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Azotobacter species as a Natural Plant Hormone Synthesizer

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Abstract

Azotobacter plays an important role in increasing the development and physical condition of plants.Azotobacter as non symbiotic nitrogen fixing bacteria was isolated from six different rhizospheric soil samples were collected from the ten different villages of Pravara area. Isolation of *Azotobacter* spp was carried out using Ashbys liquid and were purified further. Each *Azotobacter* spp culture were recognized through microscope and biochemically tests were carried out and as per consequences obtain the spp were in, *A. vinelandii*, *A.insignis, A. chroococcum , A.agilis. A. beijerinckia, A.macrocytogenes.* Results strongly supports that the *Azotobacter* spp in combinations C+M+I and B+V+A are significantly effective to improve the crop yield. Their efficiency equal to the recommended dose of fertilizers. Highest gibberllic acid in production AzT_1, kinetin in A_ZT_2, NAA in A_ZT_1 A_ZM_3 and IAA. This supported that mutual association of *Azotobacter* spp. lead to synthesis of growth hormone, nitrogen, phosphate uptake and modification in rhizospheric interactions with respect to wheat varieties. Results strongly supports that the *Azotobacter* spp combinations C+M+I and B+V+A are significantly effective to improve the crop yield. Their efficiency equal to the recommended dose of fertilizers. The *Azotobacter* spp are capable of producing two phytohormones. *A. chroococcum* is found in the rhizospheric which posses the ability to enhance plant growth when applied to roots applied on tubers seed dressed are functioning as a plant growth promoting rhizobacteria. It was further experimentally proved that plant response to inoculation with PGPR enhance nitrogen and parameters such as plant dry weight, development, morphology of root system, grain yield, protein and mineral nutrient content. They further observed that plant response to inoculation with PGPR enhance nitrogen and parameters such as plant dry weight, development, morphology of root system, grain yield, protein and mineral nutrient content.

Keywords: *Azotobacter*spp, PGPR, Rhizospheric soil, HPLC, microbial population.

Introduction

Three main groups of compounds are auxins, gibberellins (GAs) and cytokinins. Auxins, cytokinins and gibberellins are activators of growth and developmental processes. Generally, there is no direct correlation between the absolute concentrations of endogenous phytohormones their role in plants. *Azotobacter* is free living bacteria and is widely distributed in different types of soils. *Azotobacter* is a nitrogen fixer so it enhances the plant development, produces plant growth regulators and increases mineral phosphates solubility by producing hydrogen cyanide, siderophore. *Azotobacter* has antifungal activity it produces antibiotics**.** Response of *Azotobacter* varies with wheat genotypes. Such variation is generally attributed to variability in root characters, vernalization, photoperiodic response, tillering, nutrient uptake, photosynthesis duration and grain yield[1,2] The response of *Azotobacter* inoculation with varieties led to low output (yield) and to draught tolerant genotypes led to increase in grain[3]. So, proper selection of *Azotobacter* strains and wheat variety is most important. Therefore research should be intensified to establish an effective, associative/ endophytic system in wheat by identifying effective bio-inoculants strains and respective wheat genotypes for better understanding and use of PCR based molecular markers to identify genotype-microbe interactions Mutant strains of *Azotobacter* shows higher increase in yield of grain, straw and root biomass. Inoculation of wheat varieties with mutant of *Azotobacter chroococcum* led to greater uptake of NPK. Survival rate of mutant in the rhizosphere is also higher than normal strains.

Review of literature: *Azotobacter* as a Plant Growth Promoting Rhizobacteria (PGPR) Apart from nitrogen fixation, *Azotobacter* synthesizes auxins, cytokinins and gibberellins like substances[4]. Three gibberellin like substances were detected in an *Azotobacter chroococcum* strain. The amount was in between 0.1 to 1µg/ml in 14 days old culture[5]. These hormones are originating from rhizosphere of root surface and affect the growth of plants. Hormones enhance phosphate solublization[6] influence nutrient uptake by increasing phosphotase activity[7], increases water and mineral uptake[8], production of Amino acids as well as Vitamin which are biologically active substances. Apart from phytohormone synthesis, *Azotobacter* also produces certain extra cellular substances like vit.B-12, thiamine, riboflavin, pyridoxine, biotin, cyanocobelamaine, panthothenic acid and folic acid. Mutant strains of *Azotobacter* shows higher increase in yield of grain, straw and root biomass. Inoculation of wheat varieties with mutant of *Azotobacter chroococcum* led to greater uptake of NPK. Survival rate of mutant in the rhizosphere is also higher than normal strains. It has been

