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EFFECT OF INOCULATION WITH DIAZOTROPHS ON GROWTH PROMOTION OF MAIZE (*ZEA MAYS* L.) UNDER GLASS HOUSE CONDITIONS

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ABSTRACT

Biological nitrogen fixation is an environmental friendly process for improving crop yield and maintaining soil health. A study was conducted to determine the effect of inoculation with diazotrophic bacteria on growth promotion of maize under glass house conditions. Seven free living diazotrophic isolates *viz. Xanthomonas sp., Beijerinckia indica, Flavobacterium johnsoniae, Pseudoxanthomonas suwonensis, Lysinibacillus sphaericus, Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* were isolated from rhizosphere of wheat. All these isolates were able to produce ammonia and IAA, while some isolates solubilised P and produced siderophores. The inoculation of maize with diazotrophic bacteria resulted in increase in all the seed germination and plant growth promoting parameters as compared to the uninoculated control. *Stenotrophomonas maltophilia* and *Beijerinckia indica* were found to be the best for improving seed germination as well as promoting plant growth of maize. So these bacteria can be used as inoculants for improving plant growth and sustaining soil health.

KEYWORDS: Diazotrophic Bacteria, Functional Characterization, Maize, Plant Growth Promotion, Seed Germination

INTRODUCTION

Nitrogen is an essential macronutrient for plant growth and is often a yield limiting factor in crop productivity owing to the loss of a major component of mineral nitrogen from the soil through run-off and leaching [1]. Diazotrophs are the nitrogen fixing bacteria [2] which exhibit nitrogenase enzyme responsible for fixing atmospheric nitrogen. The population of diazotrophic bacteria is influenced by soil physicochemical properties. Soil microorganisms promote physicochemical changes in the soil, such as the stabilization of soil organic matter, nitrogen fixation and other alterations in soil properties necessary for plant growth. One of the major concerns in today's world is soil pollution and contamination. The potential negative effects of chemical fertilizers on the environment have led to their supplementation with microbial inoculants which provide health benefits to the plant by improving the nutrient status of soil, secretion of plant growth promoting hormones, and suppression of soil-borne pathogens. These characteristic features of diazotrophs offers an environment-friendly means to improve plant productivity and to improve nutrient quality of soil [3]. Keeping all these points in view, the objective of the present study was to study the effect of inoculation with diazotrophic bacteria on seed germination and plant growth promotion of maize under glass house conditions. Diazotrophs have a positive interaction with the host plant and can reduce the requirement for N fertilizer. However, judicious use of chemical fertilizers along with microbial inoculants can be an alternate to reduce our dependence on chemical fertilizers.

MATERIALS AND METHODS

Soil Sampling and Bacterial Isolation

The soil samples were collected from wheat rhizosphere by carefully uprooting the plants and collecting the soil sample from zone around the roots. The soil samples were analyzed for various physicochemical properties *viz*: soil texture, pH, electrical conductivity, and organic carbon (OC), ammonical and nitrate nitrogen. Serial dilution spread plate technique was used to isolate bacteria on nitrogen free media. The diazotrophs were cultured and sub-cultured on nitrogen free media to test their purity and preserved in glycerol stocks at -20˚C.

Functional Characterization of Diazotrophs

All the diazotrophs were initially screened for multiple plant growth promoting traits using ammonia production, IAA production, phosphate solubilization and siderophore production.

Ammonia Production

Ammonia production was determined by the method of [4]. The diazotrophs were grown in Nitrogen free Jensen's medium (5ml) in culture vials. The vials were incubated at 28°C for 4 days. After 4 days, 1ml of Nessler's reagent was added to each tube. The presence of faint yellow colour in the medium indicated production of small amount of ammonia and deep yellow to brown colour indicated production of large amount of ammonia.

