Effect of Associative Nitrogen Fixing Bacteria on Wheat (*Triticum aestivum* L.) Yield and Yield Component

Zakia Abdelhadi Hassan **Ahmed**[1] , Tagelsir Hassan **Mohamed Ahmed**[1]

[1] **Department of Botany, Faculty of Science and Technology, Shendi University** Tagelsir4m@gmail.com

ASTRACT

The study was carried out at the Faulty of Science and Technology, Shendi University to evaluate the isolatation and characterization of associative nitrogen fixing bacteria and their role in wheat (*Triticum aestivum* L.) Yield and Yield Component. Three isolates of *Azotobacter* were isolated from South Shendi and North Shendi. One *Azotobacter* and one *Azosperillum* strain were obtained form the National Research Center, Sdan. A pot and field experiments were conducted to assess the effect of associative nitrogen fixing bacteria on the yield and yield component of wheat. A complete block design with three replicated was adopted. Inoculation with associative nitrogen fixing bacteria or a combination of them or with half dose of phosphorus have a significant ($p \leq 0.05$) effect on wheat shoot length, shoot dry weight, number of Tiller per plant, number of Kernels per Plant, (both in pot and field experiments), 100 seeds Weight and yield per Sq Meter compared to the control untreated plots. Addition of half phosphorus recommended dose to any isolate or a combination of them showed a better effect in all tested parameters than phosphorus alone. Isolates from South Shendi gave a significant effect on all tested parameters than isolates from North Shendi or the introduced strain. The introduced *Azotobacter* and its combinations with other isolates gave the best results than *Azosperillm* alone or in a combination with any strain.

Keywords: ***Wheat, Triticum aestivum , Nitrogen Fixination ,Azotopacter***

INTRODUCTION

In Sudan, wheat (*Triticum aestivum* L.) is becoming the stable food for most of the population of urban as well as rural areas. It is the second most important cereal crop after sorghum. Increasing wheat production is becoming an essential national target to fill the gap between production and consumption. In season 2009 - 2010 the total cultivated wheat area in Sudan was 55400 feddans in the Northern state, Nile Valley

state and other schemes as a winter crop, yielding 40300 metric tons [1].

Application of biofertilizers is highly considered to limit the use of mineral fertilizers and decreasing the agricultural cost, maximizing crop yield by providing the available nutritive elements and growth promoting substances [2]. Biofertilization is one of the most of environmentally save approaches for nutrient management which can either fix atmospheric nitrogen, solubilize phosphate or the synthesis of growth promoting substances [3].

In general, biofertilizer from associative N- fixing bacteria can used specially for cereals such as rice and wheat. It is a breakthrough technology that promises very significant impact on plant productivity.

The active associative bacteria can enhance root development, growth and yield and improve soil quality. Many authors have shown the positive effect of inoculation of wheat with *Azotobacter* [4], *Azosperillum* [5] and *Bacillus* and A*zosperillum* [1].

Azosperillum as a biofertilizer is practically important in agricultural systems where fertilizer inputs are either impractical or not possible. Experiments on inoculation of wheat with *Azosperillum* often resulted in enhanced plant growth , nitrogen content and improved nutrient assimilation [2].

Azotobacter spp. represents the main group of heterotro- phic free living nitrogen-fixing bacteria associated with plants and improves productivity of the non-leguminous crops [6]. Nevertheless, wheat being a monocot, associative N2-fixing microbe like *Azotobacter* would be the key component for in situ nitrogen fortification [7].The *Azotobacter* spp.are able to fix at least 10mgN/g carbohydrate [8] and N2-fixing activity is generally more in strains associated with cultivated wheat than those of wild counterparts [9]. Besides nitrogen fixation, the *Azotobacter* spp. produce different growth hormones (IAA and other auxins, gibberllins and cytokinins), vitamins, siderophores and antifungal com- pounds to fight against many plant pathogens. However, functionality of BNF is highly location- specific, and therefore, resident strains would be better suited [10].

Objectives of the study were to Study the effects of selected Azotobacter strains on the growth and yield of wheat.

Materials and Methods

Pot Experiment

A pot experiment was carried out at the Faculty of Science and Technology, Shendi University to study the effect of Associative Nitrogen Fixing Bacteria and their role in wheat (*Triticum aestivum* L.) growth. A completely randomized block design with three replicates was adopted.

The soil used was a mixture of 70% silt and 30% sand collected from a depth of 10 – 30 cm from the River Nile Bank at Shendi City The soil mixture was crushed and cleaned from debris and plants residues and wastes. The mixture was then wetted and sterilized in an oven at 121°c for 2 hours. Pots were filled with 2 kg of the sterilized soil mixture and watered before sowing. Pots were sorted according to the design of the experiment in 3 replications.

