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SCREENING OF POTENTIAL BACTERIAL ISOLATES AS SPECIFIC BIOFERTILIZER AGENT FOR MUNGBEAN PLANTS

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The Experiment was performed under glass house condition (24°8´ N 90°0´ E) with eight rhizobial strains namely MBR-3, MBI-5, MBI-19, MBM-4, MBM-8, MBP-10, MBB-3 and MBJ-7 obtained from mungbean rhizosphere of different locations in Bangladesh and BINA MB-1 (a registered biofertilzer for mungbean) was used as standard check along with un-inoculated control to test their potentiality under glass house condition for mungbean. Result revealed that the higher plant growth, biochemical parameters, seed yield attributes and seed yield were recorded in three bacterial isolates *viz*., MBI-5, MBB-3 and biofertilizer, BINA MB-1 with being the highest in MBI-5. Therefore, the isolate MBI-5 may be used as commercial biofertilizer after few more trials in the different mungbean growing areas of Bangladesh.

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INTRODUCTION

Mungbean (*Vigna radiata* L. Wilezek) is widely grown as protein crop in Indian sub-continent for human consumption. In order to improve the yield and disease suppression farmer resort to inorganic fertilizers and possible pesticides, which often have hazardous effect and also do not fit into the frame work of organic farming? These problems are likely to become more serious in future. An alternative approach is to use Biological nitrogen fixation (BNF) resulting from a symbiosis between legume crops and root nodule bacterium, *Rhizobium* can ameliorate these problems by reducing the N-fertilizer inputs required to ensure high productivity (Gupta and Namdea, 2008).

As a legume, mungbean is capable of utilizing atmospheric nitrogen through symbiotic association with *Bradyrhizobium* sp. and thereby can meet the requirement of the N element. Inoculation of mungbean with effective *Bradyrhizobium* inoculant is necessary for soils where the organisms are ineffective, absent or scarce (Hossain *et al*., 2014). *Bradyrhizobium* strains are present in all soils of Bangladesh but they may not be equally effective in nodulation and N-fixation. In this situation, inoculation can meet the challenge by providing superior strains in the soil, so that the most effective nodulation and nitrogen fixation are obtained. Thus it was thought that there is a scope for utilizing the effective Bradyrhizobial strains for obtaining more yield of mungbean under field conditions which may play vital role in improving soil environment and agricultural sustainability. To reduce the production cost and to fulfill the demand, more pulse production could be achieved through seed inoculation with *Bradyrhizobium* strains which is known to increase biological nitrogen fixation (BNF). Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been repeatedly reported (Zhang *et al*. 1996; Pan *et al*. 1999; Bin *et al*. 2000; Asad *et al*., 2004; Figueiredo *et al*. 2008; Hayat *et al*., 2010; Sharma. and Khurana, 2012; Parvin *et al*., 2018). *Bradyrhizobium* inoculation increased mungbean seed yield from 4.3% to 26.2% (BINA, 2019). Maximum yields were obtained when fertilizers applied together with *Bradyrhizobium* inocula (Hossain *et a*l., 2014). Therefore, this study was undertaken to screen the BNF strains that are compatible with *Vigna radiata* and use as biofertilizer.

MATERIALS AND METHODS

The present investigation was carried out in glass house at Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh. The experimental material consists of one mungbean popular variety viz., Binamung-8 which were inoculated with 9 different rhizobial starins/isolates namely MBR-3, MBI-5, MBI-19, MBM-4, MBM-8, MBP-10, MBB-3 and MBJ-7 obtained from root nodules of mungbean plant of different locations in Bangladesh and BINA MB-1 (a registered biofertilzer for mungbean) was used as standard check along with un-inoculated control to test their potentiality under glass house condition for mungbean. Sixty five Bradyrhizobial strains were isolated from mungbean nodules at different locations of Bangladesh. Colony morphology of the each bacterial isolates was examined after 7 days of inoculation of each bacterial isolate on nutrient agar plate and stereoscopic microscope (Olympus, SZH 10) was used for better resolution of bacterial colony morphological characteristics. Gram staining was done to identify cell morphology. The collected strains were Gramnegative, colonies not exceeding 1 mm in diameter within 7 days in yeast extract-manitol medium at the optimal temperature of 25 to 28 ^{0}C and according to Jordan (1982), the strains were Bradyrhizobium. These strains were cultured and screened through laboratory experiments. For selection better strains, a mini pot experiment was setup in glass house following the standard protocol of Santos et al. (1999). The plants were harvested at 45 days after sowing and eight strains were selected and used in this study based on superior nodule number, plant height, shoot and root dry weight.