proved that some mutants of *Azotobacter* can fix nitrogen if surplus amount of ammonia is present which is interrelated to the industrial application of *Azotobacter*[9]. The mutant of *Azotobacter* plays an important role in because hinders mobilization in alginate beads and it also give the opportunity to generate ammonia (plant manure) and production of antibiotics proved that when maize crop is inoculated with *Azotobacter* nitrogen fixation, production of phytohormones increased the uptake of food and also one of the reason to increase crop production and yield[10,11]. It have been recommended that oak seedlings inoculated with *Azotobacter* results in alternative growth responses [12]. It have been stated that after inoculation of azotobacterin to barley (*Hordeom vulgare*) leads to growth of plant height , dry matter content, soil nitrogen content in the lands which are having the deficiency of nitrogen in soil[13].

It has also been observed that *Rhizoctonia solani* growth is inhibited by producing an antifungal antibiotics which inhibits so it mainly acts as a Plant Growth Promoting Rhizobacteria (PGPR).

Material and Methods

Collection of soil samples: Soil samples of ten different wheat fields were collected from different sites in Pravara area. These soil samples are air dried, cleaned and sieved to fine particles

Isolation of *Azotobacter* spp: 1g soil samples were inoculated in sterile Ashby's medium and incubated for 8 days at $25^0C \pm 2^0C$. *Azotobacter* spp. were identified with microscopic observations on pigment basis and purified by transfer and retransfer on the same medium[14].

Fertilizer dose: As per recommended dose of NPK (40:50:100 kg/ha.) chemical fertilizers like urea and single super phosphate, were worked out as per treatment and plot size.

Mutation: Four days mix culture of *Azotobacter* spp.were used for mutation treatment. 20 ml cultures were poured in separate sterilized petriplates in laminar air flow in aseptic condition. The plates were exposed to U.V radiation for 20 min. 5ml treated culture broth was taken out from respective cultural plates after 5 minutes interval and poured in sterile Ashbys liquid medium and incubated for 7 days. Mutants were identified on pigment basis and their effect was studied by seed dressing in the field experiment[15,16].

Field study: Field experiment was carried out to study the effectiveness of *Azotobacter* alone and in mix combination treatment with graded levels of nitrogen on growth and yield of wheat

Experiment Design: Two wild and mutated combination of C+M+I and B+V+A were selected for genotypical study. These effective formulations were freshly grown on Ashbys medium for 7 days. Four local varieties HD 2189, Lok 1, 496 and Trimbak were selected for field experiment. Seeds were sterilized with 0.1% $HgCl_2$ for 5 min and consecutively washed with sterilized distilled water. After washing seeds were dressed with selected formulations of culture broth and dried in shade. Experimental plots were laid down in randomized block design with 6 treatments and 3 replicates and sowing was done at 22.5 cm row spacing. The effect of selective formulation was studied with different parameters viz % germination, number of tillers, height of tillers, fresh weight, dry weight, number of leaves, leaf area, chlorophyll content, spike length, spike weight, grain weight nitrogen and phosphorus uptake

Harvesting of Crop: Crop was harvested at 120 days after sowing. The straw and grain production of wheat were recorded

Total dry matter: Plant samples were collected from each treatment at 30, 60 and 90 days after sowing. Samples were rolled in brown papers, kept in oven at 60^0C for12 hrs for drying and dry weight was recorded. The records acquired from field trials were subjected to statistical analysis by using standard means.

Statistical analysis: From field experiments it observations noticed were subjected to statistical analysis by using standard methods[17]. The standard error from the treatments and the critical difference at the 5 % level of significance were worked out and used for the comparison between treatments.

Results and Discussion

From the results it was observed that int able1,2 at 60 and 90 days maximum plant height, tillers, number of leaves, leaf area, fresh weight and dry weight was observed in mutated non mutated treatment. This supports that enhancement is due to mutualistic interactions of strains and synthesis of growth promoting hormones in combinations of strains. The results also indicates that effectiveness was dependant on the stages of crop growth.