Wheat variety (Wadi Elneel) was obtained from the local market in Shendi. Seeds sorted by hand to remove the up normal seeds and obtain similar size seeds. The seeds were sterilized with calcium hypochlorite.

Azotobacter strain and *Azosperillum strain* were obtained from The National Research Center. Three locally isolated strains of *Azotobacter* were selected from the isolated strains which showed the best growth rate. These were 2 from South Shendi (AzotoSSh 5 and AzotoSSh 10) and the thired one from North Shendi (AzotoNSh8).

Phosphorus fertilizer was added as DAP at a rate of 25 Kg / Feddan or half the rate as recommended in the treatments immediately after sowing.

Twenty-four treatment were used as designed in the excrement including untreated control. Phosphorus in the form of DAP was added in 25 Kg/fed or half the dose in combination with any bacterial strain or a combination of strains.

Six undamaged wheat seeds were sown per pot. After the seedling had reached 10-15 cm in length they were thinned to3 seedlings per bag. The pots were watered daily in order to maintain the moisture at approximately 75% of the field capacity.

Seedling inoculation, with either isolate was made by making 2 holes, each of 2-5 cm depth, 0.5 cm in diameter and at a distance of about 2 cm from each seedling. Five ml of the broth media were injected by a sterile disposable syringe to the halls around each seedling. Where a combination of strains was adopted 2,5 ml of each inoculum was added to make 5 ml per hole, Inoculation was done aseptically 10 days after sowing.

At harvest, 3 bags of each treatment were cut by the edges. The soil clump was then put on a 0.5cm mesh screen and washed gently with tap water to wash the soil from the root system. The hole plant was kept in labeled paper bags and taken to the laboratory.

In the Laboratory, Shoot dry weight, Shoot length, Number of tillers and Kernel per plant were measured.

Data were assessed by analysis of variance (ANOVA) [12]. Significance was accepted at $p \leq 0.05$.

Field Experiment

A field experiment was carried out at the Faculty of Science and Technology, Shendi University Experimental Farm. A completely randomized block design with three replicates was adopted.

The land was prepared by ploughing, harrowing, then levelling. The land was then divided to plots 5 x 5 meters and lines were made 60 Cm between lines and 5 north – south lines per plot.

Wheat variety (Wadi Elneel) was obtained from the local market in Shendi. Seeds were sorted by hand to remove the up normal seeds and obtain similar size seeds. Sowing was done at a rate of 25 Kg/feddan. The same treatments used in the pot experiment were adopted.

Azotobacter strain and *Azosperillum* were obtained from The National Research Center. Three locally isolated strins of *Azotobacter* were selected from the isolated strains which showed the best growth rate . These were 2 from South Shendi (AzotoSSh 5 and AzotoSSh10) and the third one from North Shendi (AzotoNSh8).

Seed inoculation, with either isolate was made by mixing wetted seeds with the appropriate isolate as recommended in the experiment. Liquid gum Arabic was added and mixed with the seed to adhere the isolates with the seeds. The seeds were left to dry under shade before sowing. Phosphorus fertilizer was added as DAP at a rate of 25 Kg / feddan or half the rate as recommended in the treatments immediately after sowing.

The plots were irrigated immediately and Then every week until harvesting. At maturity, ten plants were cut at the soil surface randomly from the middle rows. Samples from each plot were kept in a paper bag and transferred immediately to the laboratory.

In the laboratory, shoot length was measured using normal tap meter. Using

normal balance, shoot dry weight was weighed. The number of tillers and kernels were determined. The weight of 1000 seeds and yield per square meter was determined. Data were obtained in triplicates and analyzed.

Statistical analysis

Multifactor analysis of variance was performed to determine the effect of each treatment on the measured parameter. Comparison between means of treatments for various parameters measured were made by slandered error calculation . Each sample was analyzed in triplicate and figures were then averaged. Data were assessed by analysis of variance (ANOVA) [12]. Significance was accepted at $p \leq 0.05$.

Results and Discussion

Pot Experiment

Effect of Treatments on Shoot Length

Table 1, shows the effect of phosphorus fertilization and inoculation with Azotobacter and /or Azosperillum or a combination of both on the shoot length of wheat in the pot experiment. Phosphorous fertilizer or inoculation with associative nitrogen fixing bacteria have no significant effect on the shoot length. Azosperillum sp gave the highest length compared to Azotobacter or phosphorus. The locally isolated strains showed better performance than the introduced ones. Although the defferences between treatment were not significant th combination of Azosperikkum and the locally isolated strains gave the highest shoot length.

