in 2.5% sodium hypochlorite solution for 5 min, rinsed with 10 mL of 99.5% ethanol five times and washed with sterilized MHN solution five times to remove the traces of sodium hypochlorite and ethanol. Five surface sterilized seeds were sown in the pots.

The single pure colony of the *B. japonicum* strain USDA110, and indigenous strains from an A1E plate, were cultured in A1E liquid media (Kuykendall, 1979) and incubated on a rotary shaker at 30°C for 7 days. One milliliter of liquid culture of each isolate was diluted with 99 mL of sterilized Hoagland solution to prepare abacterial suspension of about 10^7cells mL^{-1}. Each seed was inoculated with 5 mL of bacterial suspension. The plants were cultivated in an environmentally controlled room (25°C and 75% relative humidity) for 4 weeks. Watering was done as necessary. Autoclaved deionized water was used in this study. The first screening experiment was performed from February 2015 to March 2015. From first screening experiment, five effective strains showing the higher nitrogenase activity compared to *B. japonicum* USDA110 and other indigenous strains in Yezin-6 (*non-Rj*) soybean cultivar were selected for second screening experiment. These selected five strains were evaluated for symbiotic efficacy on different *Rj*-

*Corresponding author. E-mail: aungzawhtwe333@gmail.com.

Table 1. The origins of Bradyrhizobium strains isolated from Myanmar.

Isolate (Accession number)	Species name	Isolated area	Location
AHY6-1 (LC037244)	*B. liaoningense*	Aungban	Shan State
SMY3-1 (LC037334)	*B. liaoningense*	Myaung	Sagaing Region
MMY3-2 (LC037285)	*B. liaoningense*	Madaya	Madalay Region
MMY6-9 (LC037292)	*B. liaoningense*	Madaya	Madalay Region
SAY6-1 (LC037304)	*B. japonicum*	Aungban	Shan State
SHY3-10 (LC037323)	*B. japonicum*	Heho	Shan State
SAY3-7 (LC037300)	*B. japonicum*	Aungban	Shan State
SMY6-4 (LC037347)	*B. yuanmingense*	Myaung	Sagaing Region
SMY6-10 (LC037353)	*B. yuanmingense*	Myaung	Sagaing Region
SAY3-5 (LC037298)	*B. elkanii*	Aungban	Shan State
SAY6-2 (LC037305)	*B. elkanii*	Aungban	Shan State
AHY3-1 (LC037234)	*B. elkanii*	Hinthada	Ayeyawaddy Region
BLY6-1 (LC037264)	*B. elkanii*	Letpandan	Bago Region
BLY3-1 (LC037254)	*B. elkanii*	Letpandan	Bago Region
MMY6-1 (LC037284)	*B. elkanii*	Madaya	Madalay Region
SAY3-4 (LC037297)	*B. elkanii*	Aungban	Shan State
SAY6-5 (LC037308)	*B. elkanii*	Aungban	Shan State
AHY6-8 (LC037251)	*B. elkanii*	Hinthada	Ayeyawaddy Region
SMY3-5 (LC037338)	*B. elkanii*	Myaung	Sagaing Region
SHY3-1 (LC037314)	*Bradyrhizobium* spp.	Heho	Shan State
SHY6-1 (LC037324)	*Bradyrhizobium* spp.	Heho	Shan State
MMY3-5 (LC037278)	*Bradyrhizobium* spp.	Madaya	Madalay Region
MMY3-7 (LC037280)	*Bradyrhizobium* spp.	Madaya	Madalay Region
AHY3-6 (LC037239)	*Bradyrhizobium* spp.	Hinthada	Ayeyawaddy Region
AHY3-9 (LC037242)	*Bradyrhizobium* spp.	Hinthada	Ayeyawaddy Region

Source: Htwe et al. (2015a).

gene-harboring soybean cultivars designated as Yezin-6 (*non-Rj*), Yezin-9 (Rj_3), Yezin-10 (Rj_2Rj_3) and Yezin-11 (Rj_4) from June 2015 to July 2015. Htwe et al. (2015b) stated that Yezin-6 (*non-Rj*), Yezin-9 (Rj_3), Yezin-10 (Rj_2Rj_3) and Yezin-11 (Rj_4) showed the higher nitrogen fixation and nodulation due to inoculation with *B. japonicum* USDA110. Therefore, these four efficient soybean cultivars for nitrogen fixation were used to evaluate the symbiotic effective of selected strains for second screening experiment. From second screening experiment, three different strains showing higher nitrogenase activity in Yezin-6 (*non-Rj*) and Yezin-11 (Rj_4) were selected for further experiment. Symbiotic effectiveness of these three selected strains was tested on Yezin-3 (Rj_4) and Yezin-8 (*non-Rj*) in August 2015. This experiment aimed to check the effectiveness of these selected strains on other Rj_4 and *non-Rj* genotypes soybean cultivars. Nodulation, nitrogenase activity, shoot dry weight and root dry weight were measured from three plants per pot at 28 days after sowing.

