Published by :
http://www.ijert.org

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

Screening of Plant Growth Promoting Rhizobacteria for Improving the Growth of Mung Bean

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***Abstract*— Plant growth promoting rhizobacteria (PGPR) are considered to have a beneficial effect on host plants and may facilitate plant growth by different mechanisms. Present study was conducted to screen the eleven rhizobacterial strains for plant growth promoting activities in mung bean under axenic conditions. Results indicated that the bacterial inoculation significantly increased the root/shoot length, fresh and dry biomass as compared to respective uninoculated control. However, the effect of bacterial strains SM3 and SM10 was statistically significant for improving the shoot growth and root growth of mung bean seedlings, respectively, compared with other strains and uninoculated control. Characterization of rhizobacteria for plant growth promoting traits revealed that SM3 and SM10 strain possess auxin, siderophore, exopolysaccharide and phosphate solublization ability. The preliminary investigation of this study suggested that these strains could be used for improving the productivity of mung bean, although the potential of plant growth promotion depend upon on the type of bacterial strain.**

Keywords—Rhizobacteria, Auxin, Exopolysaccharide, Mung bean

I. INTRODUCTION

Mung bean is an important pulse crop grown worldwide. Its importance is not only due to its nutritional value in human diet but also play vital role in improving soil fertility through biological nitrogen fixation in the nodules [1]. However, its productivity is low due to its cultivation on marginal lands in arid region [2].

Research about how soil microorganisms that may play a role in soil fertility may provide a means of reducing the quantities of fertilizers needed has been performed for several decades. Plant growth promoting rhizobacteria (PGPR) are the beneficial free living soil-borne bacteria which can colonize with plant roots and are able to improve the plant growth through multiple mechanisms of action [3, 4]. Plant growth promoting rhizobacteria have been reported to improve the plant growth via biological fixation of atmospheric nitrogen, biosynthesis of phytohormone, nutrient solublisation and increasing the host plant resistance to biotic and stress factors [5-7]. Many bacteria, including Klebsiella, Bacillus, Azospirillum, Herbaspirillum, Burkholderia and Pseudomonas spp., have been found to be associated with cereals including mung bean and other crops. The inoculation of mung bean with such bacteria has been shown to enhance crop yields [8, 9].

The large-scale application of PGPR to crop as inoculants would be attractive as it would substantially reduce the use of chemical fertilizers and pesticides, which often pollute the environment. This has a heavy impact on the natural and human environment, as well as on human health, through the pollution of soils, waters, and the whole food supply chain. Therefore, the purpose of this work was to select and characterize growth-promoting rhizobacteria and to assess their potential to increase the growth of mung bean.

II. MATERIAL AND METHODS

In this study we evaluated the response of inoculation of rhizobacterial strains for growth promoting activities in mung bean under axenic conditions. The rhizobacterial strains (SM2, SM3,,,, SM12) used in this study were collected from Environmental Microbiology lab., Department of Environmental Sciences, King Abdulaziz University.

For each strain, inoculum was prepared in flasks using Luria Bertani (LB) medium without agar. Each flask containing 125 mL of broth was inoculated with a selected strain of bacteria and incubated in a shaking incubator (100 rpm) for 72 h at 28 ± 1°C. Mung bean seeds were surface sterilized by momentarily dipping them in 95% ethanol and then in 0.2% $HgCl_2$ solution for 3 minutes and a subsequent thorough washing with sterilized distilled water. Three surface sterilized pre-germinated seeds were dipped into the inocula for 10 minutes and placed in autoclaved jiffy-7® (Jiffy Products International AS, Norway). Sterilized LB broth was used for the control treatment. The whole procedure of inoculation and sowing was executed in a laminar flow hood to eliminate or reduce the chances of contamination. Each treatment was replicated thrice following completely randomized design. Sterilized half strength Hoagland solution was used to supply water and nutrients to plants. In the growth chamber, suitable temperature is adjusted between 25-30 °C with an alternate light (275 µmol m^{-2} s^{-1}) and dark period of 10

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

and 14 h, respectively. Data regarding root/shoot length and fresh biomass was recorded at harvesting after three weeks. On the basis of plant growth promotion of mung bean in screening experiment, two most effective strains were selected for field evaluation.

