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Effect of *Azotobacter chroococcum* on Wheat as PGPR

V S Phalke[1], B T Pawar[2]
[1] Department of Microbiology, Deogiri College, Chhatrapati Sambhajinagar, Maharashtra, India
[2] Department of Botany, Shri Muktanand College, Gangapur, Chhatrapati Sambhajinagar, Maharashtra, India

Abstract
Plant Growth Promoting *Azotobacter spp.* (PGPR) improves yield by the production of phytohormones, IAA, ACC-deaminase, phosphate solubilization. *Azotobacter spp*. introduced to cereal crops as PGPR which is economically best alternative to chemical fertilizers. During the present investigation application of *Azotobacter spp*. as PGPR on non-leguminous crops *i.e.* Wheat was carried out. *A. chroococcum* was used for present investigation and the effect on vegetative growth of plants was studied. The results were observed after 63 days from the date of sowing and the difference in average results were recorded in observation tables. After studying all the parameters, overall growth of Wheat crop after the application of *A. chroococcum* were described. All the parameters were studied in replications showed increased growth in treated plants as compared to control plants. So, it can be summarized that application of *A. chroococcum* as PGPR on Wheat showed positive effects in vegetative growth after 20 days. The increase in maximum percent growth recorded after application of *A. chroococcum was* 56.02%. *A. chroococcum* can be effectively used as PGPR for Wheat crop.

Keywords: *A. chroococcum*, *Rhizobacteria*, Wheat, PGPR

Introduction

Plant Growth Promoting Rhizobacteria (PGPR) is also known as Plant Health Promoting Bacteria (PHPR). They are involved in plant growth by improving soil as they produce tolerant crops and balance biogeochemical cycles (Gouda *et al.*, 2018) [18]. PGPR improves nutrients uptake by producing various phytohormones like IAA, gibberellins, auxins, cytokinins etc. which ultimately results in increase in root, shoot length, nodulation, nitrogen content etc. (Gupta *et al.*, 2000) [19]. Sustainable agriculture requires new approaches of supplying macro-nutrients for crops to reduce excess use of chemical fertilizers. It is very important to study interaction between PGPR and host plants. *A. chroococcum* is involved in the process of nitrogen fixation through non-symbiotic association

A. chroococcum is also introduced to cereal crops as PGPR which can be economically important alternative to chemical fertilizers. Some scientist studied the use of *Rhizobium* as PGPR on popular cereal crops as Antoun *et al.*, in 1998 studied the use of *Rhizobium* and *Brady Rhizobium* together as PGPR showing potential results in non-legumes that is, here, radish. Mahmud *et al.*, (2020) explained the importance to use artificial symbiont to economically crops like rice, maize, wheat etc. Perez-Montano *et al.*, (2014) explained the use of PGPR on legumes and cereal plants, they stated PGPR stimulate the plant growth by their own metabolism or affecting the plant metabolism which ultimately result in increase in root development, increases enzymatic activity and suppresses plant pathogens. Hence, during the present investigation application of *A. chroococcum* as PGPR on non-leguminous crops *i.e.* Wheat was carried out.

Materials and methods

A. chroococcum was used for present investigation and applied on a monocot crop *i.e.* Wheat to study the effect of application of *A. chroococcum* on vegetative growth without formation of root nodules. Initially, the mother culture was prepared from this *A. chroococcum* and then, the carrier material was used for observing the PGPR activity.

1. Mass Production: Pure cultures of *A. chroococcum* were used for making starter culture. The selective liquid media *viz*. YEMA/Congo Red and YEM broth without adding agar was used for inoculation of *Azotobacter* isolates. The liquid media was poured in sterile conical flask of 250 ml capacity and sterilized at 15 lbs pressure for half an hour. The *Azotobacter* isolates from pure cultures were inoculated in conical flask containing respective liquid media aseptically on laminar air flow platform. The cultures were incubated in rotary shaker for 4 days. After incubation, cell count reached up to 108-109 cells/ml and become thicker in consistency which was called mother culture (Ismail *et al.*, 2020). Mother culture was stored under suitable condition and temperature. The pH of the inoculums was kept in between 6.0 - 7.5 pH. The liquid broth culture can be used as PGPR directly as liquid bioinoculant.