Acetylene reduction assay

The soybean plants were cut at the cotyledonary nodes and the roots with intact nodules placed in 100 mL conical flasks. Flasks were sealed with a serum stopper and injected with 12 mL of (C_2H_2) gas to replace air with acetylene. One-mL subsamples were analyzed for ethylene (C_2H_4) concentration at 5 and 65 min after injecting with C_2H_2 gas, using a flame ionization gas chromatograph (GC-14A, Shimadzu, Kyoto, Japan) equipped with a stainless steel column (3 mm diameter, 0.5 m long) as described by Soe and Yamakawa (2013a). After the assay, nodules were counted. Shoots, roots and nodules were collected separately and oven dried at 70°C for 24 h to record dry weights.

Statistical analysis

Data were analyzed using the STATISTIX 8 software package (Analytical Software, Tallahassee, FL, USA) and the means were compared by Tukey's HSD test with a P value < 0.05 taken to indicate statistical significance.

RESULTS

Screening of effective bacterial strains by Yezin-6 (*non-Rj*) for N fixation

The cultivar Yezin-6 (*non-Rj*) was inoculated with 25 *Bradyrhizobium* strains isolated in Myanmar and the strain USDA110, and compared for nodulation and nitrogenase activity. They were not significantly different in terms of shoot or root dry weight (Table 2). The highest nodule counts were found in the plants inoculated with *B. japonicum* USDA110. The highest nodule dry weights

Table 2. Effect of Myanmar Bradyrhizobial strains on the acetylene-reducing activity, nodulation and plant growth of Yezin-6 soybean cultivar at 28 days after sowing.

Strain	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1})
AHY6-1	10.00^{abcd}	28.60^{ab}	0.39^{a}	0.18^{a}	1.23^{ab}
SMY3-1	8.33^{abcd}	24.00^{ab}	0.41^{a}	0.17^{a}	**1.95^{ab}**
MMY3-2	10.67^{abcd}	24.70^{ab}	0.33^{a}	0.15^{a}	1.46^{ab}
MMY6-9	8.67^{abcd}	23.70^{ab}	0.34^{a}	0.17^{a}	1.45^{ab}
SAY6-1	10.33^{abcd}	26.60^{ab}	0.33^{a}	0.14^{a}	0.70^{ab}
SHY3-10	9.67^{abcd}	30.90^{ab}	0.37^{a}	0.15^{a}	1.12^{ab}
SAY3-7	5.67^{cd}	33.50^{ab}	0.34^{a}	0.17^{a}	**2.21^{a}**
SMY6-4	9.67^{abcd}	24.80^{ab}	0.38^{a}	0.17^{a}	1.29^{ab}
SMY6-10	8.00^{bcd}	25.00^{ab}	0.38^{a}	0.17^{a}	**1.45^{ab}**
SAY3-5	13.67^{abc}	35.80^{ab}	0.40^{a}	0.17^{a}	1.22^{ab}
SAY6-2	10.33^{abcd}	27.90^{ab}	0.34^{a}	0.16^{a}	1.15^{ab}
AHY3-1	10.33^{abcd}	36.10^{ab}	0.33^{a}	0.15^{a}	**2.00^{ab}**
BLY6-1	7.67^{bcd}	28.90^{ab}	0.38^{a}	0.16^{a}	1.48^{ab}
BLY3-1	9.00^{abcd}	32.60^{ab}	0.35^{a}	0.15^{a}	1.35^{ab}
MMY6-1	7.33^{bcd}	36.50^{ab}	0.35^{a}	0.15^{a}	1.32^{ab}
SAY3-4	15.67^{ab}	40.20^{a}	0.33^{a}	0.16^{a}	1.21^{ab}
SAY6-5	10.67^{abcd}	31.00^{ab}	0.29^{a}	0.13^{a}	1.05^{ab}
AHY6-8	10.67^{abcd}	29.90^{ab}	0.26^{a}	0.15^{a}	1.22^{ab}
SMY3-5	11.67^{abcd}	35.40^{ab}	0.30^{a}	0.13^{a}	1.34^{ab}
SHY3-1	12.67^{abcd}	38.20^{ab}	0.32^{a}	0.13^{a}	0.90^{ab}
SHY6-1	8.00^{bcd}	40.40^{a}	0.37^{a}	0.15^{a}	1.22^{ab}
MMY3-5	5.67^{cd}	25.90^{ab}	0.34^{a}	0.16^{a}	0.81^{b}
MMY3-7	6.33^{cd}	27.40^{ab}	0.33^{a}	0.19^{a}	1.47^{ab}
AHY3-6	8.00^{bcd}	23.60^{ab}	0.30^{a}	0.17^{a}	**1.57^{ab}**
AHY3-9	4.67^{d}	19.30^{b}	0.28^{a}	0.15^{a}	1.00^{ab}
USDA110	17.00^{a}	21.90^{ab}	0.30^{a}	0.14^{a}	0.86^{ab}

Mean values in each column followed by the same letters are not significantly different at $P<0.05$ (Tukey's test). NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; RDW, root dry weight; ARA, acetylene reduction activity. The selection of strains was based on nitrogenase activity. Isolates in bold were selected for second screening experiment.

were obtained from the plants inoculated with *Bradyrhizobium* spp. SHY6-1 and *B. elkanii* SAY3-4, but they were only significantly different from the strain *Bradyrhizobium* spp. AHY3-9.