The rhizobacterial strains were characterized for different plant growth promoting activities. (1) The method of Mehta and Nautiyal [10] was used to assess the potential of the selected rhizobial strains to solubilize inorganic phosphate qualitatively. Phosphate growth media i.e. NBRI-PBB (National Botanical Research Institute Phosphate Bromophenol Blue) was used. (2) Agar media of Chrome azurol S (CAS) was made following Schwyn and Neilands [11] and was taken in Petri plate (30 mL per plate). In each Petri plate, three wells were made on media using # 1 cork borer and 30 µl of the respective culture was added to the wells. To control plates, sterile broth media was added. All the plates were incubated for 48 hours at room temperature. Change in color of the medium was evidenced as positive for siderophore production. (3) Broth culture (3-day-old) of the strains tuned to 0.5 OD (107 – 108 CFU mL^{-1}) obtained by using BIOLOG® OD meter was applied on the Petri plates having RCV-glucose media [12] at five places. The plates were incubated for four days at 28 °C ± 1 after which the Petri plates were examined for mucoid growth of the inoculated strains. Mucoid colonies were considered as positive in exopolysaccharide production. (4) IAA (auxin) production in the presence or absence of its precursor L-tryptophan (L-TRP) was carried out by using the method of Sarwar et al. [13].

Data were analyzed by analysis of variance (ANOVA) [14] and means were compared using LSD test. Software used for the analysis was Statistix 8.1 (Analytical Software, USA).

III. RESULTS AND DISCUSSION

Performance of the inoculated seedlings of mung bean was significantly different from the un-inoculated control. However, the degree of plant growth promotion in mung bean by inoculation with rhizobacterail strains is different from one another but differ significantly from uninoculated control. The effect of inoculation with rhizobacteria on shoot fresh biomass of mung bean seedlings is shown in Table 1. Among the tested strains, inoculation with rhizobacterial strains SM2 and SM3 significantly improved the shoot fresh biomass of mung bean seedlings compared with their respective uninoculated control. However, bacterial strains SM10, SM11 and SM12 showed similar effect on shoot fresh biomass but differed significantly from uninoculated control. Likewise, inoculation with rhizobacterial strains significantly increased the root fresh biomass of mung bean seedlings over uninoculated control (Table 1). Root fresh biomass differed significantly over control in response to inoculation with strains SM3 and SM10 compared with control. While SM8 and SM11 produced statistically similar effect on root fresh biomass but differed significantly from uninoculated control. Data regarding shoot length showed that all the tested rhizobacterial strains improved the shoot length of mung bean seedlings compared with uninoculated control (Table 1). Maximum improvement in shoot length was observed in response to inoculation with strains SM3 and SM10 over control. While the strains SM2, SM8 and SM12 gave statistically similar results with each other but improved significantly compared with control. Data presented in Table 1 reveal the significant ability of these rhizobacteria to enhance the root length of mung bean seedlings. Among the rhizobacterial strains, inoculation of SM8 and SM10 gave significant improvement in root length compared with other strains over the un-inoculated control. However, the strains SM2 and SM11 gave similar effect with each other but improved the root length significantly compared with uninoculated control.The results of biochemical tests of selected rhizobacterial strains have been presented in Table (2). The results indicated that both strains were positive for exopolysaccaharide, siderophore and phosphate solublization. While the maximum auxin production was observed in SM10 compared with SM3 strain. Briefly speaking, all the tested rhizobacterial strains have variable potential for improving the growth and development of mung bean seedlings. However, the strains SM3 and SM10 were found more efficient for improving the seedling growth compared with other strains and uninoculated control under axenic conditions.

Table 1. Effect of PGPR inoculation on growth of mung bean seedlings under axenic conditions.