Conclusion

From the experimental finding it was proved that C+M+I treatment proved to be significant for all wheat varities followed by B+V+A. Maximum average grain yield per plot recorded in Trimbak (1.0897kg) followed by nonmutated C+M+I, B+V+A, RDF, control treatments. This supported that mutual association of *Azotobacter* spp leads to synthesis of growth harmones, nitrogen uptake and increase in growth parameters. Therefore present investigation strongly recommended that one has to investigate suitable *Azotobacter* spp.combination with respect to locally adapted climatic conditions and wheat varities to increase growth yield and economy of farmer.

Table-1
(Average plant height (cm), Average number of leaves, Average number tillers of Average leaf area, Average dry weight, Average grain yield) at 60,90 Days

Varities	***Treatment***	***Average plant height*** Replicate Mean(cm)	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***
HD 2189	*Az-T_1*	*73.80*	*7.4*	*18.4*	*30.00*	*2.40*	*4.77*
	Az-T_2	*69.90*	*6.5*	*16.0*	*18.00*	*2.05*	*4.30*
	Az-M_3	*70.87*	*7.1*	*23.8*	*29.67*	*2.15*	*5.00*
	Az-M_4	*68.40*	*6.3*	*17.7*	*23.00*	*2.05*	*3.40*
	RDF	*65.47*	*5.6*	*17.7*	*16.33*	*1.85*	*3.73*
	CONTROL	*64.13*	*5.2*	*15.6*	*12.00*	*1.60*	*2.97*

Varities	***Treatment***	***Average seed*** Replicate Mean	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***
LOK1	*Az-T_1*	72.47	*7.0*	*25.9*	*23.00*	*2.95*	*4.30*
	Az-T_2	69.07	*5.6*	*11.4*	*18.33*	*2.35*	*3.30*
	Az-M_3	77.50	*6.7*	*13.3*	*20.00*	*2.55*	*3.63*
	Az-M_4	69.67	*5.6*	*11.2*	*18.07*	*2.30*	*2.97*
	RDF	67.67	*5.6*	*13.3*	*19.00*	*2.10*	*2.87*
	CONTROL	61.07	*4.5*	*10.00*	*14.67*	*1.65*	*2.63*

Varities	***Treatment***	***Average plant height*** Replicate Mean(cm)	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean (gm***
TRIMBAK	*Az-T_1*	*73.67*	*7.7*	*14.2*	*21.67*	*2.36*	*4.50*
	Az-T_2	*70.87*	*5.8*	*12.8*	*17.00*	*1.60*	*4.17*
	Az-M_3	*72.47*	*5.9*	*14.3*	*16.00*	*2.20*	*4.73*
	Az-M_4	*65.67*	*5.5*	*12.4*	*14.67*	*1.85*	*3.17*
	RDF	*50.23*	*5.5*	*11.8*	*15.00*	*1.80*	*3.10*
	CONTROL	*40.33*	*4.5*	*9.4*	*14.00*	*1.70*	*3.07*

Varities	***Treatment***	***Average seed*** Replicate Mean	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***
LOK1	*Az-T_1*	72.47	*7.0*	*25.9*	*23.00*	*2.95*	*4.30*
	Az-T_2	69.07	*5.6*	*11.4*	*18.33*	*2.35*	*3.30*
	Az-M_3	77.50	*6.7*	*13.3*	*20.00*	*2.55*	*3.63*
	Az-M_4	69.67	*5.6*	*11.2*	*18.07*	*2.30*	*2.97*
	RDF	67.67	*5.6*	*13.3*	*19.00*	*2.10*	*2.87*
	CONTROL	61.07	*4.5*	*10.00*	*14.67*	*1.65*	*2.63*

Varities	***Treatment***	***Average plant height*** Replicate Mean(cm)	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***
TRIMBAK	*Az-T_1*	*73.67*	*7.7*	*14.2*	*21.67*	*2.36*	*4.50*
	Az-T_2	*70.87*	*5.8*	*12.8*	*17.00*	*1.60*	*4.17*
	Az-M_3	*72.47*	*5.9*	*14.3*	*16.00*	*2.20*	*4.73*
	Az-M_4	*65.67*	*5.5*	*12.4*	*14.67*	*1.85*	*3.17*
	RDF	*50.23*	*5.5*	*11.8*	*15.00*	*1.80*	*3.10*
	CONTROL	*40.33*	*4.5*	*9.4*	*14.00*	*1.70*	*3.07*