2. Carrier Material: Mother culture can be used alone as liquid bioinoculant or it can be converted in to solid form by using carrier material. In experimentation, the carrier material used was activated charcoal. Fine powder of carrier material was weighed 50 gm. and autoclaved at 15 psi at 1210C for 20 min. The mother culture was mixed with carrier in 1:3 ratio (Datta *et al.*, 2015) [16]. After proper mixing, the mixture was spread on plastic sheet in closed room for air drying. Carrier material was incubated for 4-10 days at 22-24^{0}C which help the growth of *Rhizobium* in carrier material.

3. *in-vivo* Experimental Design: Liquid inoculants were stored in sterile plastic bottles and used directly to the desired plant. Each experiment was having control and treated seeds for comparison and each experiment were repeated for three times and the average of all these 03 observation was considered for further investigation. Actual application of the PGPR or inoculants to crops was done by using two methods as seed treatment and soil treatments.

a. **Seed Treatment:** Seeds of Wheat were used for studying the effect of bioinoculant. First, 10 gm. seeds were surface sterilized before sowing using 0.1% Murcuric chloride in petriplates for 2 min. and then washed using distilled water. Sterilized seeds were soaked in respective PGPR for 1 hr. in laminar air flow chamber. Inoculated seeds were then immediately sowed in pots containing one third of soil (Purwaningsih *et al.,* 2020). Along with treated seeds, normal seeds without treatment were also sown as control. The seeds in each pot were watered daily as per requirement to retain the moisture in soil

b. **Soil Treatments:** Along with seed treatment, soil treatments were also done for getting better results. Liquid inoculum as well as water mixed solid inoculum was directly used as PGPR to soil. The liquid PGPR was taken in 20ml sterile syringe and applied drop wise to the pots containing treated seeds. The observations were recorded from the date of plantation.

The treated as well as control plants were observed for its vegetative growth in respect to the parameters like root length, shoot length, total plant length and number of leaves.

Results and discussion

Effect of *A. chroococcum* onthe vegetative growth of Wheat

Effect of *A. chroococcum* was studied onthe vegetative growth of wheatusing the various parameters like root length, shoot length, total plant length, no. of leaves and leaf length. All the recorded results were summarized in Table 01. It was clearly observed that maximum percent growth was recorded in the number of leaves (Percent growth 112.5); while minimum percent growth was recorded in root length (Percent growth 17.5). Other parameters like total plant length, shoot length and leaf length were also showed remarkable percent growth of 42.0, 53.4 and 54.7 respectively. These results indicated that *A. chroococcum* was found to be more effective in increasing the number of leaves in wheat which will ultimately result in increase in yield. Effect of *A. chroococcum*on vegetative growth of Wheat after 63 days showed that only number of leaves has more effect than that of average percent growth (56.02); while root length, shoot length, total plant length and leaf length were having less effect than that of average percent growth.

Application of *A. chroococcum on* wheat showed maximum percent growth in root length *i.e.* 53.8 after 21 days of plantation minimum percent growth *i.e.*17.5after 63 days. Application of *A. chroococcum* on wheat showed sudden increase in shoot length after 56 days of plantation *i.e.* 68.8 percent growth. Maximum percent growth in wheat recorded after inoculation with *A. chroococcum i.e.* 54.3 after 56 days of plantation. While studying number of leaves in wheat, great increase (percent growth 77.8) in number of leaf after second dose (after 35 days) sof this bio inoculant. After 63 days 54.7 percent growth was recorded regarding wheat leaf length was recorded. While studying effect of *A. chroococcum* on wheat, maximum percent growth (112.5) was recorded regarding number of leaf as compare to other parameters. Minimum percent growth (17.5) was recorded regarding root length as compare to other parameters. (As per Table No.02 and Fig.01)

Pandya *et al*., (2016) reported significant effect of *Azotobacter* on *Triticum aestivum* (wheat) from Gujarat. They recorded increase in shoot length, root length and total length of plant after treatment Esmalipour *et al*., (2013) studied integrated nutrient management strategies for wheat crop by using *Azotobacter* sp. and reported the increase in plant height as well as yield of the crop. Rajaee *et al.*, (2007) applied *A. chroococcum* as bio-inoculant for wheat crop under greenhouse conditions. Promising results were highlighting superior effect on yield, seed protein percentage, seed weight, leaf area, nutrients uptake like N, P, Fe, Zn etc. than that of chemical fertilizers Aazadi *et al*., (2014) reported effect of *Azotobacter* and *Azospirullum* on different variety of wheat growth and development as well as yield. They concluded that mix bacterial inoculation showed significant effect on plant height and yield. Tummaratti *et al*., (2014) found statistically superior effect on buckwheat after the treatment of *A. chroococcum*. They used *A. chroococcum* as bio-inoculant for buckwheat and observed effect on vegetative growth in respect to different parameter *viz.* plant height, number of leaves and leaf length.