Indole-3-Acetic Acid Production (IAA)

IAA production by diazotrophs was estimated by [5]. The diazotrophs were grown in N-free malate broth supplemented with L-tryptophan (100 μgl^{-1}) at 28°C for 3-5 days, centrifuged and 2 ml of the supernatant was transferred to a fresh test tube. If supernatant was alkaline then two drops of orthophosphoric acid were added followed by 4 ml of reagent A (1 ml of 0.4 M $FeCl_3$ in 50 ml of 35% perchoric acid). The solution was incubated for 30 min. at room temperature and the optical density was measured at 530 nm.

Phosphate Solubilization

Each diazotrophs was spot inoculated on the surface of Pikovskaya's agar medium and phosphate solubilization was estimated after 1-5 days of incubation at 28°C for 48 hours. The initial screening was made on the basis of holo zone formation and phosphorous solubilization index was calculated [6] as follows:

Solubilization Index = Z +C/C, Where, Z = Zone of solubilization (mm), C = Colony diameter (mm)

Siderophore Production

Siderophore production was done using chrome azurol S assay (CAS) method [7]. CAS (60.5 mg) was dissolved in 50 ml of deionized water and mixed with 10 ml of Fe (III) solution (1 m mol/1 $FeCl_3.6H_2O$ in 10 m mol/ 1N HCl). CAS solution was mixed with 72.9 mg of hexadecyltrimethylammonium bromide (HDTMA) previously dissolved in 40 ml of water. The solution was mixed properly by continuous stirring and sterilized. The CAS dye (100 ml) was mixed with 900 ml of succinate medium cooled to 50°C and the media was poured onto the petri plates. The diazotrophs were spot inoculated on the surface of CAS agar medium and incubated at 28°C for 48 hrs. Siderophore production was indicated by the appearance of halo zone of orange colour around the colonies.

Seed Germination Experiment

Seeds were treated with diazotrophs (Jensen's medium) for overnight. Seed germination studies were conducted using paper towel method [8]. Paper towel moistened with distilled water was stretched on a clean table and hundred seeds were arranged (10 rows and 10 seeds) on it. The paper towel was rolled and placed vertically in a seed germinator in upward position at 25-30 ºC. After eight days of incubation, seed germination parameters *viz.* per cent germination, root-shoot length and dry weight were recorded. Seed vigor I and seed vigor II [9] were calculated as follows:

Seed vigor I = (Mean plumule length+ Mean radical length) × Per cent germination

Seed vigor II = Seedling dry weight × Per cent germination

Glass House Experiment

The plant growth promoting potential of functionally efficient diazotrophs was studied under glass house conditions using maize (*Zea mays* L.) variety PMH-2 as host plant in glass house of Department of Microbiology, Punjab Agricultural University (PAU), and Ludhiana. The experiment was performed in clay pots of 3 kg capacity having soil with pH 7.7, EC 0.18, OC 0.37 %, NH_4-N 59 ppm and NO_3-N 35 ppm, using randomized block design in triplicates. The diazotrophs were supplied @ 1.5 ml/pl containing 5.8×10^6 cells/ml along with an uninoculated control. Various plant growth parameters *viz.* root-shoot length, root-shoot biomass, chlorophyll content [10], nitrogen (Kjeldahl's method), phosphorous and potassium [11] were recorded at 45 days after sowing (DAS).

RESULTS AND DISCUSSIONS

The texture of soil samples varied from loam to silt loam, sandy loam, loamy sand and clay loam, pH ranged from 6.0 - 7.8, electrical conductivity from 0.12 - 0.58 dSm^{-1} , organic carbon from 0.17 - 0.86 per cent, ammonical nitrogen from 14ppm -119ppm and nitrate nitrogen from 14 ppm - 105 ppm [12]. Assessment of soil quality is dependant on a large number of physical, chemical, biological, microbiological and biochemical properties, the last two being the most sensitive since, as they respond rapidly to changes in soil environment [13].

