Evaluation of Nodulation Efficiency of Indigenous Rhizobium Isolates on Green Gram Varieties

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Abstract

Studies were conducted at Department of Plant Pathology, Yezin Agricultural University from July 2016 to April 2017 to evaluate the nodulation efficiency of indigenous rhizobium isolates on green gram varieties. Fifty-two soil samples were collected from green gram fields in Yinmarpin, Palae, Salingyi, Butalin and Monywa Townships in Sagaing Region. Nodulation efficiency of 52 rhizobium isolates was measured on plant growth of Yezin-11 variety by using Completely Randomized Design (CRD) with four replications. All tested isolates showed great variation in their capacity to produce nodules and shoot dry matter. YMP 1, YMP 7, YMP 11, PLE 1 and PLE 5 were found to be effective isolates to enhance the growth of Yezin-11 variety. These isolates were evaluated their effectiveness on five green gram varieties - MAS-1, Yezin-1, Yezin-9, Yezin-11 and Yezin-14 by using 7 x 5 x 3 factorial CRD arrangement (including N^+ and N^- control treatments). This study revealed that the responses of five rhizobium isolates were varied depending on green gram varieties. In all tested green gram varieties, YMP 11 isolate resulted in higher symbiotic effectiveness and increased shoot dry weight (%) over N^+ control than other tested isolates. The higher symbiotic effectiveness of YMP 11 isolate is an indication that such isolate may be better compatible and thus may have potential as source of rhizobium inoculant.

Key words: green gram, indigenous, nodulation, rhizobium, symbiotic effectiveness

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Introduction

In Myanmar, green gram, an important pulse crop for ecologically and economically, covers nearly 28% of total pulse area with annual production of 1.60 million metric tons in 2015-2016 (MOALI 2016). It is one of the popular pulse crops in the other countries as a cheap, high quality and protein-rich food (Delic et al. 2011). Besides, it retains and repairs soil fertility by fixing atmospheric nitrogen through symbiotic nitrogen fixation with rhizobium (Mansoor 2007). Due to these benefits, nowadays, its cultivated area has been progressively expanded from 0.95 million ha to 1.21 million ha and the average yield increased from 1.05 to 1.32 metric tons ha^{-1} during 2005 to 2015 (MOALI 2016). The green gram potential yield of the world was 1.90 metric tons ha^{-1} (Chauhan and Williams 2018). One major constraint in green gram yield increase is the absence of specific and effective rhizobia in soil (Funga et al. 2016).

Fortunately, green gram has the ability to establish a symbiotic partnership with rhizobium that may supply needed N to the plant (Mandal et al. 2009). This ability leads to decrease or absence of N fertilizer application in the field (Abbas et al. 2011). Green gram in symbiosis with effective *Rhizobium* and *Bradyrhizobium* spp. can fix 30-60 kg N ha^{-1} depending on agro-ecological conditions (Mansoor 2007). Although it is capable of fixing atmospheric nitrogen through rhizobia, green gram's yield is low because of its poor nodulation. Although rhizobia are present in most soils, variation in number, effectiveness in nodulation and nitrogen fixation were observed (Anjum et al. 2006). Hence, inoculation with efficient rhizobia at planting time is often recommended in environments where compatible rhizobia are absent, soil rhizobial population density has been reduced, or where rhizobia are less effective (Cheminingwa and Vessey 2006). Than (2010) stated that indigenous rhizobium isolates play an important role because they are adapted to local environmental conditions. Moreover, they were more effective than the exotic isolate. Investigation of

different strains of rhizobium isolates on each and every crop is important to select the best suitable green gram *Rhizobium* strain for the optimum growth and productivity under a particular environmental condition (Saleh et al. 2014). With the release of improved varieties of green gram, it is of prime importance to find out the most efficient host-rhizobium combination to attain the maximum yield benefits. Therefore, the evaluation on the effectiveness of indigenous rhizobium isolates on specific host plant should be investigated. For these reasons, the present study was conducted to evaluate the nodulation efficiency of indigenous rhizobium isolates on green gram varieties.