In this screening experiment, the strains were compared for their ability to fix N, in terms of acetylene reduction activity (ARA) per plant. The nitrogenase activity of each bacterial strain on the cultivar Yezin-6 (non-*Rj*) is described in Table 2. The highest ARA per plant was observed for the *B. japonicum* strain SAY3-7. The top four *Bradyrhizobium* strains, designated as *B. japonicum* SAY3-7, *B. elkanii* AHY3-1, *B. liaoningense* SMY3-1 and *Bradyrhizobium* spp. AHY3-6, had superior N fixing ability compared to the other strains. Nitrogenase activities of *B. liaoningense* MMY3-2, *B. elkanii* BLY6-1 and *Bradyrhizobium* spp. MMY3-7 were higher than that of *B. yuanmingense* SMY6-10, but they were not significantly different. Therefore, we selected the *B. yuanmingense* SMY6-10 because it belonged to different species from top four strains. Moreover, *B. yuanmingense* SMY6-10 was superior in terms of N fixation ability among the *B. yuanmingense* strains. Therefore, these five strains were selected to study their symbiotic effectiveness on different *Rj* genotypes.

Evaluation of the effectiveness of selected *Bradyrhizobium* strains on different *Rj*-gene soybean cultivars

Five *Bradyrhizobium* strains, designated as *B. japonicum* SAY3-7, *B. elkanii* AHY3-1, *B. liaoningense* SMY3-1, *Bradyrhizobium* spp. AHY3-6 and *B. yuanmingense* SMY6-10, and the control strain *B. japonicum* USDA110, were compared for symbiotic effectiveness on four soybean cultivars. For the cultivar Yezin-6 (non-*Rj*) inoculated with different *Bradyrhizobium* strains, the nitrogenase activity and nodule number per plant differed significantly among the strains (Table 3). Similarly, inoculation with *B. liaoningense* SMY3-1 showed

Table 3. Effect of six different bradyrhizobial strains on acetylene reduction activity, nodulation and plant growth of Yezin-6 soybean cultivar at 28 DAS.

Strain	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1})
SAY3-7	3.67^{c}	26.00^{a}	0.45^{a}	0.22^{a}	1.25^{ab}
AHY3-1	17.33^{a}	38.40^{a}	0.51^{a}	0.26^{a}	0.90^{b}
SMY3-1	13.00^{ab}	23.80^{a}	0.46^{a}	0.22^{a}	1.67^{a}
SMY6-10	10.33^{abc}	20.60^{a}	0.44^{a}	0.17^{a}	0.82^{b}
AHY3-6	6.33^{bc}	23.50^{a}	0.48^{a}	0.22^{a}	0.82^{b}
USDA110	15.00^{a}	17.90^{a}	0.37^{a}	0.19^{a}	0.64^{b}

Mean values in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; RDW, root dry weight; ARA, acetylene reduction activity.

Table 4. Effect of six different bradyrhizobial strains on acetylene reduction activity, nodulation and plant growth of Yezin-9 soybean cultivar at 28 DAS.

Strain	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1})
SAY3-7	8.33^{a}	31.10^{a}	0.43^{a}	0.20^{a}	1.13^{a}
AHY3-1	7.67^{a}	24.50^{b}	0.37^{a}	0.18^{a}	0.82^{a}
SMY3-1	7.67^{a}	13.40^{d}	0.33^{a}	0.17^{a}	1.01^{a}
SMY6-10	6.00^{a}	19.20^{bc}	0.39^{a}	0.18^{a}	0.97^{a}
AHY3-6	7.00^{a}	18.60^{cd}	0.36^{a}	0.15^{a}	0.63^{a}
USDA110	6.67^{a}	21.20^{bc}	0.40^{a}	0.19^{a}	0.97^{a}

Mean values in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; RDW, root dry weight; ARA, acetylene reduction activity.

significantly higher nitrogenase activity among tested strains, but the activity was not significantly different from *B. japonicum* SAY3-7. *Bradyrhizobium elkanii* AHY3-1 and *B. japonicum* USDA110 produced a significantly greater number of nodules than the other strains, but nodule production was not significantly different from that of *B. liaoningense* SMY3-1 and *B. yuanmingense* SMY6-10. In the cultivar Yezin-6 (non-*Rj*), dry weights of nodules, shoots and roots were not significantly different among the *Bradyrhizobium* strains (Table 3). These results confirmed that *B. liaoningense* SMY3-1 and *B. japonicum* SAY3-7 were superior in terms of the ability to fixN, and *B. elkanii* AHY3-1 was superior for nodulation.