Effect of Treatments on Shoot Dry Weight

As shown in table 1, plant shoot dry weight followed the same pattern as the shoot length.

Effect of Treatments on Number of Tiller per plant

The effect of phosphorus fertilization and inoculation with Azotobacter or Azosperillum or a combination of both on the number of kernel per wheat plant in the pot experiment was shown in table 2. Inoculation with associative nitrogen fixing bacteria a have a significant ($p \geq 0.05$) effect on the kernel per plant compared to the control. The locally isolated strains showed better performance as the isolates from South Shendi either alone or in combination with other treatments showed significant differences to the other

treatments. Isolates from South Shendi in combination with phosphorus gave the highest number of tillers per plant.

Effect of Treatments on Number of Kernels per Plant

As shown in table2, the effect of treatments on the number of kernels per plant shows the same performance as the treatments effect on the number of tillers per plant.

Table1: Effects of treatments on plant shoot length(Cm) and shoot dry weight(gm) in potted soil

Treatment	Shoot length (Cm)	Shoot dry weight(gm)
Control	36.00	2.05
Phosphorus (P)	32.77	1.37
Azotobacter spp (B1)	38.50	1.80
Azosperillum spp (B2)	32.33	1.77
Azoto NSh (B3)	40.50	2.10
Azoto SSh 1 (B4)	40.50	2.70
Azoto SSh 2 (B5)	36.00	2.77
B1 + B3	32.77	2.17
B1 + B4	34.00	1.73
B1 + B5	39.50	2.77
B2 + B3	35.50	2.57
B2 + B4	32.50	1.10
B2 + B5	35.67	1.70
½ P + B1	36.50	2.27
½ P + B2	32.00	1.17
½ P + B3	39.77	2.27
½ P + B4	37.00	3.23
½ P + B5	34.17	1.53
½ P + B1 + B3	36.67	2.40
½ P + B1 + B4	36.33	1.80
½ P + B1 + B5	34.00	2.00
½ P + B2 + B3	36.17	1.90
½ P + B2 + B4	33.33	1.93
½ P + B2 + B5	38.00	2.10
LSD	±	± 0.17 6

Table 2: Effects of treatments on tiller number/plant and kernel number/plant in potted soil

Treatment	Tiller number / plant	Kernel number / plant
Control	1.50	1.00
Phosphorus (P)	1.50	1.00
Azotobacter spp (B1)	1.50	1.00
Azosperillum spp (B2)	2.00	1.67
Azoto NSh (B3)	1.50	1.00
Azoto SSh 1 (B4)	2.50	2.00
Azoto NSh 2 (B5)	2.50	1.50
B1 + B3	2.50	1.50
B1 + B4	2.33	2.00
B1 + B5	2.50	1.50
B2 + B3	3.00	1.00
B2 + B4	2.00	1.00
B2 + B5	2.00	1.33
½ P + B1	3.00	1.00
½ P + B2	2.00	1.00
½ P + B3	3.00	1.50
½ P + B4	2.67	1.33
½ P + B5	2.33	1.67
½ P + B1 + B3	3,67	2.00
½ P + B1 + B4	2.67	1.00
½ P + B1 + B5	2.30	1.33
½ P + B2 + B3	2,50	1.50
½ P + B2 + B4	2.00	1.00
½ P + B2 + B5	2.33	1.00
LSD	± 0.180	± 0.111

Field Experiment

Effect of Treatments on Shoot Length

Table 3, shows the effect of phosphorus fertilization and inoculation with Azotobacter isolates and /or Azosperillum or a combination of them on the shoot length of wheat in the field experiment. Inoculation with associative nitrogen fixing bacteria or a combination of them or with half dose of phosphorus have a significant ($p \geq 0.05$) effect on wheat shoot length compared to the control untreated plots. Addition of phosphorus alone had no significant differences with the inoculums. Significant differences ($p \geq 0.05$) was observed between introduced and locally isolated strains but not between the introduced ones. The combination of introduced *Azotobacter spp*

with any of the locally isolated strains showed significand differences with their combination with *Azosperillum spp.* Biofertilization of wheat with non-symbiotic N-fixing bacteria resulted in a significant increase in plant height compared to untreated plats.[11]. This result was in accordance with the result obtained in the study.