Broth culture were prepared by taking a loopful of the respective Rhizobacterial isolates and transferred to the liquid medium of 100 ml conical flask which were incubated for 24 hours on a rotatory shaker. When the culture in the flask showed dense milky white growth, the broth cultures were ready for seed inoculation. Pots were filled with 8 kg sterilized sand and arranged in a Completely Randomized Design with 8 replications for each treatment. Four replications were used for nodulation count, growth and biochemical study. Before sowing, the seeds of mungbean were surface sterilized with 90 per cent alcohol for 30 second and followed by immersion in 32 per cent H_2O_2 for one minutes and ten times washing in sterile distilled water and then sown in sterile sand. Five treated seeds were sown in each pot at a depth of 3-4 cm. The pots were watered regularly with the tap water upto the retention capacity of the pot. After germination, 2 seedlings were maintained in each pot and 2ml of inoculum broth culture (10^8 bacteria/ml) of individual Bradyrhizobia was applied once below the surface of the root zone of the seedlings, in the afternoon. The pots were kept under vector free and optimal growth conditions [temperature of 28/25³C (day/night, with a standard deviation of ± 2.8 ^{0}C) with air humidity 60-70%]. The plants were regularly supplied with one-fourth strength sterilized Long Ashton nutrient solution (Hewilt, 1966) twice a week until harvest.

During present investigation, the influence of bacterial isolates on plant growth and biochemical parameters and at harvest seed yield and yield attributes were recorded. For growth parameters, two harvests were recorded at 40 and 55 days after sowing (DAS). The whole plants were oven drying at 80 ± 2 ^{0}C for 72 hours and dry weights were recorded. The growth analysis was carried out following the formulae of Hunt (1978). Nodule number was recorded 2 times, at 30 and 50 DAS. Leaf chlorophyll, nitrate reductase and photosynthesis were determined at 55 DAS. Leg-hemoglobin was determined at 50 DAS. Leaf area of each sample was measured at 70 DAS (at maturity stage) by automatic leaf area meter (Model: LI 3000, LI-COR Biosciences, USA). Leaf chlorophyll was determined following the method of Yoshida et al. (1976). Leaf photosynthesis was measured by photosynthesis meter (LI 6400XT, LI-COR Biosciences, USA). Nitrate reductase activity was determined following the methods of Stewart and Orebamjo (1979). Leg-haemoglubin was determined by the Cyanmethaemoglobin method (Schifftmann and Lobel, 1970). Grain protein per cent was estimated by Micro-Kjeldhal method (AOAC, 1980). The yield contributing characters were recorded at harvest of each pot. All data were analyzed statistically as per the used design following the one way analysis of variance technique and the mean differences were adjusted with Duncan's Multiple Range Test using the statistical computer package programme, MSTAT-C (Russell, 1986).

RESULTS AND DISCUSSION

The effect of bacterial isolates on plant height, leaf area (LA), biological yield (BY) and nodule production both at 30 and 50 DAS was significant (Table 1). Results indicated that plant height, LA, BY and nodule production was greater in bacterial isolates treated plant than in control plant. This result indicated that application of bacterial isolates had tremendous effect on growth and development in mungbean plants. The highest plant height (41.8 cm), BY (15.13 g plant^{-1}) and nodule number plant^{-1} was recorded in MBI-5 followed by MBJ-7 and BINA MB-1 with same statistical rank. On the other hand, the check bio-fertilizer, BINA MB-1 produced the highest LA (773 cm^2 plant^{-1}) followed by MBI-5 (746 cm^2 plant^{-1}) with same statistical rank. The isolate MBI-5 also showed higher seed yield (Table 3). This result indicates that higher LA and BY is desirable for getting higher seed yield in mungbean. Plant growth and yield are represented by the crop's early ability to intercept solar radiation and its subsequent utilization for biomass production (Hanlan *et al*., 2006). In crop plant, increase interception of solar radiation at early seedling stages enable plant to make rapid early growth, resulting in high yield (Purcell *et al*., 2002). In the present experiment, the isolates which produced greater number of nodules also showed higher LA and BY. Similar result was also reported by Dutta *et al.* (1998) who observed that the isolate which had capacity to increase nodule number also showed higher LA and BY in lentil. Generally, high TDM and LA producing genotypes showed higher seed yield (Mondal *et al*. 2011). In the present experiment, the Bradyrhizobial strain MBI-5 treated plants showed higher LA and TDM also showed higher seed yield.