In cultivar Yezin-9 (Rj_3), nodule dry weight differed significantly among the inoculated *Bradyrhizobium* strains (Table 4), although nodule number, shoot dry weight and root dry weight N activity were not significantly different. Among the tested strains, *B. japonicum* SAY3-7 produced a significantly greater nodule mass. *Bradyrhizobium japonicum* SAY3-7 had the highest N fixing ability among inoculated strains, although it was not superior for nitrogenase activity. These results indicated that the *B. Japonicum* strain SAY3-7 was more effective in terms of nodulation, N fixation and plant growth for the cultivar Yezin-9 (Rj_3).

There was significant difference in nodule number and nodule dry weight for the cultivar Yezin-10 (Rj_2Rj_3), inoculated with *Bradyrhizobium* strains. *Bradyrhizobium elkanii* AHY3-1 produced a sgnificantly greater number of nodules and higher nodule dry weight, although there were no significant differences with *B. liaoningense* SMY3-1. Nitrogenase activity and the shoot and root dry weights were not significantly different among the inoculated strains (Table 5). These results confirmed that *B. elkanii* AHY3-1 was more effective in nodule formation on the roots of Yezin-10 (Rj_2Rj_3).

For Yezin-11 (Rj_4), the nitrogenase activity, nodule number and nodule dry weight were significantly different among the *Bradyrhizobium* strains (Table 6). A higher level of N fixation, in term of nitrogenase activity, was observed for Yezin-11 (Rj_4) inoculated with *B. japonicum* SAY3-7and *B. elkanii* AHY3-1, although they were not significantly different from Yezin-11 (Rj_4) inoculated with *B. liaoningense* SMY3-1, *B. yuanmingense* SMY6-10 or *B. japonicum* USDA110. *B. elkanii* AHY3-1 produced a significantly greater number of nodules compared to the other strains, but it was not significantly different from *B. japonicum* USDA110. Moreover, *B. elkanii* AHY3-1 produced a significantly higher nodule dry weight compared with the other strains. Shoot and root dry weights were not significantly different among the *Bradyrhizobium* strains (Table 6). These results demonstrated that the strains *B. elkanii* AHY3-1 and *B. japonicum* SAY3-7 were more effective in terms of

Table 5. Effect of six different bradyrhizobial strains on acetylene reduction activity, nodulation and plant growth of Yezin-10 soybean cultivar at 28 DAS.

Strain	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW(g plant^{-1})	ARA (µmol C_2H_4 h^{-1} plant^{-1})
SAY3-7	4.33^{c}	11.20^{b}	0.35^{a}	0.15^{a}	0.44^{a}
AHY3-1	15.67^{a}	23.60^{a}	0.42^{a}	0.19^{a}	0.54^{a}
SMY3-1	13.33^{ab}	16.40^{ab}	0.43^{a}	0.18^{a}	0.77^{a}
SMY6-10	8.67^{bc}	11.80^{b}	0.41^{a}	0.17^{a}	0.48^{a}
AHY3-6	10.33^{bc}	11.00^{b}	0.41^{a}	0.17^{a}	0.59^{a}
USDA110	9.00^{bc}	13.70^{b}	0.36^{a}	0.17^{a}	0.56^{a}

Mean values in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; RDW, root dry weight; ARA, acetylene reduction activity.

Table 6. Effect of six different bradyrhizobial strains on acetylene reduction activity, nodulation and plant growth of Yezin-11 soybean cultivar at 28 DAS.

Strain	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (µmol C_2H_4 h^{-1} plant^{-1})
SAY3-7	5.67^{b}	27.20^{ab}	0.39^{a}	0.20^{a}	1.41^{a}
AHY3-1	19.00^{a}	33.50^{a}	0.46^{a}	0.24^{a}	1.41^{a}
SMY3-1	11.67^{ab}	23.20^{abc}	0.43^{a}	0.22^{a}	1.03^{ab}
SMY6-10	7.33^{b}	11.90^{c}	0.48^{a}	0.24^{a}	0.67^{ab}
AHY3-6	5.67^{b}	18.60^{bc}	0.45^{a}	0.20^{a}	0.59^{b}
USDA110	14.00^{ab}	25.10^{abc}	0.42^{a}	0.22^{a}	1.23^{ab}

Mean values in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; RDW, root dry weight; ARA, acetylene reduction activity.

Table 7. Effect of three different bradyrhizobial strains on acetylene reduction activity, nodulation and plant growth of Yezin-3 soybean cultivar at 28 DAS.