Effect of Treatments on Shoot Dry Weight

The effect of phosphorus fertilization and inoculation with Azotobacter and /or Azosperillum or a combination of both on the shoot dry weight of wheat in the field experiment was shown in Table 3. As in the shoot length, Inoculation with associative nitrogen fixing bacteria or a combination of them or with half dose of phosphorus have a significant ($p \geq 0.05$) effect on wheat shoot length compared to the control untreated plots. Addition of phosphorus significantly over under weighed Azotobacter isolates wither introduced or locally isolated ones but has no significant differences with Azosperillum.

Addition of half phosphorus recommended dose to any isolate or a combination of them resulted in a higher shoot dry weight than phosphorus alone. The introduced Azotobacter and its combinations with other isolates gave the best results. Many research workers obtained the same result showing a significant increase in plant dry matter upon inoculation with N-fixing associative bacteria and enhanced plant growth [5].

Effect of Treatments on Number of Tiller per plant

Table 4, shows the effect of phosphorus fertilization and inoculation with Azotobacter isolates and /or Azosperillum or a combination of them on the number of tillers of wheat in the field experiment. Azosperillum alone has no significant($p \geq 0.05$) effect with any treatment whereas all the isolates of Azotobacter significantly ($p \geq 0.05$) over numbered the control, phosphorus and Azosperillum. The locally isolated strains of Azotobacter from South Shendi performed better than the other isolates. Addition of half recommended dose of phosphorus has no significant differences either to the isolates alone or a combination of them. [11] showed that wheat inoculation with non-symbiotic nitrogen fixing microorganisms increased the numbers of tillers and kernels per plant over chicken manure fertilizer.

Table 3: Effects of treatments on plant shoot length(Cm) and shoot dry weight(gm)

Treatment	Shoot length (Cm)	Shoot dry weight(gm)
Control	79.00	10,15
Phosphorus (P)	78.50	8.40
Azotobacter spp (B1)	68.50	6.35
Azosperillum spp (B2)	71.75	7.05
Azoto NSh (B3)	75.50	11.25
Azoto SSh 1 (B4)	69.25	10.35
Azoto SSh 2 (B5)	64.50	6.15
B1 + B3	60.50	7.85
B1 + B4	58.00	10.20
B1 + B5	64.50	6.75
B2 + B3	78.00	8.85
B2 + B4	78.50	7.35
B2 + B5	72.50	5.45
½ P + B1	95.50	13.60
½ P + B2	78.50	7.00
½ P + B3	67.50	7.80
½ P + B4	63.75	6.40
½ P + B5	69.50	9.65
½ P + B1 + B3	75.25	12.30
½ P + B1 + B4	80.00	8.60
½ P + B1 + B5	87.50	11.20
½ P + B2 + B3	64.75	8.60
½ P + B2 + B4	71.25	9.65
½ P + B2 + B5	76.00	11.85
LSD	± 1.279	± 0.241

Effect of Treatments on Number of Kernels per Plant

Table 4, shows the effect of phosphorus fertilization and inoculation with Azotobacter isolates and /or Azosperillum or a combination of them on the number of tillers of wheat in the field experiment. As in the pot experiment, kernel number per plant followed the same pattern as the number of tiller per plant. As Azotobacter significantly over numbered the control, phosphorus and Azosperillum. The locally isolated strains of Azotobacter from South Shendi performed better than the other ($p \geq 0.05$) isolates. Addition of half recommended dose of phosphorus has no significant differences either to the isolates alone or a combination of them. [11] showed that wheat inoculation with non-symbiotic nitrogen fixing microorganisms increased the numbers of tillers and kernels per plant over chicken manure fertilizer.

Table 4: Effects of treatments on tiller number/plant and kernel number/plant

Treatment	Tiller number / plant	Kernel number / plant
Control	4.00	3.50
Phosphorus (P)	5.50	5.00
Azotobacter spp (B1)	8.00	6.00
Azosperillum spp (B2)	5.50	5.00
Azoto NSh (B3)	5.50	5.50
Azoto SSh 1 (B4)	4.00	4.00
Azoto SSh 2 (B5)	7.50	6.00
B1 + B3	4.00	3.50
B1 + B4	5.00	4.00
B1 + B5	7.00	6.00
B2 + B3	6.50	6.00
B2 + B4	4.50	4.50
B2 + B5	7.50	7.00
½ P + B1	6.50	6.00
½ P + B2	6.00	3.50
½ P + B3	7.50	7.00
½ P + B4	7,00	6.00
½ P + B5	5.00	4.54
½ P + B1 + B3	6.50	4.50
½ P + B1 + B4	8.50	6.50
½ P + B1 + B5	5.50	5.50
½ P + B2 + B3	7.50	6.00
½ P + B2 + B4	6.50	4.50
½ P + B2 + B5	8.00	5.50
LSD	± 0.374	± 0.385

inoculation with non-symbiotic nitrogen fixing microorganisms increased the numbers of tillers and kernels per plant over chicken manure fertilizer.

