The Journal of Animal & Plant Sciences, 23(1): 2013, Page: 251-255
ISSN: 1018-7081

EVALUATION OF ROCK PHOSPHATE SOLUBILIZATION CAPACITY BY ISOLATED STRAINS AND THEIR EFFECT ON MUNGBEAN

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ABSTRACT

We determined the degree of P-solubilization using phosphate solubilizing isolates and their effect on growth and yield of mungbean by both laboratory and pot experiments. For phosphate solubilization study, thirty four isolates including control was tested the degree of phosphate solubilization using rock phosphate. In pot condition, four levels of P (0, 20, 40 and 60 kg P ha^{-1}) were tested with seven effective phosphate solubilizers on mungbean. Each experiment was laid out in a complete randomized design with four replications. The effective phosphate solubilizing isolate was obtained from rice rhizosphere soil at Old Brahmaputra Floodplain (AEZ-9). Phosphorus solubilization ranged from 0.21 to 7.34 mg P L^{-1} in I_{21} and I_{16}, respectively. Inoculation of phosphate solubilizing isolates considerably enhanced the nodulation and yield of mungbean while the effect was more pronounced when they were applied with phosphatic fertilizer compared to un-inoculated with no phosphatic fertilizer. The highest nodule number (24.00 and 96.17 per plant) and dry weight (40.00 and 84.75 mg per plant) was produced by I_1 and I_5 at Bogra and I_7 at Pabna, respectively. Among the phosphorus levels, 20 and 60 kg P ha^{-1} showed the maximum seed yield of mungbean at Pabna and Bogra. Based on the results, it may be concluded that phosphorus @ 20 and 60 kg P ha^{-1} along with I_4 and I_1 isolates were the best treatments for increasing mungbean yield at Pabna and Bogra, respectively. However, a comprehensive approach to employ the isolates in mungbean should be carried out to explore the hidden potential of isolates and to promote the growth and yield of mungbean under field conditions.

Key words: Mungbean, agroecological zones, isolates, phosphate solubilization, nodulation and yield.

INTRODUCTION

Mungbean is one of the important pulse crops in Bangladesh and it ranks fifth position in respect of acreage and production (BBS, 2008). The farmers of Bangladesh usually do not follow improved management packages like phosphatic fertilizer and phosphatic biofertilizers for maximizing yield and reduce the use of chemical fertilizer. Poor socio-economic condition of the farmers and suitable technology are the main barrier behind it. Phosphorus is a non-renewable source and a major plant nutrient for higher crop yield. It limits the crop production because only about 20% of applied phosphorus is utilized by crop and remaining part is converted into insoluble forms in acid and alkaline soils (Rodriguez and Fraga, 1999). In Bangladesh, most of the soils are acidic or alkaline in nature as a result, the availability of phosphorus for the nutrition of crops is very low due to its fixation. Adequate supply of phosphatic biofertilizer with less cost could play a vital role in improving the mungbean yield. In this context, P-solubilization ability of micro-organisms is considered to be one of the most important traits associated with plant P nutrition (Chen *et al*., 2006; Adesemoye and Kloepper, 2009). In this regard, we need to conduct research for the isolation of phosphate solubilizing bacteria (PSB) from different agro-ecological zones (AEZ) of Bangladesh and tested its efficiency in respect of phosphate solubilization. Several bacterial species are referred to as phosphate solubilizing bacteria and have been considered to have potential use as biofertilizer to improve the plant growth and yield (Vessey, 2003). The beneficial effects of phosphate solubilizing bacteria on crop productivity have been widely described but the use of PSB as biofertilizer is scarcely documented in mungbean. Therefore, the objectives of the research studies were to test the degree of phosphorus solubilization and their effect on mungbean.