Materials and Methods

Investigation of indigenous rhizobium isolates

Soil samples collection

Fifty-two soil samples were collected from five green gram growing Townships of Sagaing Region - Yinmarpin (14 soil samples from 4 villages), Palae (12 soil samples from 4 villages), Salingyi (10 soil samples from 3 villages), Butalin (5 soil samples from 1 village) and Monywa (11 soil samples from 3 villages). Green gram fields which have no previous history of inoculation with rhizobia produced by Department of Agricultural Research (DAR) were selected from each site and 30 g of soil samples were collected from the upper 15 to 20 cm depth by using composite sampling pattern. Then these samples were pooled together and composite samples (150 g) were put in plastic bags (Jida and Assefa 2011).

Preparation of test plant

Surface-sterilized Yezin-11 green gram seeds were germinated in Petri dishes. The pots were filled with 500 g of sterilized sand and 50 ml of N free nutrient solution.

Inoculum preparation and inoculation

Collected soil samples were air-dried, ground and passed through a sieve to remove stones and large pieces of organic matter. Two grams of each composite soil sample was

diluted with 98 ml of sterilized yeast mannitol broth (YMB) solution in a 200 ml conical flask. The flask was shaken on a rotary shaker at 120 rpm for one hour to prepare a well-mixed soil suspension. A 5 ml aliquot of soil suspension was inoculated beside the pre-germinated seed (Htwe 2016). Then the surface of the substrate was covered with sterilized gravel (about 2 cm) as an anti-contamination layer. Un-inoculated nitrogen (N^+ control) (70 ppm KNO_3 in nutrient solution) and un-inoculated (N^- control) treatments were also provided. N free nutrient solution was poured at the rate of 50 ml $plant^{-1}$ at three day interval.

Experimental design and data recording

The experiment was laid out in a Completely Randomized Design (CRD) with four replications (54 x 4). Plants were harvested at 35 days after sowing (DAS). The plants were uprooted and gently washed with water not to remove the root hairs and nodules. Nodules were carefully separated from the roots by hand. The plants were cut at the root crown to separate the shoot and root portions. The nodules and shoots were oven dried at 60˚C for 48 hours and 72 hours respectively (Cheminingwa and Vessey 2006). Nodule dry weight (g), shoot fresh weight (g) and shoot dry weight (g) were recorded. Symbiotic effectiveness percent (SE%) of the isolates was also calculated by using the following formula. SE values were rated as highly effective (>80%), effective (51-80%), lowly effective (35-50%) and ineffective (<35%) (Beck et al. 1993).

$$\text{SE \%} = \frac{\text{Shoot dry weight of inoculated plants}}{\text{Shoot dry weight of un-inoculated } (N^+) \text{ control plants}} \times 100\ \%$$

Percent differences in shoot dry weights (SDW) between inoculated and N^+ control plants were calculated as the following formula described by Zerihun and Fassil (2010).

$$\text{Increased SDW (\%)} = \frac{\text{SDW of inoculated plants} - \text{SDW of } N^+ \text{ control plants}}{\text{SDW of } N^+ \text{ control plants}} \times 100\ \%$$

Evaluation of symbiotic effectiveness of effective rhizobium isolates on different green gram varieties

Preparation of test plants

The varieties used in this experiment were MAS-1, Yezin-1, Yezin-9, Yezin-11 and Yezin-14. Surface-sterilized green gram seeds were germinated in Petri dishes. The pots were filled with 500 g of sterilized sand and 50 ml of N free nutrient solution

Isolation of effective-indigenous rhizobium isolates

The nodules were surface-sterilized in 5% sodium hypochloride solution (NaOCl) for 3 minutes and rinsing 3 times in sterilized distilled water. The nodules were crushed in small bottles containing 1 ml of sterilized distilled water. A loopful of the bacterial suspension was streaked on plated yeast mannitol agar media (YMA) containing congo-red (25 µg ml^{-1}) and single colonies were selected after incubation at room temperature for 3-5 days (Bala et al. 2011). Single colonies were sub-cultured on YMA slants to obtain pure cultures.

