Range Mgmt. & Agroforestry 34 (2) : 171-174, 2013
ISSN 0971-2070

Application of bacterial endophytes as bioinoculant enhances germination, seedling growth and yield of maize (*Zea mays* L.)

N. P. Singh*, R. K. Singh, J. P. Shahi, H. K. Jaiswal and T. Singh[1]
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi- 221005, India
[1]Present address: Indian Grassland and Fodder Research Institute; Jhansi-284003, India
*Corresponding author e-mail norang40@gmail.com
Received: 24th July, 2012 Accepted: 10th December, 2013

Abstract
Ten endophytic bacteria isolated from maize plants at farmer fields were characterized for their endophytic association and various biochemical activities such as production of phytohormones, siderophore and phosphate solubilization. All the selected isolates produced indole acetic acid. However, four isolates (BHU3, BHU8, BHU9 and BHU10) have ability for phosphate solubilization. Siderophore production was shown by six isolates (BHU1, BHU2, BHU3, BHU5, BHU8 and BHU10). Activates of endophytic bacterial isolates under field conditions indicated significant increase in seed germination, chlorophyll content, cob length, plant height, test weight, grain yield and harvest index over control. Isolates BHU3, BHU8 and BHU10 performed relatively better than the other isolates.

Keywords: Biochemical activities, Endophyte, Maize, PGPR

Abbreviations: **IAA**: Indole Acetic Acid, **PGPR**: Plant Growth Promoting Rhizobacteria

Introduction

Maize (*Zea mays* L.) is one of the three important cereal crops after wheat and rice in world. Increasing demand for food and livestock feed caused maize to be introduced as an important crop in temperate and semi-arid regions. Intensive farming practices that aim to produce higher yield, require extensive use of agrochemicals which are costly and create environmental pollution (Kozdro *et al.,* 2004).

Microorganisms play pivotal role in recycling of plant nutrients and have considerable potential to reduce the need for chemical fertilizers (Diep and Hieu, 2013). Plant growth promoting rhizobacteria have more significance in this regard because they can enhance the availability of plant nutrients as well as promote plant growth (Verma *et al.,* 2013). Direct effects of PGPR have been attributed to the production of plant hormones such as auxins (Patil *et al.,* 2011), cytokinins (Arkhipova *et al.,* 2005), and gibberellins (Patel *et al.,* 2012) as well as solubilization of phosphate (Verma *et al.,* 2013). Indirect mechanisms for promotion of plant growth includes suppression of bacterial, fungal and nematode pathogens by production of cyanide, siderophores, ammonia (Marques *et al.,* 2010), antibiotics and volatile metabolites *etc*. The present study reports the characterization of endophytic bacterial isolates from maize plants and effect of their inoculation on germination, growth parameters and yield of maize.

Materials and Methods

Isolation and Identification of bacterial endophytes

Maize roots from North-eastern part of Uttar Pradesh (Varanasi, Mirzapur and Jaunpur) and hilly region of Uttrakhand (Chamoli and Joshimath), India were collected at the age of 15 days after sowing. Roots were cleaned thoroughly with tap water, rinsed with sterile distilled water and cut into 2-5 cm long pieces. Five grams of root pieces of each sample were transferred to a sterile 250 ml Erlenmeyer flask containing 50 ml of sterile water, shaken for 15 min, and washed six times in 50 ml of sterile distilled water. Pieces of root were then aseptically transferred to another sterile 250 ml Erlenmeyer flask and surface sterilized by shaking in 95% ethanol for 1 min and then in 0.1% $HgCl_2$ for 5 min (Chaintreuil *et al.,* 2000). The 0.1 ml suspension of sample was spread on Yeast extract manitol medium (Vincent, 1970), Luria Broth medium (Bertani, 1951), Azelic acid medium (Santos *et al.,* 2001), and King s B medium (King *et al.,* 1954) and incubated at 28^{0}C for 48-72 hr. Well developed colonies were selected and stored in the same media at 4 ^{0}C.

Characterization of isolates

Morphological characteristics of the endophytic colonies such as shape, size, elevation, surface, margin and colour were recorded on YEM agar plates after 3 days of incubation. Siderophore production by bacterial isolates was detected as described by Schwyn and Neilands (1987) with some modifications. IAA production was estimated by growing the bacterial strains in YEM broth supplemented with 100 µl tryptophan ml^{-1} (Gordon and Weber, 1951). Phosphate solubilising activity of the isolates was assessed on Pikovskyaõs medium.