MATERIALS AND METHODS

Pot and laboratory trials were conducted to determine phosphorus solubilizing capacity and their effect on growth and yield of mungbean. For laboratory study, isolates from rhizosphere soils of healthy rice, jute, blackgram, mustard, *Dhaincha*, soybean, garlic, maize, brinjal and lettuce were collected. In all, 34 phosphate solubilizing isolates were obtained from different agroecological zones of Bangladesh (Fig. 1). Phosphate solubilization efficiency of these isolates was determined by using Pikovskaya medium with tricalcium phosphate. Ingredient of this Pikovskaya's broth (g L^{-1}) is yeast extract, 0.05; dextrose, 10.0; tricalcium phosphate, 5.0; ammonium sulfate, 0.50; potassium chloride, 0.20; magnesium sulfate, 0.10; manganese sulfate 0.0001; ferrous sulfate 0.0001. Phosphate solubilizing isolates were used in 200 ml Pikovskaya's broth culture. Cultures were harvested 96 hr after inoculation. Centrifugation was done at 8000 rpm for 30 min. Dissolved phosphorus concentration in broth culture filtrate was filtrated and phosphorus was determined by Vanado-molybdate method (APHA, 1995). It was expressed in terms of mg L^{-1} of phosphorus in culture medium.

Pot experiments were conducted during 2004 at Bogra and Pabna with four levels of P (0, 20, 40 and 60 kg P ha^{-1}) and seven phosphomicrobial isolates including un-inoculated control. Factorial experiment was laid out in a complete randomized design and mungbean was used as test crop with four replications. Bacterial isolates were incubated on nutrient agar. A single colony was transferred to 1 L conical flask containing nutrient broth and incubated aerobically on a rotating shaker (240 rpm) overnight at 28^0C. The bacterial suspension was diluted with sterile distilled water to a final concentration of 10^8 colony forming unit (CFU) mL^{-1}. Seeds were mixed with liquid broth culture of phosphate solubilizing bacteria as per treatment in a polyethylene bag and were kept in a cool dry place. Intercultural operations such as weeding, thinning and pesticide spraying were done as and when necessary. Data on yield contributing parameters like total nodule number $plant^{-1}$ and nodule dry weight $plant^{-1}$ were recorded from 10 randomly selected plants and yield was noted from the harvested plot. Data were statistically analyzed and Duncan's Multiple Range Test was applied to examine significant differences between the treatment means (Gomez and Gomez, 1994).

RESULTS AND DISCUSSION

Effect of isolates on phosphate solubilization: Results on phosphate solubilization capacity from different agro ecological zones with crop's rhizosphere are presented in Table 1. Phosphorus solubilization ranged from 0.21 to 7.34 mg P L^{-1} in I_{21} and I_{16}, respectively. The highest phosphorus solubilization was observed in Old Brahmaputra Floodplain soil (AEZ-9) and the lowest phosphorus solubilization was found in High Ganges River Floodplain (AEZ-11). According to phosphate solubilization capacity, bacterial isolates from Old Brahmaputra Floodplain, Tista Mender Floodplain and Old Meghna Estuarine Floodplain soil performance was better than the Active Ganges River Floodplain and High Ganges River Floodplain soils. Isolates from rice except High Ganges River Floodplain soil and blackgram for all AEZs rhizosphere soils performed better results than the other crop's rhizosphere isolates. Similar trend was observed in respect of net solubilized phosphorus and %phosphorus solubilization. Isolates from rhizosphere soil had good capacity to solubilize unavailable phosphorus against control. In this regard, the efficiency of phosphate solubilizing bacteria at Old Brahmaputra Floodplain soils was better than those of other agroecological zones of Bangladesh. Old Brahmaputra Floodplain soils are neutral in reaction. So, bacteria can easily survive in neutral soil. These results are in agreement with the findings (Gupta *et al*., 2007). From these results we may conclude that isolation of PSB from different AEZ's is essential for the development of effective biofertilizer.