The growth parameters like total dry mass (TDM) and absolute growth rate (AGR) and biochemical parameters like chlorophyll content, nitrate reductase (NR) activity and phosynthesis (Pn) in leaves, and leg-hemoglobin (LHB) content in nodule was significantly higher than that of uninoculated plant (Table 2). Even amongst the bacterial isolates, there were significant variation in growth parameters and biochemical parameters. The highest TDM both at 40 and 55 DAS, and AGR was recoded in MBI-5 followed by MBB-3 and biofertilizer, BINA MB-1 with same statistical rank. The lowest TDM production both at 40 and 55 DAS, and AGR was recorded in uninoculated plant. These three isolates (MBI-5, MBB-3 and BINA MB-1) also showed higher seed yield (Table 3). This result indicates that higher growth rate at flowering and fruiting stage is desirable for getting higher seed yield in mungbean. Plant growth and yield are represented by the crop's early ability to intercept solar radiation and its subsequent utilization for biomass production (Mondal *et al*., 2013). In field crop, increase interception of solar radiation at early seedling stages enable plant to make rapid early growth, resulting in high yield (Purcell *et al*., 2002). In the present experiment, the isolate treated plants showed higher leaf area which intercept more solar radiation and also showed high TDM as well as AGR. Similar result was also reported by Dutta *et al.* (1998) in lentil who observed that the bacterial isolate treated genotypes which had capacity to early higher growth rate as well as also showed higher seed yield.

Table 1. Effect of bacterial isolates on morphological characters and nodule production in mungbean

Strains	Plant height (cm)	Leaf area (cm^2 $plant^{-1}$)	Biological yield (g $plant^{-1}$)	Number of nodules $plant^{-1}$ at	
				30 DAS	50 DAS
No strain (cont.)	35.8 c	548 f	7.510 e	1.25 f	2.30 g
MBR-3	38.6 b	655 d	11.13 d	13.2 c	18.6 d
MBI-5	41.8 a	746 ab	15.13 a	18.0 a	24.5 ab
MBI-19	37.3 c	613 e	10.79 d	8.50 e	11.1 f
MBM-4	40.6 ab	713 bc	13.52 c	17.1 ab	22.9 bc
MBM-8	38.1 b	676 c	13.17 c	14.8 c	16.9 e
MBP-10	37.8 b	672 cd	14.10 a	17.0 ab	20.8 cd
MBB-3	38.8 b	692 cd	14.62 ab	11.8 d	25.3 a
MBJ-7	39.9 ab	740 ab	13.55 abc	17.9 a	22.9 ab
BINA MB-1 (Biofertilizer)	40.6 ab	773 a	14.11 abc	16.4 b	21.8 c
F-test	**	**	**	**	**

In a column, figures with same letter (s) do not differ significantly at P ≤ 0.05; ** indicate significant at 1% level of probability; DAS = days after sowing

Table 2. Variation in total dry mass production and biochemical parameters due to bacterial isolates application in mungbean

Strains	Total dry mass production at (g $plant^{-1}$)		Absolute growth rate (mg p^{-1} d^{-1})	Chloro-phyll (mg g^{-1} fw)	Nitrate reductase (µ mol NO_2^- g^{-1}fw h^{-1})	Photo-synthesis (µmol CO_2 $m^{-2}s^{-1}$)	Leg-haemoglobin (mg g^{-1} fw)
	40 DAS	55 DAS	40-55 DAS				
No strain (cont.)	2.41 d	4.64 g	149 e	1.46 e	2.14 e	13.14 d	12.30 cd
MBR-3	3.68 cd	7.05 de	225 c	1.65 de	2.39 d	13.95 cd	12.24 cd
MBI-5	4.34 a	9.20 a	331 a	2.12 a	2.66 a	15.81 ab	13.04 a
MBI-19	3.57 d	6.04 f	173 d	1.72 cd	2.33 d	14.0 c	12.15 d
MBM-4	4.06 ab	7.98 c	261 b	1.86 bc	2.46 bc	15.40 ab	13.00 a
MBM-8	3.61 cd	6.83 de	215 c	1.77 cd	2.40 d	14.21 bc	12.51 b
MBP-10	3.95 bc	7.29 d	223 c	1.82 c	2.50 abc	15.19 a	12.66 bc
MBB-3	4.14 ab	8.99 ab	323 a	1.80 cd	2.62 a	14.22 bc	13.12 a
MBJ-7	3.91 bc	8.54 b	309 a	1.90 b	2.42 cd	15.19 ab	12.34
BINA MB-1 (Biofertilizer)	4.00 b	8.77 ab	318 a	2.02 ab	2.55 ab	16.00 a	12.84 ab
F-test	**	**	**	**	*	**	*