Field experiment

The field experiment was conducted at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India in 2011. The soil was sandy clay loam in texture having 40.8 % water holding capacity, pH 7.2, electrical conductivity 0.155 dSm^{1}, 0.78 % organic C and 213.2, 27.2 and 254.7 kg available N, P_2O_5 and K_2O ha^{-1}, respectively. The microbial population of total bacteria, fungi and actinomycetes were 4.5×10^{-8}, 3.1×10^{-8} and 3.4×10^{-8} CFU g^{1} soil), respectively. Data were recorded on various plant growth parameters and yield attributes were analysed by mean of one way ANOVA procedure and means were compared by the F-test ($P<0.05$).

Results and Discussion

Morphological and biochemical characterization

Endophytic association by plant growth promoting rhizobacteria (PGPR) exert beneficial effects on plant growth and development by a wide variety of mechanisms. Among the ten isolates BHU3 formed bluish colonies where as BHU6 formed pink and rest of the isolates shown white watery colonies (Table 1). Plant growth promoting activities of IAA production, siderophore and phosphate solubilization were tested. Among ten endophytic bacterial isolates, only six isolates (BHU1, BHU2, BHU3, BHU5, BHU8 and BHU10) showed the production of siderophore. All endophytic bacterial isolates have the ability to production of IAA, higher IAA production was observed with BHU3, BHU8, BHU9 and BHU10 as compared with BHU1, BHU2, BHU5 and BHU6. In case of phosphorous solubilizing ability among ten isolates only BHU3, BHU8, BHU9 and BHU10 isolates had the ability for solubilization of phosphorus (Table 1). Similar findings were also reported by (Alia *et al.,* 2013).

Field experiment

The results showed that inoculation with all endophytic bacterial treatments had a stimulated significant increase in seed germination, chlorophyll content, cob length, test weight, plant dry weight, grain yield and harvest index (Table 2). BHU8 showed maximum increase (29.36%) in plant height over control. Seed germination was significantly increased by 22.70%, 22.70%, 20.25% and 18.25% respectively due to inoculation with endophytic bacterial isolates BHU3, BHU8, BHU9 and BHU10. A significant increase in chlorophyll content was recorded in plants inoculated with BHU8 (32.08%) followed by BHU3 (31.09%), BHU10 (28.33%) and BHU9 (25.57%) over control. Maximum cob length of maize significantly influenced with BHU8 (26.78%), followed by BHU3 (23.71%), BHU10 (21.67%) and BHU9 (20.64%) control. Maximum dry matter production was recorded with BHU8 (32.84%) over the control. Maximum grain yield was recorded with BHU8 (72.22%) followed by BHU3 (58.89%), BHU10 (52.50%) and BHU9 (50.00%) over control. Maximum test weight was also recorded with BHU8 (29.75 g). Harvest index ranged from 27.67 to 37.41% and maximum harvest index was recorded with BHU8. Many reports have shown a contribution to the growth of maize plants by plant growth promoting rhizobacteria. Some studies showed that the extent of positive bacterial effects on plant growth may vary between the species or on different genotypes of the maize crop (Mitter *et al.,* 2013).

Table 1. Morphological and plant growth promoting activities (Siderophore, IAA and P-solubilization) by endophytic bacterial isolates.

Isolates	Colour	Pigmentation	Siderophore production	IAA production	P-solubilisation
BHU1	White watery	No	+	+	ND
BHU2	White watery	No	+	+	ND
BHU3	Blue green	Blue	++	+++	+++
BHU4	White mucilageous	No	ND	+ +	ND
BHU5	White mucilageous	No	++	+	ND
BHU6	Pink	Pink	ND	+	ND
BHU7	White mucilageous	No	ND	+ +	ND
BHU8	White watery	No	+++	+ ++	+++
BHU9	White watery	No	ND	+ ++	+++
BHU10	White watery	No	+++	+++	+++

ND = Not detected; + = weak producer; ++ = medium producer; and +++ = good producer.

Table 2: Effect of endophytic bacterial isolates on growth and yield attributes of maize under field conditions