Effect of phosphatic fertilizer on mungbean: The results of P fertilizer on total nodules and dry weight of nodules at Bogra and Pabna are presented in Table 2. Nodule number ranged from 8.25 to 19.71 and 72.79 to 96.17 at Bogra and Pabna, respectively. The highest nodules number (19.71 and 96.17 $plant^{-1}$) was observed in 20 and 40 kg P ha^{-1} at Bogra and Pabna, respectively. At Bogra, 20 kg P ha^{-1} produced the highest total nodules $plant^{-1}$ (19.71) followed by 40 and 60 kg P ha^{-1}. Data on nodule number was not statistically significant in different treatments at Pabna. The highest nodule weight (36.88 and 86.79 gm $plant^{-1}$) was observed in 60 and 40 kg P ha^{-1} at Bogra and Pabna, respectively. The lowest total nodules $plant^{-1}$, nodules dry weight $plant^{-1}$ and seed yield $plant^{-1}$ was observed in control plots. From these results it may be concluded that different doses of P performed different on nodulation at different locations. Bogra soil is acidic in mature, on the other hand, Pabna soil is alkaline. So, calcium content was higher in Pabna soil than the Bogra soil. From these results we may be concluded that Pabna soils had good capacity to release applied phosphatic fertilizer using phosphate solubilizers. Grain yield increased significantly with the increase of phosphorus level (Table 2). However, an increasing trend was noted up to 60 kg P ha^{-1}, which was significantly superior to 20 kg P ha^{-1} at Bogra. On the other hand, highest grain yield was observed in 20 kg P ha^{-1} at Pabna. Highest dose of phosphorus performed better results than the lower doses of phosphorus at Bogra but in Pabna lowest dose (20 kg P ha^{-1}) showed better result. Because Bogra soils are acidic in nature so excessive amount of phosphorus needed for getting optimum mungbean yield. In Bogra soils, application of 20 kg P ha^{-1} phosphorus got fixed into insoluble compounds as Fe-P and Al-P. For example, the average concentration of available phosphorus in soil ranges from 0.05 to 10 ppm, out of which only as infinitesimal part is available to plant. Apart from this, P in soils moves mainly by diffusion and its rate of diffusion is very slow. During mungbean production, P uptake rate was quite high; it