The diazotrophic bacteria were isolated on nitrogen free media so as to obtain bacteria specifically able to fix atmospheric nitrogen. A total of 169 diazotrophic cultures obtained on different media were sub-cultured on their respective medium and preserved in 30% glycerol stock at -20˚C. Screening of 169 diazotrophic cultures was done for different functional traits, out of which, 77 isolates produced ammonia (31 low, 26 medium and 20 high), 100 produced IAA (**43** (0-10µg/ml); **36** (10-20 µg/ml); **18** (20-30 µg/ml) and **3** (30-40 µg/ml), 44 isolates could solubilize phosphate (**31** (<1.0cm zone); **13** (>1.0cm zone)) and 15 could produce siderophores (**6** (<1.0cm zone) and **9** (>1.0cm zone)). The cultures with multiple plant growth promoting activities were characterized as *Xanthomonas sp.*, *Beijerinckia indica*, *Flavobacterium johnsoniae*, *Pseudoxanthomonas suwonensis*, *Lysinibacillus sphaericus*, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* using partial sequencing of 16S rDNA [12]. The functional attributes of diazotrophs such as ammonia production, IAA production, P solubilization and siderophore production are presented in Table 1.

Table 1: Functional Characteristics of Identified Diazotrophs

Diazotrophic Cultures	Ammonia Production	IAA Production (MG/Ml)		PSI	Siderophore Production (Cm)
		3 Days	5 Days		
Xanthomonas sp.	++	8.19	12.47	-	1.4
Beijerinckia indica	++	22.17	27.22	-	-
Flavobacterium johnsoniae	++	3.45	9.25	-	-
Pseudoxanthomonas suwonensis	++	6.37	13.58	-	-
Lysinibacillus sphaericus	++	7.28	12.61	-	1.6
Stenotrophomonas maltophilia	++	25.52	31.09	1.58	-
Pseudomonas aeruginosa	+++	21.26	23.05	1.72	-

++ Medium**,** +++ High ammonia production**,** PSI: Phosphate solubilization index

Maize (*Zea mays* L.) is an important C_4 plant grown for both grain and green fodder. It requires significant amounts of N to satisfy its potential for rapid growth and biomass production [14].These isolates were further tested for plant growth promotion of maize using seed germination and under glass house experiment. Bacterization of maize seeds with diazotrophis resulted in a significant increase in per cent seed germination as compared to the un-bacterized seed control. Maximum seed germination was obtained by inoculation with *P. suwonensis* (87 %) followed by *B. indica* (86.5 %), total seedling length with *B. indica* (33.73 cm) followed by *S. Maltophilia* (33.13 cm), seedling dry weight by *S. maltophilia* (1.043 g) and *B. indica* (0.81 g), seed vigor I by *B. indica* (2917.60) followed by *P. suwonensis* (2869.27) and seed vigor II by *S. maltophilia* (80.311) and *B. indica* (70.065). The results obtained in this study showed that majority of seedling growth parameters were affected with application of diazotrophs Figure 1.

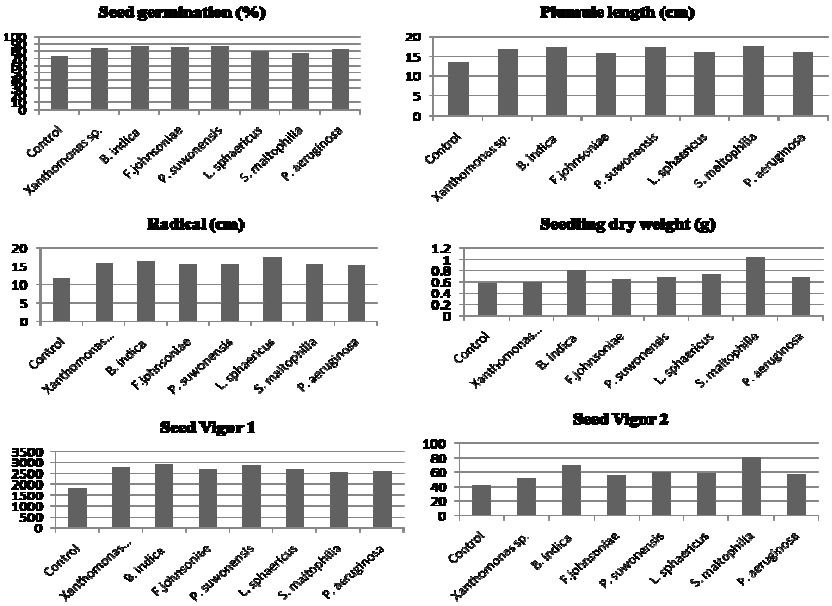

Figure 1: Effect of Priming of Maize Seeds with Diazotrophs on Seed Germination, Seedling Length, Dry Weight and Seed Vigor