Isolates	Plant height (cm)	Seed Germination (%)	Chlorophyll (SPAD)	Cob length (cm)	100 Grain weight (g)	Dry matter (g)	Grain yield (g)	Harvest index (%)
Control	163.50	81.50	36.24	81.50	20.00	305.25	90.00	27.67
BHU1	174.75	93.00	39.10	83.33	23.50	325.50	95.75	29.41
BHU2	182.75	84.00	39.45	91.66	24.25	327.00	109.25	33.40
BHU3	211.50	100.00	47.86	103.33	30.25	405.50	155.00	38.22
BHU4	187.75	92.00	40.95	93.33	24.50	346.50	113.00	32.65
BHU5	189.50	83.00	41.50	94.66	23.25	341.25	111.25	32.60
BHU6	192.00	84.63	42.00	95.70	24.75	359.25	122.20	35.85
BHU7	191.75	91.13	40.50	92.50	24.00	353.25	117.00	32.65
BHU8	202.00	100.00	47.50	100.83	29.75	382.25	143.00	37.41
BHU9	196.00	96.38	45.50	98.33	27.00	370.00	135.00	36.48
BHU10	200.50	98.00	46.50	99.16	28.00	377.00	137.25	36.60
LSD 5%	1.37	0.60	0.89	1.02	0.66	3.35	2.02	0.77

Conclusion

The present research shows a high bacterial diversity in the roots and rhizosphere soil of the maize plants cultivated in different regions of Uttar Pradesh. Ten bacterial isolates demonstrated the positive effects of these strains on germination percentage, plant height, grain yield and chlorophyll content under field conditions. Among ten endophytic bacterial isolate, highest producers of IAA and siderophore and efficient phosphate solubilizing ability, BHU3, BHU8 and BHU10 were found to be most efficient plant growth promoter on the maize cultivars used in this study. These strains were therefore recommended for the use as bio fertilizer for field trials in future.

Acknowledgement: Authors were thankful to Head, Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University for providing the necessary facilities.

References

Alia, A. A., S. N. Khokhar, B. Jabeen and S. A. Asad. 2013. Phosphate solubilizing bacteria associated with vegetables roots in different ecologies. *Pak. J. Bot*. 45: 535ï 544.

Arkhipova, T. N., S. U. Veselov, A. I. Melantiev, N. E. V. Marty and G. R. Kudoyerova. 2005. Ability of bacterium Bacillus to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. *Plant Soil*. 272: 201ï 209.

Bertani, G. 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J. Biotech*. 62: 293ï 300.

Chaintreuil, C., E. Giraud, Y. Prin, J. B. A. Lorquin, M. Gillis, P. de Lajudie and B. Dreyfus. 2000. Photosynthetic *Bradyrhizobia* are natural endophytes of the African wild rice *Oryza breviligulata*. *Appl Envi Microbiol*. 66: 5437ï 5447.

Diep, C. N. and T.N. Hieu. 2013. Phosphate and potassium solubilizing bacteria from weathered materials of denatured rock mountain, Ha Tien, Kiên Giang province Vietnam. *Am. J. Life. Sci*. 1: 88ï 92.

Gordon, S. A. and R. P. Weber. 1951. Colorimetric estimation of indole acetic acid. *Plant Physiol*. 26: 192ï 195.

King, E. O., M. K. Ward and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Labo. Clini. Medi*. 44: 301ï 307.

Kozdro, J., J. T. Trevors and J. D. Van Elsas. 2004. Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. *Soil Biol. Biochem*. 36: 1775ï 1784.

Marques, A. P. G. C., C. Pires, H. Moreira, A. O. S. S. Rangel and P. M. I. Castro. 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biol. Bioch*. 42: 1229ï 1235.

Mitter, B., G. Brader, M. Afzal, S. Compant, M. Naveed, F. Trognitz and A. Sessitsch. 2013. Advances in elucidating beneficial interactions between plants, soil and bacteria. *Adv. Agron*. 121: 381ï 445.

Patel, D., C. K. Jha, N. Tank and M. Saraf. 2012. Growth enhancement of chickpea in saline soils using plant growth-promoting rhizobacteria. *J. Plant Growth Regul*. 31: 53ï 62.

Patil, N. B., M. Gajbhiye, S. S. Ahiwale, A. B. Gunjal and B. P. Kapadnis. 2011. Optimization of Indole 3 acetic acid (IAA) production by *Acetobacter diazotrophicus* L1 isolated from Sugarcane. *Inter. J. Env. Sci*. 2: 0976ï 4402.

Santos, P. E. D. L., R. B. Cristales and J. C. Mellado. 2001. *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental and geographic distribution. *Appl. Env. Microbiol*. 67: 2790ï 2798.

Schwyn, B. and J. B. Neilands. 1987. Universal assay for the detection and determination of siderophores. *Anal. Bioch*. 160: 47ï 56.

Verma, J. P., J. Yadav, K. N. Tiwaric and A. Kumar. 2013. Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. *Ecol. Eng*. 5: 282ï 286.

Vincent, J. M. 1970. *A manual for the practical study of root-nodule bacteria*. International Biological Programme handbook no. 15. Blackwell Scientific Pub. Ltd., Oxford.