Table 1: Effect of *A. chroococcum* on the vegetative growth of Wheat

Sr. No.	No. of Days	Root Length		Shoot Length		Total Length		No. of Leaves		Leaf Length	
		Difference against Control	% Growth	Difference against Control	% Growth	Difference against Control	% Growth	Difference against Control	% Growth	Difference against Control	% Growth
1	14 Days	2.8	34.1	5.2	46.0	8	41.0	01	50	2.1	20.6
2	21 Days	5.0	53.8	6.0	42.6	11	47.0	03	60	4.8	38.7
3	28 Days	4.9	47.6	8.3	49.7	13.1	48.5	04	50	3.9	23.8
4	35 Days	4.8	42.9	8.1	40.9	12.9	41.6	07	77.8	5.7	30.5
5	42 Days	4.7	38.8	8.6	36.1	13.4	37.3	08	80	6.4	32.3
6	49 Days	4.6	34.6	11.0	40.0	15.7	38.5	10	83.3	10.0	48.8
7	56 Days	3.7	23.3	23.4	68.8	27.1	54.3	12	85.7	11.1	52.4
8	63 Days	3.2	17.5	21.0	53.4	24.2	42.0	18	112.5	12.2	54.7

Table 2: Summery of *A. chroococcum* effect on vegetative growth of Wheat after 63 days

Sr. No.	Parameters	Average results After Treatment	Average results in Control	Difference	Percent Growth
1	Root length	21.5	18.3	3.3	17.5
2	Shoot Length	60.3	39.3	21	53.4
3	Total plant length	81.8	57.6	24.2	42.0
4	No. of leaves	34	16	18	112.5
5	Leaf length	34.5	22.3	12.2	54.7
Average		46.42	30.7	15.74	56.02

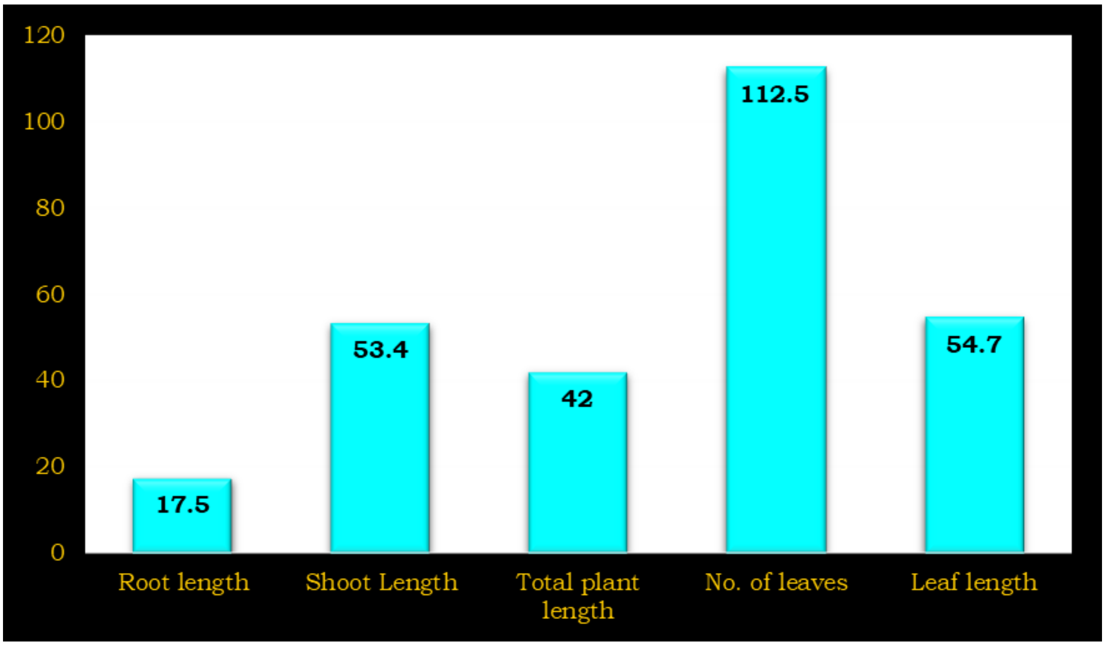

Fig 1: Summery of *A. chroococcum* effect on vegetative growth of Wheat after 63 days