S. maltophilia, *B. indica* and *L. sphaericus* were found to be best in terms of increase in majority of seed germination parameters. Production of IAA by bacterial cultures is responsible for a variety of functions including cell

division and root elongation. The increase in the seed germination and root shoot biomass of inoculated plants could be attributed to the production of IAA by these bacterial cultures which might have resulted in stimulation of seed germination, acceleration of root growth, modification in root architecture and increase in root biomass [15].

Under glass house conditions, maximum root length (57.25 cm) was obtained in the plants inoculated with *B. indica*, however, *S. maltophilia* inoculation resulted in increase in majority of growth parameters *viz.* root fresh weight (5.7 g), dry weight (1.24 g), shoot fresh weight (26.3 g) and dry weight (6.5 g) Figure 2.

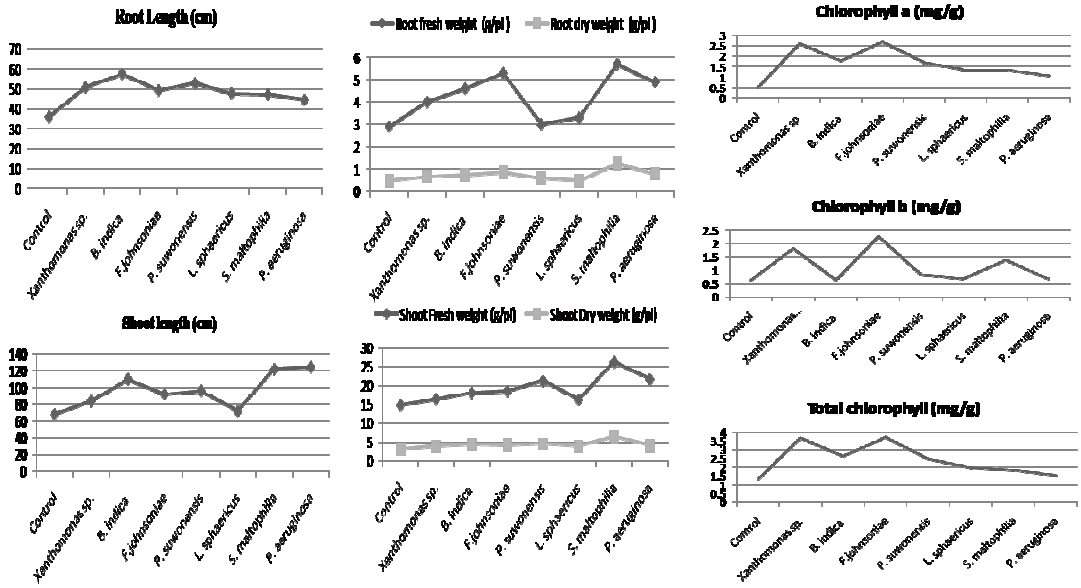

Figure 2: Effect of Inoculation with Diazotrophs on Root-Shoot Biomass and Chlorophyll Content

In inoculated plants, the content of chlorophyll 'a' ranged from 1.08 - 2.70 mg/g of plant tissue, chlorophyll 'b' ranged from 0.63 - 2.27 mg/g of plant tissue and chlorophyll 'total chlorophyll' ranged from 1.54 - 3.73 mg/g of plant tissue with highest chlorophyll 'a' (2.70 mg/g) chlorophyll 'b' (2.27 mg/g) and total chlorophyll (3.73 mg/g) obtained by inoculation with *F. johnsoniae*. An increase in N, P and K content of the maize plants was obtained in inoculated plants as compared with uninoculated control Table 2. Highest values of N, P and K were observed in plants inoculated with *S. maltophilia* and *Xanthomonas sp*. The diazotrophs were able to produce ammonia and some could solubilise phosphorus which could be one of the reasons for increasing the nitrogen and phosphorus.