PGPR	Shoot length (cm)	Root length (cm)	Shoot fresh biomass (mg)	Root fresh biomass (mg)
Control	13.4 f	14.83 ef	86.67 h	15.66 j
SM2	21 a-c	18.33 bc	190 a	85 f
SM3	22.33 a	15.73 e	196.67 a	105 e
SM4	21.67 ab	19 b	156.66 b-d	81.66 f
SM5	18 de	14 f	150 de	28.33 i
SM6	16.6 e	18 c	143.33 e	65 g
SM7	22.33 a	17 d	160 bc	111.66 d
SM8	19.67 b-d	21.33 a	123.33 f	118.33 bc
SM9	18.4 de	10.83 g	103.33 g	41.33 h
SM10	20 b-d	20.76 a	163.33 b	135 a
SM11	17.1 e	18.83 bc	163.33 b	121.66 b
SM12	19.33 cd	14.67 f	153.33 cd	115 cd
LSD	2.06	1.00	9.76	5.01

Means followed by different letter are significantly different according to LSD ($p \leq 0.05$)

Rhizobacteria have been extensively studied in different plant species to understand how to use them wisely to maximize the crop production. Several bacterial genera including *Arthrobactor, Azospirillum, Bacillus, Enterobactor, Pseodomonas, Rhizobium, Acinetobactor, Burkholderia* and many others have been confirmed for plant growth promoting (PGP) activities in different crop plants [15, 16, 17, 18, 19]. In our study, 11 PGPR strains were tested for PGP activities in mung bean. The stains SM3 and SM10 among the tested strains were found the most promising for growth promotion of mung bean in terms of root or shoot length and root or shoot fresh weight. Both these strains were found to have IAA production, phosphate solublization and exopolysaccharide activity which might be the most plausible causes of plant growth promotion [17, 20-22]. These results are confirmed by findings of Arruda et al. [17] where inoculation of maize seedlings with Achromobacter sp. and Burkholderia sp. possessing IAA and phosphate solublization activity significantly enhanced the dry weight of root and shoot. Similar effect of PGPR inoculation on plant growth promotion

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 5 Issue 04, April-2016

have been observed by various researchers in other crops i.e., wheat [23], corn [24], mung bean [9] and peanut [25].

Table 2. Plant growth promoting characteristics of selected rhizobacterial isolates

PGPR strain	IAA Production (mg L^{-1})		EPS	PS	Sid
	Without L-TRP	With L-TRP			
SM3	2.56	22.36	+	+	+
SM10	2.79	26.88	+	+	+

IAA (Indole acetic acid), L-TRP (L-Tryptophan), EPS (Exopolysaccharide activity), PS (Phosphate solublization), Sid (Siderophore activity).

IV. CONCLUSION

The present study shows a high potential of plant growth promoting rhizobacteria for improving the growth and development of mung bean. Therefore, the use of PGPR inoculants may be an effective approach for improving the crop productivity.

ACKNOWLEDGMENT

This article was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah. The authors, therefore, acknowledge with thanks DSR technical and financial support.

REFERENCES

[1] Elahi, N.N., S. Mustafa and J.I. Mirza. 2004. Growth and nodulation of mung bean (Vigna radiata L.) as affected by sodium chloride. J. Res. Sci. 15:139-143.

[2] Ahmad M, Zahir ZA, Khalid M, Nazli F, Arshad M (2013) Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. Plant Physiol Biochem 63:170-176. doi:10.1016/j.plaphy.2012.11.024

[3] Patten CL, Glick BR (2002) Role of Pseudomonas putida indole acetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–801. doi: 10.1128/AEM.68.8.3795-3801.2002

[4] Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571–586.

[5] Kang SM, Joo GJ, Hamayun M, Na, CI, Shin, DH, Kim, YK, Hong JK, Lee IJ (2009) Gibberellin production and phosphate solubilization by newly isolated strain of Acinetobacter calcoaceticus and its effect on plant growth. Biotechnol Lett 31:277-281. doi:10.1007/s10529-008-9867-2

[6] Kang S, Radhakrishnan R, Khan AL, Kim M, Park J, Kim B, Shin D, Lee I (2014) Gibberellin secreting rhizobacterium, Pseudomonas putida H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. Plant Physiol Biochem 84:115-124. doi:10.1016/j.plaphy.2014.09.001