References

1. Afzal A, Bano A. Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). *Int J Agric Biol*,2008:10(1):85-88.
2. Afzal A, Saleem S, Iqbal Z, Jan G, Faisal M, Malik A, *et al*. Interaction of rhizobium and *Pseudomonas* with wheat (*Triticum aestivum* L.) in potted soil with or without P2O5. *J Plant Nutr*,2014:37(13):2144–2156.
3. Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R. Potential of rhizobium and *Bradyrhizobium* species as plant growth promoting rhizobacteria on nonlegumes: effect on radishes (*Raphanus sativus* L.). *Plant Soil*,1998:204:57-67.
4. Baral BR, Adhikari P. Effect of *Azotobacter* on growth and yield of maize. *SAARC J Agri*,2013:11(2):141-147.
5. Barik AK, Goswami A. Efficacy of biofertilizers with nitrogen levels on growth, productivity and economics in wheat (*Triticum aestivum*). *Indian J Agron*,2003:48(2):100-102.
6. Behl RK, Ruppel S, Kothe E, Narula N. Wheat x *Azotobacter* x VA mycorrhiza interactions towards plant nutrition and growth – a review. *J Appl Bot Food Qual*,2007:81:95-109.
7. Bhaduri D, Pal S, Purakayastha TJ, Chakraborty K, Yadav RS, Akhtar MS. Soil quality and plant-microbe interactions in the rhizosphere. In: Lichtfouse E, ed. *Sustainable Agriculture Reviews*. Springer International Publishing, 2015.
8. Bhaduri J, Kundu P, Debarpan M, Roy SK. Isolation and characterization of nitrogen fixing bacteria (*Azotobacter* sp.) from tea field soil of Dooars and Darjeeling region of North Bengal, India. *Int J Eng Sci Invention*,2016:5(8):46-51.
9. Bisset KA, Hale CMF. The cytology and life-cycle of *Azotobacter chroococcum*. *J Gen Microbiol*,1953:8:442-448.
10. Bjelic DD, Marinkovic JB, Tintor BB, Tancic SL, Nastasic AM, Mrkovacki NB. Screening of *Azotobacter* isolates for PGP properties and antifungal activity. *Matica Srpska J Nat Sci Novi Sad*,2015:129:65-72.
11. Chaudhary D, Narula N, Sindhu SS, Behl RK. Plant growth stimulation of wheat (*Triticum aestivum* L.) by inoculation of salinity tolerant *Azotobacter* strains. *Physiol Mol Biol Plants*,2013:19(4):515–519.
12. Chen SL, Tsai MK, Huang YM, Huang CH. Diversity and characterization of *Azotobacter* isolates obtained from rice rhizosphere soils in Taiwan. *Ann Microbiol*,2017. doi:10.1007/s13213-017-1312-0.
13. Chennappa G, Adkar-Purushothama CR, Naik MK, Suraj U, Sreenivasa MY. Impact of pesticides on PGPR activity of *Azotobacter* sp. isolated from pesticide flooded paddy soils. *Greener J Agric Sci*,2014:4(4):117-129.
14. Dasgupta D, Brahmaprakash GP. Soil microbes are shaped by soil physico-chemical properties: A brief review of existing literature. *Int J Plant Soil Sci*,2021:33(1):59-71.
15. Din M, Neloferb R, Salmanc M, Abdullaha, Khan FH, Khan A, *et al*. Production of nitrogen fixing *Azotobacter* (SR-4) and phosphorus solubilizing *Aspergillus niger* and their evaluation on *Lagenaria siceraria* and *Abelmoschus esculentus*. *Biotechnol Rep*,2019:20:1-5.