In a column, figures with same letter (s) do not differ significantly at P ≤ 0.05; *, ** indicate significant at 5% and 1% level of probability, respectively

Table 3. Effect of bacterial isolates on yield and yield contributing characters in mungbean

Strains	Pods plant^{-1} (no.)	Seeds pod^{-1} (no.)	100-seed weight (g)	Seed weight plant^{-1} (g)	Protein percentage in seed
No strain (cont.)	8.77 e	8.20 c	4.57 b	3.29 g	23.30 c
MBR-3	11.8 c	8.88 b	4.72 ab	4.95 e	23.55 bc
MBI-5	14.8 a	9.82 a	4.81 a	6.79 a	23.90 ab
MBI-19	10.3 d	8.71 b	4.66 ab	4.18 f	23.53 bc
MBM-4	13.8 ab	9.70 a	4.87 a	6.53 ab	24.34 a
MBM-8	11.7 c	9.27 b	4.77 a	5.22 e	23.67 b
MBP-10	12.6 b	9.60 ab	4.80 a	5.80 d	23.59 b
MBB-3	13.7 ab	9.54 ab	4.78 a	6.14 cd	24.20 a
MBJ-7	13.0 b	9.46 ab	4.94 a	6.09 cd	23.60 b
BINA MB-1 (Biofertilizer)	13.4 ab	9.41 b	4.88 a	6.20 bc	23.97 a
F-test	**	*	*	**	*

In a column, figures with same letter (s) do not differ significantly at $P \leq 0.05$; *, ** indicate significant at 5% and 1% level of probability, respectively.

The highest/higher chlorophyll content, NR activity and Pn in leaves, and LHB in nodule was recorded in MBI-5, MBB-3 and biofertilizer, BINA MB-1 and these three isolate treated plants also showed higher seed yield which indicated seed yield is positively correlated with chlorophyll, NR, Pn in leaves and LHB in nodule. On the other hand, the uninoculted plants showed inferiority in chlorophyll, NR and Pn and also gave lower yield performance. These results are consistent with Peter and Satish (2015) who reported that Chlorophyll and Pn was greater in bacterial isolate treated plants than uninoculated plant.

The effect of Rhizobial strains on seed yield and yield contributing characters was significant (Table 3). Results indicated that seed yield, number of pods plant^{-1}, number of seeds pod^{-1}, 100-seed weight and protein percentage in seed were greater in bacterial isolates treated plant than in uninoculated plant. This result indicating that application of bacterial isolates had tremendous effect on yield contributing characters and thereby seed yield in mungbean. The higher number of pods plant^{-1}, seed number pod^{-1} and 100-seed weight was recorded in MBI-5, MBB-3 and biofertilizer, BINA MB-1 with being the highest in MBI-5. The seed weight was higher in these three (MBI-5, MBB-3 and biofertilizer, BINA MB-1) isolates treated plants due to production of higher number of pods plant^{-1} and seeds pod^{-1}. Many researchers reported that seed yield increased 10-40% in leguminous plant when applied biofertilzer than uninoculated plant (Asad *et al*., 2004; Dey *et al*., 2004; Gupta and Namdeo, 2008; Hossain *et al*., 2014; Peter and Satish, 2015, Parveen *et al*., 2018) that supported the present experimental results. In crop plant, increase leaf area, TDM, chlorophyll content and photosynthesis rate in leaves are positively correlated with seed yield (Mondal *et al*., 2011). In the present experiment, the isolates treated plants showed increased leaf area, chlorophyll content and photosynthesis rate in leaves resulting increase TDM, thereby increase seed yield. Protein content in seed was higher in Rhizobia inoculated plants than uninoculated plants are also supported by many workers (Dey *et al*., 2004; Laranjo *et al*., 2014; Peter and Satish, 2015).

CONCLUSION

Bacterial isolates treated plants improve growth, biochemical parameters and yield in mungbean than in uninoculated plant. Amongst nine tested bacterial isolates, MBI-5 performed the best regarding growth, morphological and biochemical parameters and yield contributing characters resulting the highest seed yield. This isolate may be used as commercial biofertilizer agent after few more trials.

CONFLICT OF INTEREST

There is no conflict of interest between the authors about the research

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