Varities	**Treatment**	**Average plant height** Replicate Mean(cm)	**Average number of leaves** Replicate Mean	**Average leaf area** Replicate Mean(cm)^2	**Average number of tillers** Replicate Mean	**Average dry weight** Replicate Mean(gm)	**Average spike weight Replicate Mean(gm**
496	Az-T_1	73.13	7.4	18.2	24.00	2.50	5.13
	Az-T_2	72.07	6.4	11	13.33	2.00	3.67
	Az-M_3	76.53	6.3	17.1	16.33	2.30	4.20
	Az-M_4	71.53	6.0	11.0	12.67	2.00	3.60
	RDF	70.93	4.4	9.6	12.00	1.85	3.17
	CONTROL	60.20	4.0	14.2	11.00	1.55	2.93

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Table-2
At 90 Days

Varities	***Treatment***	***Average plant height*** Replicate Mean	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***	***Average grain weight Replicate Mean(gm***
HD2189	*Az-T_1*	84.0	*7.4*	*21.7*	*30.00*	*2.80*	*6.10*	*0.978*
	Az-T_2	82.13	*6.6*	*11.8*	*18.00*	*2.35*	*6.00*	*0.897*
	Az-M_3	92.60	*7.1*	*21.2*	*29.67*	*2.60*	*6.90*	*0.947*
	Az-M_4	75.89	*6.3*	*14.6*	*23.00*	*2.20*	*5.20*	*0.892*
	RDF	75.1	*5.6*	*13.5*	*16.33*	*2.00*	*5.00*	*0.793*
	CONTROL	67.33	*5.2*	*12.5*	*12.00*	*1.70*	*4.80*	*0.752*

Varities	***Treatment***	***Average plant height*** Replicate Mean(cm)	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***	***Average grain weight Replicate Mean(gm***
LOK1	*Az-T_1*	*89.73*	*7.0*	*24.5*	*23.00*	*2.65*	*6.30*	*0.967*
	Az-T_2	*83.13*	*6.0*	*17.5*	*18.33*	*2.45*	*5.33*	*0.811*
	Az-M_3	*86.60*	*6.3*	*24.0*	*20.00*	*2.70*	*6.20*	*0.888*
	Az-M_4	*73.50*	*5.6*	*17.1*	*18.07*	*2.25*	*5.20*	*0.824*
	RDF	*67.53*	*5.0*	*14.4*	*19.00*	*2.05*	*5.10*	*0.726*
	CONTROL	*65.37*	*4.5*	*10.5*	*14.67*	*1.90*	*4.00*	*0.701*

Varities	***Treatment***	***Average plant height*** Replicate Mean(cm)	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***	***Average grain weight Replicate Mean(gm***
TRIMBAK	*Az-T_1*	*88.40*	*6.8*	*19.5*	*21.67*	*2.40*	*7.10*	*1.087*
	Az-T_2	*73.00*	*5.8*	*16.8*	*17.00*	*1.95*	*5.50*	*o.898*
	Az-M_3	*85.93*	*5.9*	*16.0*	*16.00*	*2.20*	*6.00*	*0.965*
	Az-M_4	*88.53*	*5.5*	*14.7*	*14.67*	*2.00*	*5.20*	*0.829*
	RDF	*78.37*	*5.0*	*12.5*	*15.00*	*1.90*	*5.00*	*0.819*
	CONTROL	*70.33*	*4.5*	*15.5*	*14.00*	*1.65*	*4.70*	*0.780*

	Treatment	***Average seed*** Replicate Mean	***Average number of leaves*** Replicate Mean	***Average leaf area*** Replicate Mean(cm)^2	***Average number of tillers*** Replicate Mean	***Average dry weight*** Replicate Mean(gm)	***Average spike weight Replicate Mean(gm***	***Average grain weight Replicate Mean(gm)***
496	*Az-T_1*	80.47	*8.1*	*19.6*	*24.00*	*2.60*	*6.90*	*0.839*
	Az-T_2	72.80	*6.0*	*13.6*	*13.33*	*2.05*	*5.50*	*0.781*
	Az-M_3	83.13	*6.7*	*16.7*	*16.33*	*2.35*	*6.50*	*0.844*
	Az-M_4	75.13	*5.2*	*11.3*	*12.67*	*2.05*	*5.00*	*0.746*
	RDF	69.33	*5.0*	*11.8*	*12.00*	*2.00*	*4.50*	*0.655*
	CONTROL	66.33	*4.6*	*14.1*	*11.00*	*1.70*	*4.00*	*0.599*