Table 2: Effect of Inoculation of Identified Diazotrophs on Nitrogen, Phosphorous and Potassium of Maize Plants at 45 DAS

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Control	1.24	0.21	1.49
Xanthomonas sp.	2.59	0.48	2.87
Beijerinckia indica	1.76	0.39	2.36
Flavobacterium johnsoniae	1.92	0.33	2.83
Pseudoxanthomonas suwonensis	1.45	0.44	2.69
Lysinibacillus sphaericus	2.39	0.26	2.27
Stenotrophomonas maltophilia	2.97	0.45	2.91
Pseudomonas aeruginosa	2.83	0.49	2.67
CD @ (5%)	**0.13**	**0.12**	**0.11**

Values represent mean of triplicates

Several researchers have also reported similar findings in which inoculation with bacterial strains resulted in

increased the biomass of plants and also had a significant impact on the total N, P and K in the plant tissues over the uninoculated control [16, 17].

In conclusion, the application of diazotrophic cultures resulted in increase in the plant growth promoting parameters improves soil fertility and helps in maintaining soil health. The future prospects include the application of exceptionally potential isolates under field conditions and to evaluate their effect on growth and yield parameters.

REFERENCES

1. Bhattacharjee, R.B., Singh, A. and Mukhopadhayay, S.N. "***Appl. Microbiol. Biotechnol.*** 80,199-209 **(2008)**.
2. Burr, R.H. and Roberts, G.P. "***Ann. Rev. Nut.,*** 13, 317-35 **(1993)**.
3. Roesch, L.F.W., Camargo, F.A.O., Bento, F.M. and Triplett, E.W. "***Pl. Soil,*** 302, 91-104 **(2008)**.
4. Lata and Saxena, A.K. In training manual on Biofertilizer technology (eds.) A K Saxena IARI New Delhi pp. 24-25 **(2003)**.
5. Gordon, A.S. and Weber, R.P. "***Pl. Physiol.*** 26,192-95 **(1951)**.
6. Edi-Premono, M, Moawad, A.M. and Vlek, P.L.G. "***Indones J. Crop Sci.*** 11, 13–23 **(1996)**.
7. Schwyn, B. and Neilands, J.B. "***Anal Biochem***. 160, 47-56 **(1987)**.
8. ISTA. "***Seed Sci. Tech.*** 27, 175 **(1999)**.
9. Abdul-Baki, A.A. and Anderson, J.D. "***Crop Sci.*** 13,630-33 **(1973)**.
10. Anderson, J. M. and Boardman, N. K. ***Aus. J. Biol. Sci.*** 17:93-101 **(1964)**.
11. Jackson, M.L. "***Soil Chem. Analy***., pp 134-82. Prentice hall, New Delhi (India) **(1967)**.
12. Pathania N, Gosal S.K., Saroa G.S. and Vikal Y. "***Af. J. Microbiol. Res.*** 8, 862-871 **(2014)**.
13. Kumar, S., Chaudhuri, S. and Maiti, S.K. "***Middle-East J. Sci. Res.*** 13, 898-906 **(2013)**.
14. Kennedy, I.R., Choudhary, A.T.M.A. and Kecskes, M.L. "***Soil Biol. Biochem.*** 36, 1229-1244 **(2004)**.
15. Martinez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G. and Moraa, M.L. "***J. Soil Sci. Plant Nut.***10, 293-319 **(2010)**.
16. Biswas, J.C., Ladha, J.K. and Dazzo, F.B. "***Soil Sci. Soc. Am. J.*** 64, 1644-50 **(2000)**.
17. Yim, G., Zhang, G., Kang, J.W., Staley, J.T. and Doty, S.L. "***Biol. Fertil. Soils*** 45,669-74 **(2009)**.