Inoculum preparation and inoculation

YMP 1, YMP 7, YMP 11, PLE 1 and PLE 5 isolates were used as the inocula. Inocula were prepared by suspending the bacterial mass in sterilized water (about 10^8 cells ml^{-1} by dilution plate count method) and pre-germinated seeds were inoculated with 1 ml of bacterial suspension by using sterilized syringe (Somasegaran and Hoben 1985). Then the surface of the substrate in the plastic pot was covered with sterilized gravel (about 2 cm) as an anti-contamination layer. Un-inoculated N^+ control and N^- control treatments were also provided. N free nutrient solution was poured at the rate of 50 ml $plant^{-1}$ at three day interval.

Experimental design and data recording

Factorial experiment was laid out in a Completely Randomized Design (CRD) with three replications. There were two factors involving five rhizobium isolates and two un-inoculated controls as the first factor, and five green gram varieties as the second factor. Data

recording was done as in the previous experiment.

Statistical analysis

Analysis of variance was performed using Statistix Version 8.0 program and means were separated by Least Significant Difference (LSD) test at 5% probability level.

Results and Discussion

Investigation of indigenous rhizobia isolates

All tested 52 indigenous rhizobium isolates collected from Yinmarpin (YMP), Palae (PLE), Salingyi (SLG), Batalin (BTL) and Monywa (MWA) soils were able to nodulate Yezin-11 green gram variety. Nodule dry weight and shoot dry weight of green gram inoculated with 52 rhizobium isolates were significantly different from each other at 1% level (Table 1). These differences pointed out rhizobia isolates performed differently in their efficiency in nodulation and plant growth of Yezin 11 green gram variety. Vijila and Jebaraj (2008) also reported that all tested strains nodulated their host very well with different level of infectivity in green gram. Rhizobial strains within a species vary in their ability in nodulation and nitrogen fixation. In this study, YMP and PLE isolates gave better performance in nodulation and plant growth than SLG, BTL and MWA isolates.

Nodule dry weight may be considered as a usual character to select effective rhizobium isolates (Saleh et al. 2014). Somasegaran and Hoben (1985) explained that shoot dry matter is a good indicator of relative isolate effectiveness. Out of 52 tested isolates, the nodule dry weight of the plants inoculated with YMP 11 was significantly higher than those of the plants inoculated with the rest isolates. The lowest nodule dry weight was observed in the plant inoculated with MWA 2, but not significantly different from YMP 4, YMP 8, YMP 9, YMP 10, PLE 6, PLE 12, SLG 5 isolates, BTL isolates and MWA isolates. The highest shoot dry weight was recorded in plant inoculated with YMP 11, followed by those with PLE 5, YMP 1, PLE 1, YMP 7, PLE 7, YMP 3, YMP 6, YMP 14, YMP 2, PLE 10, YMP 4, PLE 9

and PLE 2 inoculated plants. The lowest shoot dry weights were shown by the hosts inoculated with MWA 1, SLG 5, MWA 2 and BTL 3 isolates. The correlation analysis result showed that nodule dry weight was highly significant and positively correlated (r = 0.81**) with shoot dry weight. When SE% was calculated, all tested isolates gave higher SE% (>80%) ranging from 83.49% to 309.62%. It was observed that YMP 11 gave the highest SE% followed by PLE 5, YMP 1, PLE 1 and YMP 7. BTL 3 produced the lowest value. According to the percentage differences in shoot dry weight of rhizobium inoculated and N^+ control plants, SLG 5, BTL 3, MWA 1 and MWA 2 isolates were inferior to N^+ control but superior to N^- control (Table 2). And thus, 92% of tested isolates performed better but 8% of them were lower than un-inoculated (N^+) control. Kawaka et al. (2014) stated that 42% of common bean nodulating indigenous rhizobium isolates were effective nitrogen fixers and 16% performed as good as the positive controls. As five indigenous rhizobium isolates - YMP 1, YMP 7, YMP 11, PLE 1 and PLE 5 - had the above indicators, they could be effective-indigenous rhizobium isolates. These isolates were selected to authenticate their symbiotic effectiveness on five green gram varieties.

Evaluation of symbiotic effectiveness of selected rhizobium isolates on different green gram varieties

Significant differences in nodule dry weights and shoot dry weights were observed among the isolates at 1 % level. The nodule dry weights of the isolates ranged from 18.73 to 33.27 mg $plant^{-1}$. The nodule dry weight of the plants treated with YMP 11 was found to be the highest and significantly different from other tested isolates. The lowest nodule dry weight was found in plants inoculated with PLE 1 but statistically similar to that of YMP 7.