[7] Richardson AE, Barea, JM, McNeill AM, Combaret CP (2009) Acquisition of phosphorous nitrogen in the rhizosphere and plant growth promotion by microorganism. Plant Soil 321:305-339. doi: 10.1007/s11104-009-9895-2

[8] Mehnaz, S., Kowalik, T., Reynolds, B., Lazarovitz, G., 2010. Growth promoting effects of corn (Zea mays) bacterial isolates under greenhouse and field conditions. Soil Biol. Biochem. 42, 1848–1856

[9] Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through co-inoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate-deaminase. Can J Microbiol 57:578-89. doi: 10.1139/W11-044

[10] Mehta S, Nautiyal CS (2001) An efficient method for qualitative screening of phosphate solubilizing bacteria. Curr. Microbiol. 43: 51-58. doi:10.1007/s002840010259

[11] Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160: 47-56. doi:10.1016/0003-2697(87)90612-9

[12] Ashraf M, Berge SH, Mahmood OT (2004) Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40:157–162. doi: 10.1007/s00374-004-0766-y

[13] Sarwar M, Arshad M, Martens DA, Frankenberger Jr WT (1992) Tryptophan dependent biosynthesis of auxins in soil. Plant Soil 147:207-215. doi: 10.1007/BF00029072

[14] Steel RGD, Torrie JH, Dicky DA (1997) Principles and procedures of statistics—A biometrical approach. 3rd ed. McGraw-Hill, New York, NY.

[15] Beneduzi A, Peres D, Vargas LK, Bodanese-Zanettini MH, Passaglia LMP (2008) Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil. Appl Soil Ecol 39:311–320. doi:10.1016/j.apsoil.2008.01.006

[16] Won-I K, Cho WK, Kim SN, Chu H, Ryu KY, Yu JC, Park CS (2011) Genetic Diversity of Cultivable Plant Growth-Promoting Rhizobacteria in Korea. J Microbiol Biotechnol 21:777–790. doi: 10.4014/jmb.1101.01031

[17] Arruda L, Beneduzi A, Martins A, Lisboa B, Lopes C, Bertolo F, Passaglia LMP, Vargas LK (2013) Screening of rhizobacteria isolated from maize (Zea mays L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. Appl Soil Ecol 63:15– 22. doi: 10.1016/j.apsoil.2012.09.001

[18] Hussain MB, Zahir ZA, Asghar HN, Mahmood S (2014) Scrutinizing Rhizobia to Rescue Maize Growth under Reduced Water Conditions. Soil Sci Soc Am J 78:538–545. doi: 10.2136/sssaj2013.07.0315

[19] Kumar A, Maurya BR, Raghuwanshi R, (2014) Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (Triticum aestivum L.). Biocatal Agric Biotechnol. doi: 10.1016/j.bcab.2014.08.003

[20] Zahir ZA, Arshad M, Frankenberger Jr WT (2004) Plant growth promoting rhizobacteria application and perspectives in agriculture. Adv Agron 81:96-168. doi: 10.1016/S0065-2113(03)81003-9

[21] Nadeem, SM, Zahir ZA, NaveedM, Asghar HN, Arshad M (2010) Rhizobacteria capable of producing ACC-deaminase may mitigate the salt stress in wheat. Soil Sci Soc Am J 74: 533–42. doi:10.2136/sssaj2008.0240

[22] Günes A, Turan M, Güllüce M, Sahin F (2014) Nutritional content analysis of plant growth-promoting rhizobacteria species. Eur J Soil Biol 60:88-97. doi: 10.1016/j.ejsobi.2013.10.010

[23] Kudoyarova GR, Melentiev AI, Martynenko EV, Timergalina LN, Arkhipova TN, Shendel GV, Kuz'mina LY, Dodd IC, Veselov SY (2014) Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. Plant Physiology and Biochemistry 83:285-291. doi: 10.1016/j.plaphy.2014.08.015

[24] Piromyou P, Buranabanyat B. Tantasawat P, Tittabutr P, Boonkerd N, Teaumroong N (2011) Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. Eur J Soil Biol 47:44–54. doi:10.1016/j.ejsobi.2010.11.004

[25] Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. Microbiological Research 159:371-394.