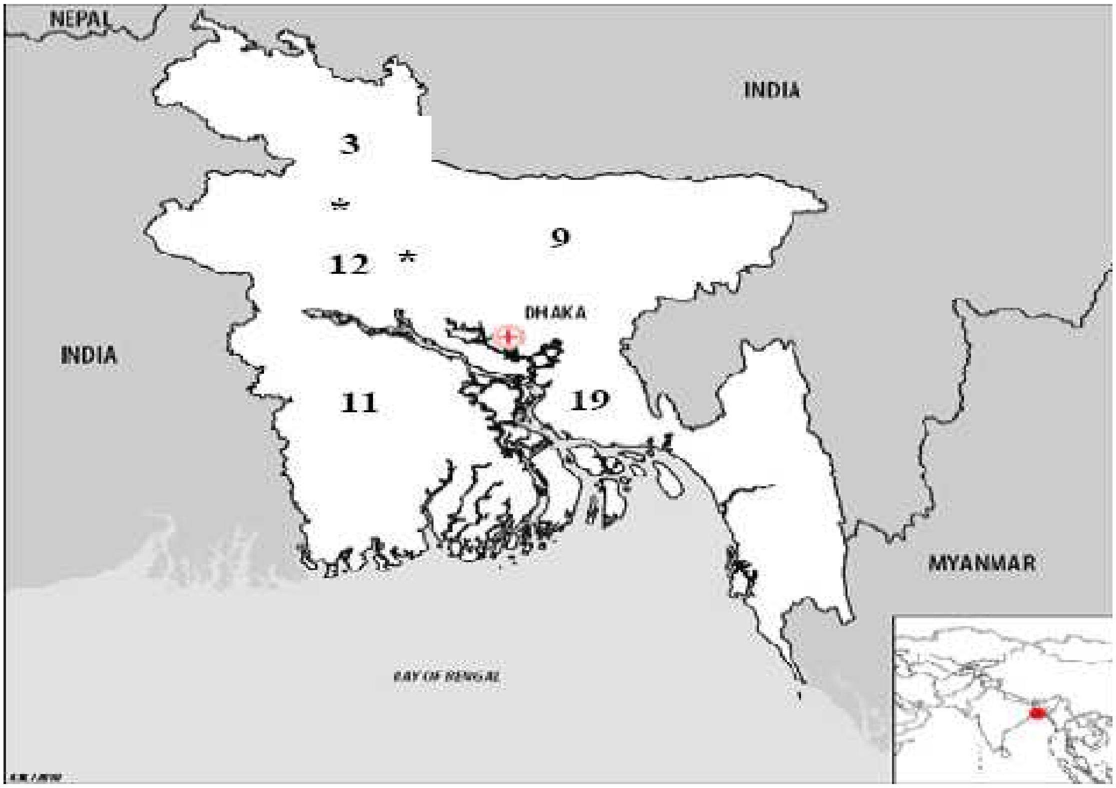

Fig 1. Map of Bangladesh (Agro-ecological zones of collected rhizosphere soils:AEZ-3, 9, 11, 12 & 19; *-Field experimental locations)

Table 1. Effect of different phosphorus solubilizing isolates on phosphorus solubilization in rock phosphate.

Isolates	AEZs, Location	Rhizosphere	Amount solubilized (mg P L^{-1})	Net solubilized (mg P L^{-1})	% solubilization
I_0	-	-	0.40	-	-
I_1	Tista Meander Floodplain (AEZ-3)	*Dhaincha*	5.03	4.63	42.48
I_2			7.23	6.84	62.73
I_3			5.88	5.48	50.23
I_4			4.01	3.61	33.12
I_5	Old Brahmaputra Floodplain (AEZ-9)	Blackgram	4.70	4.30	39.45
I_6			4.44	4.04	37.06
I_7			3.75	3.35	30.73
I_8			7.45	7.05	64.68
I_9			4.65	4.25	38.99
I_{10}		Soybean	5.04	4.64	42.57
I_{11}			2.95	2.55	23.40
I_{12}			5.24	4.84	44.40
I_{13}			1.66	1.25	11.46
I_{14}		Rice	4.86	4.46	40.92
I_{15}			5.53	5.13	47.06
I_{16}			7.74	7.34	67.34
I_{17}			6.71	6.31	57.89
I_{18}		Mustard	5.72	5.39	49.45

I_{19}	High Ganges River Floodplain (AEZ-11)	Rice	3.58	3.18	29.17
I_{20}			0.89	0.49	4.50
I_{21}			0.61	0.21	1.93
I_{22}		Blackgram	2.54	2.14	19.63
I_{23}		Mustard	2.06	1.66	15.23
I_{24}		Brinjal	1.65	1.25	11.47
I_{25}		Lettuce	1.04	0.64	5.87
I_{26}		Garlic	1.90	1.50	13.76
I_{27}		Maize	1.84	1.44	13.21
I_{28}	Active Ganges River Floodplain (AEZ-12)	Rice	3.58	3.18	29.17
I_{29}			4.75	4.35	39.91
I_{30}			4.44	4.04	37.06
I_{31}			1.85	1.45	13.30
I_{32}	Old Meghna Estuarine Floodplain (AEZ-19)	Rice	3.92	3.52	32.30
I_{33}		*Dhaincha*	4.76	4.36	40.00
I_{34}		Blackgram	6.79	6.39	58.62
$LSD_{0.05}$			0.39	-	-
CV(%)			5.73	-	-

Table 2. Effect of phosphorus and bacterial isolates on yield and yield contributing characters of mungbean