The shoot dry weights (SDW) of isolates ranged from 288.60 to 547.93 mg $plant^{-1}$. The maximum shoot dry weight was obtained in plants inoculated with YMP 11 isolate, which was significantly different from plants inoculated with the rest isolates and un-inoculated control plants. The minimum shoot dry weight was found in N^- control plants,

which was significantly lower than that of N^+ control plants (393.47 mg plant^{-1}). The results indicated that nitrogen fixing ability of five indigenous rhizobium isolates differed from each other. Some strains of rhizobia are more effective than others to a particular host plant. This property should be attributed to the genetic variation of strains used in chickpea (Mandhare et al. 2005). The difference in shoot dry weight observed with selected rhizobium isolates was due to the difference in the genetic but also in effectiveness of each isolate (Ndusha 2011).

Green gram varieties exhibited a significant effect on the nodule dry weight and shoot dry weight at 1 % level. The nodule dry weight ranged from 13.86 to 22.29 mg plant^{-1}. The highest nodule dry weight was found in Yezin-11 variety, which significantly varied from those of other tested varieties. The lowest nodule dry weight was observed in Yezin-1, but not significantly different from that of Yezin-14. The shoot dry weight ranged from 324.71 to 492.76 mg plant^{-1}. The maximum shoot dry weight was observed in Yezin-11 variety, which was statistically similar to that of MAS-1. Yezin-1 produced the minimum shoot dry weight. The differences in nodule and shoot dry weights produced by different varieties could be attributed to highly specific interactions between green gram varieties and rhizobium isolates. Scheffer (2007) reported that leguminous species were very specific in their rhizobial requirement and could nodulate and increase nodule dry weight only by their own rhizobia.

There was an interaction between isolates and green gram varieties for nodule dry weight at 1 % level. The highest nodule dry weight was found in Yezin-11 variety inoculated with YMP 11 while the lowest nodule dry weight observed in Yezin-14 variety treated with PLE 1. In all tested green gram varieties, YMP 11 inoculated plant gave the maximum nodule dry weight (Table 2). There was also an interaction between isolates and green gram varieties for shoot dry weight at 5 % level. Inoculation led to occurrence of higher shoot dry weight compared to un-inoculated N^- control. N^+ control was also effective in obtaining higher shoot dry weight over N^- control. The maximum shoot dry weight was observed in YMP 11

inoculated Yezin-11 variety while the minimum shoot dry weight found in un-inoculated (N^-) Yezin-1 control plant (Table 2). All tested isolates gave higher symbiotic effectiveness percent (SE%) (>80%) ranging from 88.03% to 125.73% on MAS-1, 87.22% to 144.34% on Yezin-1, 100.71% to 152.09% on Yezin-9, 104.10% to 126.01% on Yezin-11 and 86.58% to 155.31% on Yezin-14. Almost all YMP isolates were compatible to all green gram varieties. PLE isolates did not perform to increase in SDW over N^+ control in most of green gram varieties. Among the tested isolates, YMP 11 gave the highest SE % on all tested varieties. There was no net increased SDW over N^+ control in Yezin-1 when inoculated with YMP 7, PLE 1 and PLE 5 isolates. No net increased SDW was also observed in MAS-1 which treated with PLE and PLE 5 isolates. Moreover, net increased SDW did not occur when YMP 1 and PLE 1 isolates were inoculated in Yezin-14. Therefore, all tested green gram varieties preferred YMP 11 isolate to YMP 1, YMP 7, PLE 1 and PLE 5 isolates. In the present study, the nodulation efficiency depends on the compatibility between rhizobium isolates and green gram genotypes. Dhar (2010) described that symbiotic nitrogen fixation is a complex physiological process influenced by interaction of genetic elements in host genotype and rhizobia. Effective rhizobia must have highly effective nitrogen fixing ability with the intended host species (Romero 2003). In the present study, YMP 11 was compatible with all tested green gram varieties.