Treatment	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (µmol C_2H_4 h^{-1} plant^{-1})
SAY3-7	9.33^{b}	28.00^{a}	0.57^{a}	0.23^{a}	0.85^{a}
AHY3-1	27.00^{a}	39.70^{a}	0.75^{a}	0.32^{a}	0.53^{a}
SMY3-1	14.33^{ab}	31.50^{a}	0.65^{a}	0.24^{a}	0.95^{a}

Mean values in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; RDW, root dry weight; ARA, acetylene reduction activity.

nitrogen fixation on Yezin-11 (*Rj*$_4$). Moreover, it was found that *B. elkanii* AHY3-1 has very efficientnodulation, as indicated by superior nodule counts and higher nodule dry weight.

Effectiveness of selected *Bradyrhizbium* strains on Yezin-3 (*Rj*$_4$) and Yezin-8 (*non-Rj*)

Three *Bradyrhizobium* strains, designated as *B. japonicum* SAY3-7, *B. elkanii* AHY3-1, and *B. liaoningense* SMY3-1, were evaluated on the soybean cultivars Yezin-3 (*Rj*$_4$) and Yezin-8 (*non-Rj*) for symbiotic effectiveness and compatibility. On Yezin-3 (*Rj*$_4$), nodule number was significantly different among these inoculated *Bradyrhizobium* strains (Table 7). Nodule dry weight, shoot dry weight, root dry weight and nitrogenase activity did not differ significantly among the tested isolates. *B. elkanii* AHY3-1 produced more nodules than the other strains, but nodule production was not significantly different from *B. liaoningense* SMY3-1. *B. elkanii* AHY3-1 had greater nodule dry weight compared to the other strains. These results indicated that *B. elkanii* AHY3-1 is superior for nodule production efficiency on Yezin-3 (*Rj*$_4$).The nitrogenase activities of *B. liaoningense* SMY3-1 and *B. japonicum* SAY3-7 was relatively greater than for *B. elkanii* AHY3-1, although they were not significantly different. These results suggest that *B. liaoningense* SMY3-1 and *B. japonicum* SAY3-7 are more effective, compared to the other strains, in terms of N

Table 8. Effect of three different bradyrhizobial strains on acetylene reduction activity, nodulation and plant growth of Yezin-8 soybean cultivar at 28 DAS.

Treatment	NN (No. plant^{-1})	NDW (mg plant^{-1})	SDW (g plant^{-1})	RDW (g plant^{-1})	ARA (μmol C_2H_4 h^{-1} plant^{-1})
SAY3-7	17.00^a	50.30^a	0.54^a	0.29^a	1.31^a
AHY3-1	19.00^a	41.70^a	0.46^{ab}	0.27^a	0.55^b
SMY3-1	19.67^a	23.10^b	0.42^b	0.25^a	0.74^b

Mean values in each column followed by the same letters are not significantly different at $P < 0.05$ (Tukey's test). NN, nodule number; NDW, nodule dry weight; SDW, shoot dry weight; RDW, root dry weight; ARA, acetylene reduction activity.

fixation on Yezin-3 (Rj_4).

For Yezin-8 (non-*Rj*), nodule dry weight, shoot dry weight and nitrogenase activity were different among the *Bradyrhizobium* strains (Table 7). Inoculation with *B. japonicum* SAY3-7 resulted in significantly higher nitrogenase activity among the tested strains. Significantly greater nodule dry weight and shoot weight were obtained from the plants inoculated with *B. japonicum* SAY3-7. Nodule number and root dry weight were not significantly different among the *Bradyrhizobium* strains (Table 8). These results highlighted *B. japonicum* SAY3-7 as having greater N fixing ability.

DISCUSSION

Nitrogen is an essential nutrient for plant growth. However, its availability is one of the major limiting factors in plant growth and development (Newbould, 1989). Therefore, current agricultural practices primarily rely on chemical fertilizers to increase productivity (Peoples et al., 2009; Jensen et al., 2012). The heavy use of chemical fertilizers in agriculture is of global concern: alternatives to chemical fertilizers are urgently needed (Sharma and Kumawat, 2011). Biological nitrogen fixation technology is now one such alternative, as it reduces the need for chemical nitrogenous fertilizer (Stacey et al., 2006). Therefore, rhizobia have been used extensively in agriculture to enhance nitrogen fixation (Elkan, 1992).

Soybean, in symbiosis with *Bradyrhizobium*, has the ability to fix nitrogen at a rate of up to 300 kg N ha^{-1} under favorable conditions (Smith and Hume, 1987). Optimizing this symbiosis can both increase yields and improve soil fertility, thus reducing the costs and environmental impacts caused by nitrogen fertilizer application (Peoples et al., 2009; Canfield et al., 2010). Therefore, increasing the use of legume crops and identifying important factors related to nodulation are needed to reduce the use of nitrogenous fertilizers and improve the sustainability of agriculture (Peoples et al., 2009; Canfield et al., 2010; Jensen et al., 2012). Selection of an efficient strain is considered to be one of the major factors affecting symbiotic N fixation.