Treatment	Bogra				Pabna			
	Total nodule (no. plant^{-1})	Nodule dry wt. (mg plant^{-1})	Stover yield (g plant^{-1})	Grain yield (g plant^{-1})	Total nodule (no. plant^{-1})	Nodule dry wt. (mg plant^{-1})	Stover yield (g plant^{-1})	Grain yield (g plant^{-1})
Phosphorus levels (P)								
P_0	8.25	20.13	1.52	0.46	72.79	66.88	1.98	0.97
P_{20}	19.71	28.46	1.31	0.60	91.33	82.13	2.72	1.14
P_{40}	17.21	26.46	1.81	0.65	96.17	86.79	3.16	1.03
P_{60}	19.25	36.88	1.55	0.77	86.67	67.17	3.18	0.95
Isolates (I)								
Uninoc.	17.25	11.25	1.62	0.55	86.67	76.92	2.39	0.62
I_1	24.00	37.33	1.62	0.71	90.75	78.42	2.80	1.18
I_2	12.42	25.17	1.54	0.69	79.25	57.83	2.61	1.23
I_3	10.58	21.83	1.22	0.61	93.92	71.92	2.97	0.97
I_4	10.67	23.83	1.54	0.57	94.75	84.50	3.02	1.33
I_5	14.92	40.00	2.02	0.63	78.08	78.08	2.75	0.83
I_6	20.75	33.67	1.36	0.61	74.33	73.50	2.63	1.09
I_7	18.25	30.75	1.45	0.59	96.17	84.75	2.91	0.93
$LSD_{0.05}$								
P	2.93	NS	NS	0.06	NS	NS	0.13	NS
I	2.23	1.78	NS	0.06	NS	NS	0.16	0.16
P × I	2.18	1.56	NS	0.05	2.43	2.37	NS	0.03

LSD=Least significant difference at 5% level of probability and NS=Non-significant

created a zone around the roots that was depleted of phosphorus in soil. In order to maintain optimum P concentration in the root zone, it becomes necessary to regularly supplement the phosphorus with the help of PSB or higher dose of phosphatic fertilizer. As a result, 60 kg P ha^{-1} performance was better than the 20 kg P ha^{-1} in Bogra soils. Similar observations were recorded by (Muralidharan and Ratan, 2001; Qureshi *et al*., 2012).

Effect of isolates on mungbean: In respect of phosphate solubilizing inoculum significantly affected nodules plant^{-1}, dry weight of nodules plant^{-1}, stover yield and yield of mungbean at Pabna and Bogra. Highest number of nodules (24.00 and 96.17 per plant) was produced when inoculated with I_1 and I_7 compared to I_3 (10.58 per plant) and I_6 (74.33 per plant) at Bogra and Pabna, respectively. At Pabna, nodulation capacity was higher than Bogra. Soils of Pabna, P use efficiency was better than the Bogra soils. In this regard, phosphate sorption decreased in high pH soil. More P was sorbed in soils having low pH than the high pH soils. These results are in agreement with the findings (Jobe *et al*., 2007; Haque *et al*., 2010).

Interaction effect of isolates and phosphatic fertilizer on mungbean: The interaction effect of phosphorus doses and isolates was significant in respect of total nodule plant^{-1}, nodule dry weight plant^{-1} and grain yield at Bogra and Pabna. Such effect for hay yield was not significant at both the locations. Similar result was also obtained by (Valverde *et al*., 2006; Qureshi *et al*., 2011). Isolates of rice rhizosphere at Old Brahmaputra Floodplain soils were effective for phosphate solubilization. It may be concluded that application of PSB isolate with 20 kg P ha^{-1} had beneficial effects on nodulation and yield of mungbean at Pabna. Phosphate solubilizing bacteria helped to improve the use efficiency of chemical phosphatic fertilizer and as a result increased yield of mungbean.

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