In conclusion, the soil in Yinmarpin, Palae, Salingyi, Butalin and Monywa Townships of Sagaing Region harboured green gram nodulating rhizobia with good symbiotic properties because all 52 tested isolates were highly effective. Among them, YMP 1, YMP 7, YMP 11, PLE 1 and PLE 5 isolates were found to be the most effective isolates to enhance the growth of Yezin-11 variety. Response of green gram varieties varied with rhizobium isolates. Thus, the higher symbiotic effectiveness of YMP 11 isolate is an indication that such isolate may be better compatible and thus may have potential as source of rhizobium inoculant.

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Table 1. Nodule dry weight, shoot dry weight, symbiotic effectiveness (SE%) and percent shoot dry weight increase over N^+ control of Yezin-11 green gram variety inoculated with 52 rhizobia isolates

Sr. No.	Isolate No.	Nodule dry weight (mg plant^{-1})^x	Shoot dry weight (SDW) (mg plant^{-1})^x	SE%	% increase in SDW over N^+ control
1	**YMP 1**	67.75 bcd^y	1448.3 abc^y	283.00	183.00
2	**YMP 2**	55.25 b-i	1280.8 a-h	250.27	150.27
3	**YMP 3**	45.25 f-q	1356.3 a-g	265.02	165.02
4	**YMP 4**	38.75 h-r	1249.0 a-h	244.06	144.06
5	**YMP 5**	64.00 b-f	1145.3 b-j	223.79	123.79
6	**YMP 6**	45.00 f-q	1349.3 a-g	263.65	163.65
7	**YMP 7**	66.75 b-e	1403.0 a-e	274.16	174.16
8	**YMP 8**	37.50 h-r	1201.0 b-i	234.68	134.68
9	**YMP 9**	36.75 i-r	1061.8 d-m	207.47	107.47
10	**YMP 10**	40.25 g-r	1142.8 b-j	223.30	123.30
11	**YMP 11**	114.5 a	1584.5 a	309.62	209.62
12	**YMP 12**	43.00 f-q	1149.5 b-j	224.62	124.62
13	**YMP 13**	53.75 b-i	1196.8 b-i	233.85	133.85
14	**YMP 14**	44.75 f-q	1339.5 a-g	261.75	161.75
15	**PLE 1**	68.25 bc	1427.8 a-d	278.99	178.99
16	**PLE 2**	52.00 b-k	1215.8 a-i	237.57	137.57
17	**PLE 3**	54.75 b-i	1107.5 b-k	216.41	116.41
18	**PLE 4**	44.00 f-q	1083.0 c-l	211.63	111.63
19	**PLE 5**	69.50 b	1461.8 ab	285.64	185.64
20	**PLE 6**	38.50 h-r	924.75 h-p	180.70	80.70
21	**PLE 7**	63.50 b-f	1369.5 a-f	267.61	167.61
22	**PLE 8**	52.75 b-j	1149.8 b-j	224.67	124.67
23	**PLE 9**	54.00 b-i^y	1247.8 a-h	243.82	143.82
24	**PLE 10**	60.75 b-g	1261.3 a-h	246.46	146.46
25	**PLE 11**	46.75 d-o	1017.5 f-n	198.83	98.83
26	**PLE 12**	40.50 g-r	990.5 g-o	193.55	93.55
27	**SLG 1**	43.25 f-q	684.75 n-t	133.81	33.81
28	**SLG 2**	52.50 b-k	868.25 i-q	169.66	69.66
29	**SLG 3**	48.00 c-n^y	912.75 h-p^y	178.36	78.36