Selection of *Rhizobium* strains suitable for each legume cultivar is essential in inoculant production. To produce inoculants for each recommended cultivar of soybean, several strains and isolates of rhizobia were initially screened in the greenhouse for their symbiotic effectiveness and nitrogen fixation ability. Then, selected strains and isolates were produced as inoculants and tested for nodulation, nitrogen fixation ability and yield response (Boonkerd and Singleton, 2002). Screening experiments were performed in a greenhouse, under controlled conditions, to select the most effective strains for each cultivar.

The symbiotic effectiveness of 25 *Bradyrhizobium* strains on Yezin-6 (non-*Rj*) was determined, as cultivars harboring non-*Rj* genes are compatible with all types of *Bradyrhizobial* strains (Ishizuka et al., 1991). The top four strains showing higher nitrogenase activity were *B. japonicum* SAY3-7, *B. elkanii* AHY3-1, *B. liaoningense* SMY3-1 and *Bradyrhizobium* spp. AHY3-6. Moreover, *B. yuanmingense* SMY6-10 belonged to different species from top four strains was also selected. Since the effectiveness of *Rhizobium* strains varies, the first step for inoculant production is to obtain the most effective strain for N fixation for the legumes to be inoculated (Kucey et al., 1988). Therefore, these five strains were selected for the next experiment.

Five strains were evaluated for their symbiotic effectiveness on four soybean cultivars: Yezin-6 (*non-Rj*), Yezin-9 (Rj_3), Yezin-10 (Rj_2Rj_3) and Yezin-11 (Rj_4). When they were compared fornitrogenase activity, it was found that all tested Myanmar *Bradyrhizobial* strains were more effective on Yezin-6 (*non-Rj*)and Yezin-11 (Rj_4), but not on Yezin-9 (Rj_3) and Yezin-10 (Rj_2Rj_3). This might be due to regulatory genes, as *Rj* genes have the ability to control the compatibility of hosts and rhizobia (Ishizuka et al., 1991; Saeki et al., 2000). In this study, *Bradyrhizobium* spp. AHY3-6 and *B. yuanmingense* SMY6-10 strains showed lower N fixation efficiency, in terms of ARA, on all cultivars when compared with other strains. It was clear that the N fixation rates depended on not only cultivars and inoculated bacterial strains, but also on the interaction between cultivar and strain. Graham (2000) showed that nitrogen fixation levels vary among legume cultivars; therefore, the higher efficiency with respect to nitrogen fixation is also dependent on particular combinations of strains and cultivarsin some crops. It has also been reported that the effectiveness of *Bradyrhizobium* strains varies with soybean genotype (Okereke et al., 2001; Tien et al., 2002).

The top three strains, *B. japonicum* SAY3-7, *B. liaoningense* SMY3-1 and *B. elkanii* AHY3-1,which were shown to be more effective on Yezin-6 (*non-Rj*) and Yezin-11 (Rj_4) cultivars, were tested on other Rj_4 and *non-Rj* cultivars, identified as Yezin-3 and Yezin-8, respectively, to determine their compatibility and effectiveness. These results indicated that *B. japonicum* SAY3-7 had the highest nitrogen fixing ability on Yezin-8 (*non-Rj*), but the nitrogenase activity of that strain was not significantly different from those of *B. liaoningense* SMY3-1 and *B. elkanii* AHY3-1 strains on Yezin-3 (Rj_4). In Yezin-3 (Rj_4) soybean cultivar, *B. elkanii* AHY3-1 produced a greater number of nodules and nodule mass although nitrogenase activity did not differ among inoculated strains. Adhikari et al. (2013) also stated that strains producing greater nodule mass do not increase N fixation, in terms of ARA.

B. japonicum SAY3-7 had consistently higher nitrogenase activity, especially on *non-Rj*- and Rj_4- gene harboring soybean cultivars was selected for *Rhizobium* inoculant production. Israel et al. (1986) reported that selection of host cultivar-compatible inoculantsis important for increasing nitrogen fixation in soybean. The extent of nitrogen fixation by soybean cultivars may vary depending on the symbiotic effectiveness of *Rhizobium* strains and their compatibility. Our results supported the findings of other studies which is clearly stated that increases in biological nitrogen fixation in soybean production can be obtained by selecting effective strains and efficient soybean cultivars as cultivar-strain pairs (Duong et al., 1984; Kucey et al., 1988; Thi, 2007; Soe and Yamakawa, 2013a).