16. Datta A, Singh RK, Tabassum S. Isolation, characterization and growth of *Rhizobium* strains under optimum conditions for effective biofertilizer production. *Int J Pharm Sci Rev Res*,2015:32(1):199-208.
17. Gothandapani S, Sekar S, Padaria JC. *Azotobacter chroococcum*: Utilization and potential use for agricultural crop production: An overview. *Int J Adv Res Biol Sci*,2017:4(3):35-42.
18. Gouda S, Kerry RG, Samal D, Mahapatra GP, Das G, Patra JK. Application of plant growth promoting rhizobacteria in agriculture. In: Advances in Microbial Biotechnology, 2018, 73-86.
19. Gupta A, Gopal M, Tilak KVB. Mechanism of plant growth promotion by rhizobacteria. *Indian J Exp Biol*,2000:38:856-862.
20. Ismail S, Dhamak A, Mohanty SR. *Rhizobium* biofertilizer technology for legumes of Maharashtra. Technical bulletin - *Rhizobium* biofertilizer technology for legumes of Maharashtra. AINP SBB technical bulletin VNMKV, Parbhani, India,2020. pp,1-10.
21. Jadhav PN, Gangavane LV. Cultural, morphological and biochemical characteristics of *Azotobacter* isolates from the rhizosphere soils of crop plants irrigated with sewage. *J Emerg Technol Innov Res*,2018:5(11):249-263.
22. Jimenez DJ, Montana JS, Martinez MM. Characterization of free nitrogen fixing bacteria of the genus *Azotobacter* in organic vegetable-grown Colombian soils. *Braz J Microbiol*,2011:42:846-858.
23. Jnawali AD, Ojha RB, Marahatta S. Role of *Azotobacter* in soil fertility and sustainability- A review. *Adv Plants Agric Res*,2015:2(6):1-5.
24. Kasa P, Modugapalem H, Battini K. Isolation, screening, and molecular characterization of plant growth promoting rhizobacteria isolates of *Azotobacter* and *Trichoderma* and their beneficial activities. *J Nat Sci Biol Med*,2015:6(2):360-363.
25. Kaviyarasan G, Shricharan S, Kathiravan R. Studies on isolation, biochemical characterization and nitrogen fixing ability of *Azotobacter* sp. isolated from agricultural soils. *Int J Sci Eng Appl Sci*,2020:6(11):118-125.
26. Kavimandan SK. Influence of rhizobial inoculation on yield of wheat (*Triticum aestivum* L.). *Plant Soil*,1986:95(2):297-300.
27. Latef AA, Alhmad MF, Kordrostami M, Baker AE, Zakir A. Inoculation with *Azospirillum lipoferum* or *Azotobacter chroococcum* reinforces maize growth by improving physiological activities under saline conditions. *J Plant Growth Regul*,2020. doi:10.1007/s00344-020-10065-9.
28. Mahmud K, Makaju S, Ibrahim R, Missaoui A. Current progress in nitrogen fixing plants and microbiome research. *Plants*,2020:9(1):1-17.
29. Mehboob I, Zahir ZA, Arshad M, Tanveer A, Azam F. Growth promoting activities of different *Rhizobium* spp., in wheat. *Pak J Bot*,2011:43(3):1643-1650.
30. Malik BS, Sharma RK, Sethi AP, Verma OP. Effect of *Azotobacter chroococcum* on wheat (*Triticum aestivum*) yield and its attributing components. *Indian J Agric Sci*,2005:75(9):600-602.
31. Nawadkar RB, Jadhav DB, Shaikh NR. Isolation of *Azotobacter* spp. from saline soil and its applications on wheat (*Triticum aestivum*) plant for future use in reclamation of saline soil with wheat plant. *Res J Life Sci Bioinformatics Pharm Chem Sci*,2015:1(2):62-73.
32. Perez-Montano F, Bellogin RA, Cerro PD, Espuny MR, Jimenez-Guerrero I, Lopez-Baena FJ, *et al*. Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol Res*,2014:169(5):325-336.
33. Purwaningsih O, Kusumastuti CT, Nugroho YS, Morib CY. The effect of *Rhizobium japonicum* inoculation on the growth of soybean cultivars in coastal area. *Ilmu Pertanian*,2020:4(1):33–39.
34. Razmjooei Z, Etemadi M, Eshghi S, Ramezanian A, Abarghuei FM, Alizargar J. Potential role of foliar application of *Azotobacter* on growth, nutritional value and quality of lettuce under different nitrogen levels. *Plants*,2022:11(406):1-1.