Table 1. Continued

Sr. No.	Isolate No.	Nodule dry weight (mg plant^{-1})^{x}	Shoot dry weight (mg plant^{-1})^{x}	SE%	% increase in SDW over N^+ control
30	**SLG 4**	49.25 b-m	807.75 j-r	157.84	57.84
31	**SLG 5**	39.75 g-r	495.00 q-t	96.73	-3.27
32	**SLG 6**	58.00 b-h	1004.5 f-o	196.29	96.29
33	**SLG 7**	46.50 e-p	706.75 m-t	138.10	38.10
34	**SLG 8**	49.25 b-m	1047.0 e-n	204.59	104.59
35	**SLG 9**	51.00 b-l	852.25 i-q	166.54	66.544
36	**SLG 10**	49.75 b-m	1065.3 d-m	208.16	108.16
37	**BTL 1**	31.75 j-r	564.50 p-t	110.31	10.31
38	**BTL 2**	26.50 o-r	686.75 n-t	134.20	34.20
39	**BTL 3**	25.50 pqr	427.25 st	83.49	-16.51
40	**BTL 4**	32.50 j-r	698.50 m-t	136.49	36.49
41	**BTL 5**	32.00 j-r	612.25 p-t	119.64	19.64
42	**MWA 1**	24.50 qr	498.50 q-t	97.41	-2.59
43	**MWA 2**	21.50 r	442.00 rst	86.37	-13.63
44	**MWA 3**	27.25 n-r	591.50 p-t	115.58	15.58
45	**MWA 4**	36.75 i-r^{y}	764.00 k-s	149.29	49.29
46	**MWA 5**	29.50 m-r	635.25 o-t	124.13	24.13
47	**MWA 6**	26.50 o-r	567.50 p-t	110.89	10.89
48	**MWA 7**	31.25 k-r	705.75 m-t	137.91	37.91
49	**MWA 8**	30.25 l-r	556.50 p-t	108.74	8.74
50	**MWA 9**	25.75 o-r	611.75 p-t	119.54	19.54
51	**MWA 10**	26.75 o-r	603.75 p-t	117.98	17.98
52	**MWA 11**	32.00 j-r	721.50 l-t	140.99	40.99
	N^+ control	0.00 s	511.75 q-t	100	-
	N^- control	0.00 s	365.75 t	-	-
	Pr>F	****	****	-	-
	$LSD_{0.05}$	**21.13**	**374.42**	-	-
	CV(%)	**34.83**	**28.30**	-	-

^{x} = Means of 4 replications, ^{y} = Means followed by the same letter in the same column are not significantly different at 5% level, **** Significant at 1 % level

YMP = Yinmarpin, PLE = Palae, SLG = Salingyi, BTL = Butalin, MWA = Monywa

Table 2. Effect of rhizobia isolates and variety on nodule dry weight and shoot dry weight of green gram at 35 DAS

Isolate	Variety					
	MAS-1	**Yezin-1**	**Yezin-9**	**Yezin-11**	**Yezin-14**	**Mean**
Nodule dry weight (mg plant^{-1})^{x}						
YMP 1	28.67 c-f^{y}	24.33 d-g	23.00 e-h	34.00 abc	22.00 f-i	26.40 B
YMP 7	29.00 c-f	14.33 jkl	14.00 kl	28.67 c-f	15.33 i-l	20.27 CD
YMP 11	33.00 abc	27.00 c-g	36.67 ab	40.00 a	29.67 b-e	33.27 A
PLE 1	23.00 e-h	15.33 i-l	21.00 g-k	21.67 f-j	12.67 l	18.73 D
PLE 5	15.67 h-l	16.00 h-l	29.00 c-f	31.67 bcd	22.67 e-i	23.00 C
N^{+} control	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 E
N^{-} control	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 E
Mean	18.48 B	13.86 C	17.67 B	22.29 A	14.62 C	
CV (%)	26.10					
Shoot dry weight (mg plant^{-1})^{x}						
YMP 1	513.00 a-d^{y}	358.33 i-p	498.33 a-e	536.33 abc	336.67 k-p	448.53 B
YMP 7	515.00 a-d	300.33 nop	377.00 g-n	483.33 a-f	459.67 c-h	427.07 BC
YMP 11	563.67 ab	497.00 a-e	569.33 ab	573.33 a	536.33 abc	547.93 A
PLE 1	394.67 f-n	304.33 m-p	428.67 d-k	527.00 a-d	299.00 nop	390.73 C
PLE 5	438.00 c-j	306.67 m-p	517.00 a-d	473.67 b-g	407.67 e-l	428.60 BC
N^{+} control	448.33 c-i	344.33 j-p	374.33 h-o	455.00 c-i	345.33 j-p	393.47 C
N^{-} control	328.33 l-p	162.00 q	277.00 op	400.67 e-m	275.00 p	288.60 D
Mean	457.29 AB	324.71 D	434.52 B	492.76 A	379.95 C	
CV (%)	14.48					

^{x}Means of 3 replications, ^{y}Means followed by the same letter in the same column are not significantly different at 5 % level