Conclusion

In this study, 25 *Bradyrhizobium* strains were selected and studied for their symbiotic effectiveness on the cultivar Yezin-6 (non-*Rj*).The five *Bradyrhizobium* strains, *B. japonicum* SAY3-7, *B. elkanii* AHY3-1, *B. liaoningense* SMY3-1, *Bradyrhizobium* spp*.* AHY3-6 and *B. yuanmingense* SMY6-10, were selected for their greater nitrogen fixing capacity. They were tested on different *Rj*-gene-harboring Myanmar soybean cultivars. *B. japonicum* SAY3-7, *B. liaoningense* SMY3-1, and *B. elkanii* AHY3-1 were more effective on Yezin-6 (*non-Rj*) and Yezin-11 (Rj_4), whereas they were not effective on Yezin-9 (Rj_3) or Yezin-10 (Rj_2Rj_3). These three strains were superior in terms of nitrogen fixing ability compared with other indigenous strains and the most widely used exotic strain, *B. japonicum* USDA110. They were tested on Yezin-8 (*non-Rj*) and Yezin-3 (Rj_4) to evaluate their effectiveness and compatibility. In this experiment, *B. japonicum* SAY3-7 was found to be the most effective strain for N fixationon Yezin-8 (non-*Rj*). Over all of the experiments, *B. japonicum* SAY3-7 was the most effective strain for N fixation, as it showed superior nitrogenase activity on Yezin-6 (non-*Rj*) in the first screening experiment, on Yezin-6 (*non-Rj*), Yezin-9 (Rj_3) and Yezin-11 (Rj_4) cultivars in the second experiment, and on Yezin-8 (non-*Rj*) in the third experiment. According to the results of this study, the most effective strain, *B. japonicum* SAY3-7, was selected to be used as an inoculant for specific cultivars to increase soybean production in Myanmar. This study was conducted in pots under controlled conditions. *B. japonicum* SAY3-7 should also be evaluated on Yezin-6 (*non-Rj*), Yezin-8 (*non-Rj*) and Yezin-11 (Rj_4) and Yezin-3 (Rj_4) for symbiotic effectiveness under field conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work was supported by Ministry of Education, Culture, Sports, Sciences and Technology (MEXT) of Japan.

REFERENCES

Adhikari D, Itoh K, Suyama K (2013). Genetic diversity of common bean (*Phaseolus vulgaris* L.) nodulating rhizobia in Nepal. Plant Soil 368:341-353.

Aung TT (2007). Selection of effective *Bradyrhizobium* strains for soybean (*Glycine max*). MSc thesis, Yezin Agricultural University (YAU), Yezin, Nayyitaw, Myanmar.

Boonkerd N, Singleton P (2002). Production of rhizobium biofertilizer. In: Kannaiyan (Ed.) Biotechnology of biofertilizers. Narosa Publishing House, New Delhi, India. pp. 122-128.

Canfield DE, Glazer AN, Falkowski PG (2010). The evolution and future of earth's N cycle. Science 330:192-196.

CSO (2006). Myanmar Agricultural Statistics (1992-1993 to 2004-2005). Central Statistical Organization, Ministry of National Planning and Economic Development, Yangon. The Republic of the Union of Myanmar.

Devine TE, Kuykendall LD (1996). Host Genetic control of symbiosis in soybean (*Glycine max* L.). Plant Soil 186:173-187.

Duong TP, Diep CN, Khiem NT, Hiep NH, Toi NV, Nhan LTK (1984).*Rhizobium* inoculant for soybean (*Glycine max* (L.) Merrill) in Mekong Delta. Response of soybean to *Rhizobium* inoculation. Plant Soil 79:235-240.

Elkan GH (1992). Taxonomy of the rhizobia. Can. J. Microbiol. 38:46-450.

Graham PH (2000).N transformations in Hand Book of Soil Science.In: Sumner ME (Ed.) CRC Press, London, UK. pp. C-141.

Htwe AZ, Saeki Y, Moe K, Yamakawa T (2015b).Determining nodulation regulatory (*Rj*) genes Myanmar soybean cultivars and their symbiotic effectiveness with *Bradyrhizobium japonicum* USDA110. Am. J. Plant Sci. 6:2799-2810.

Htwe AZ, Yamakawa T, Sarr PS, Sakata T (2015a). Diversity and distribution of soybean-nodulation bradyrhizobia isolated from major soybean-growing regions in Myanmar. Afr. J. Microbiol. Res. 9(43):2183-2196.

Ishizuka J, Suemasu Y, Mizogami K (1991). Preference of *Rj*-soybean cultivars for *Bradyrhizobium japonicum* for nodulation. Soil Sci. Plant

Nutr. 37:15-21.

Israel DW, Mathis JN, Barbour WM, Elkan GH (1986). Symbiotic Effectiveness and host-strain interactions of *Rhizobium fredii* USDA 191 on different soybean cultivars. Appl. Environ. Microbiol. 51:898-903.

Jensen ES, Peoples MB, Boddey RM, Gresshoff PM, Hauggaard-Nielsen H, Alves BJR, Morrison MJ (2012). Legumes for mitigation of climate change and feedstock in a bio-based economy: A review. Agron. Sustain. Dev. 32:329-364.

Kucey RMN, Snutwongse P, Chaiwanakupt P, Wadisirisuk P, Siripaibool C, Aryangkool T, Boonkerd N and Rennie RJ (1988).Nitrogen fixation (N-15 dilution) with soybeans under Thai field conditions. Developing protocols for screening *Bradyrhizobium Japonicum* strains. Plant Soil 108:33-41.

Kuykendall LD (1979). Transfer of R factors to and between genetically marked sublines of *Rhizobium japonicum*. Appl. Environ. Microbiol. 37:862-866.

MOAI (2013). Ministry of Agriculture and Irrigation,Myanmar agriculture in brief, Naypyitaw, The Republic of the Union of Myanmar.

Nakano Y, Yamakawa T, Ikeda M, Ishizuka J (1997). Nodulation of *Rj*-soybean varieties with *Rhizobium fredii* USDA193 under limited supply of nutrients. Soil Sci. Plant Nutr. 43:929-932.

Newbould P (1989). The use of N fertilizers in Agriculture. Where do we go practically and ecologically? Plant Soil 115:297-311.

Okereke GU, Onochie C, Onyeagba E (2001). Effectiveness of foreign bradyrhizobia strains in enhancing nodulation, dry matter and seed yield of soybean (*Glycine max* L.) cultivars in Nigeria. Biol. Fertil. Soils 33:3-9.

Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskery SL, Sampet C, Rerkasem B, Khans DF, Hauggaard-Nielsen H, Jesen BS (2009).The contributions of N-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48:1-17.

Saeki Y, Akagi I, Takaki H, Nagatomo Y (2000). Diversity of indigenous *Bradyrhizobium* strains isolated from three different *Rj*-soybean cultivars in terms of randomly amplified polymorphic DNA and intrinsic antibiotic resistance. Soil Sci. Plant Nutr. 46:917-926.

Sharma MK, Kumawat DM (2011). Co-inoculation study of *Bradyrhizobiumjaponicum* and Aspergillus niger insoybean for N fixation. J. Microbiol. Biotechnol. Food Sci. 1(3):383-394.

Smith DL, Hume DJ (1987). Comparison of assay methods for N_2 fixation utilizing white bean and soybean. Can. J. Plant Sci. 67:11-19.

Soe KM, Yamakawa T (2013a). Evaluation of effective Myanmar *Bradyrhizobium* strains isolated from Myanmar soybean and effects of coinoculation with *Streptomycesgriseoflavus* P4 for N fixation. Soil Sci. Plant Nutr. 59:361-370.

Soe KM, Yamakawa T (2013c). Low-density co-inoculation of Myanmar *Bradyrhizobiumyuanmingense* MAS34 and *Streptomyces griseoflavus* P4 to enhance symbiosis and seed yield in soybean varieties. Am. J. Plant Sci. 4:1879-1892.

Soe KM, Yamakawa T, Hashimoto S, Sarr P (2013b). Phylogenetic diversity of indigenous soybean bradyrhizobia from different agro-climatic regions in Myanmar. ScienceAsia 39:574-583.

Stacey G, Libault M, Brechenmacher L, Wan J, May GD (2006). Genetics and functional genomics of legume nodulation. Curr. Opin Plant Biol*.* 9:110-121.

Than H, Aung NN, Kyi PP (1987). Response of rhizobial peat inoculants on five important legumes. Proc. Myanmar Agri. Sci. Res. Div. 18th Congress. pp. 1-12.

Than H, Han T (1988). Contribution of N fixation to crop production in Myanmar. Proc. Myanmar Agri. Sci. Res. Div. 19th Congress. pp. 131-144.

Tien HH, Hien TM, Son MT, Herridge D (2002). Rhizobial inoculation and N_2 fixation of soybean and mungbean in the Eastern region of South Vietnam. In: Herridge (Ed.), Inoculants and N Fixation of Legumes in Vietnam. ACIAR Proceedings No. 109e. pp. 29-36.

Van K, Kim MY, Lee SH (2007). Genomic of Root Nodulation. In Genomics Assisted Crop Improvement, vol 2. pp. 435-452.

Yamakawa T, Eriguchi M, Hussain AKMA, Ishizuka J (1999). Soybean preference for *Bradyrhizobium japonicum* for nodulation: Nodulation by $Rj_2Rj_3Rj_4$-genotypes isolated from the progenies of a cross between soybean cv. ICA-2 (Rj_2Rj_3) and Hill (Rj_4). Soil Sci. Plant Nutr. 45:461-469.

Yamakawa T, Hussain AKMA, Ishizuka J (2003). Soybean preference for *Bradyrhizobium japonicum* for nodulation occupation of serogroup USDA110 in nodules of soybean plants harboring various *Rj*-genes grown in a field. Soil Sci. Plant Nutr. 49(6):835-841.