Effect of Treatments on 100 seeds Weight

As showed in Table 5, addition of any of the locally isolated strains of associative nitrogen fixing bacteria or a combination of them or with half dose of phosphorus have a significant ($p \geq 0.05$) effect on 100 seed weight. The combination of half phosphorus dose with any isolates alone or a combination of them significantly ($p \geq 0.05$) increased the weight of 100 seed.

Effect of Treatments on Yield per Sq Meter

Table 5, shows the effect of phosphorus fertilization and inoculation with

Azotobacter isolates and /or Azosperillum or a combination of them on the number of tillers of wheat in the field experiment. As in the pot experiment. Microbial inoculation has a significant ($p \geq 0.05$) differences with the control un treated plots but not with phosphorus application. No significant differences were observed between the introduced Azotobacter strain and the locally isolated ones. The combination of half phosphorus dose with Azotobacter alone or its combination with the locally isolated strains significantly ($p \geq 0.05$) increased wheat yield. The increase of crop yield due to the effect of Azotobacter was stated by [4] .

Table 5: Effects of treatments on 100 seed weight (gm) and yield (g)/M^2

Treatment	100 seed Wt (g)	Yield (g) / M2
Control	3.35	50.30
Phosphorus (P)	2.90	53.77
Azotobacter spp (B1)	3.,30	32.80
Azosperillum spp (B2)	3.40	70.70
Azoto NSh (B3)	3.45	93.80
Azoto SSh 1 (B4)	3.45	58.57
Azoto SSh 2 (B5)	3.35	70.90
B1 + B3	3.55	52,70
B1 + B4	3.50	40.00
B1 + B5	3.85	77.60
B2 + B3	3.35	26.50
B2 + B4	3.35	30.60
B2 + B5	3.10	50.27
½ P + B1	3.30	29.77
½ P + B2	3.40	49.50
½ P + B3	3.20	35.10
½ P + B4	2.90	55.87
½ P + B5	3.30	47.70
½ P + B1 + B3	3.25	44.00
½ P + B1 + B4	3.35	39.77
½ P + B1 + B5	3.35	73.13
½ P + B2 + B3	3.25	87.10
½ P + B2 + B4	3.20	45.17
½ P + B2 + B5	3.10	46.40
LSD	± 0.075	± 4.951

CONCULOTION

Inoculation with associative nitrogen fixing bacteria or a combination of them or with half dose of phosphorus have a significant ($p \geq$

0.05) effect on wheat shoot length, shoot dry weight, number of Tiller per plant, number of Kernels per Plant, 100 seeds Weight and yield per Sq Meter compared to the control untreated plots. Addition of half phosphorus recommended dose to any isolate or a combination of them showed a better effect in all tested parameters than phosphorus alone. The introduced Azotobacter and its combinations with other isolates gave the best results. Previous studies showed that inoculation of wheat with any sp. of the assosiative nitrogen fixing bacteria increased the yield and the parameters of yield component. [13] showed that inoculation of wheat with effective strains of associative N-fixing bacteria significantly increased wheat yield and yield component. It was observed that inoculation with some PGPR strains resulted in growth and yield promotion in wheat [14].

REFERENCES

1. Mohamed SS, Osman AG, Abdalla AS, Rugheim AME (2010). Response of wheat to biological and nitrogen fertilization. *U. of K. J. Agric. Sci*. 18 (1): 31-42.
2. Metin TA, Medine GB, Ramazan CC, Taskin OF, Sahin D (2010). The effect of PGPR strain on wheat yield and quality parameters. Proceeding of World Congress of Soil Science, Soil Solutions for a Changing World.1 – 6 August 2010, Brisbane, Australia.
3. We SC, Cao ZH, Li ZG, Cheung KC (2005). Effect of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma.125: 155- 166.
4. Bahrani AJP, Hagh Joo M (2010). Response of winter wheat to co-inoculation with Azotobacter and Arbescular Mycorrhizal Fungi (AMF) under Different Sources of Nitrogen Fertilizer. *Am.-Eurasian J. Agric. Environ. Sci*. 8 (1): 95-103.
5. Bashan Y, Levanony H (1990). Current status of Azospirillum inoculation technology: Azospirillum as a challenge for agriculture. *Can. J. Microbiol*. 36:591–599.
6. Hong JS, Geun TP, Mi SC, Moon SH (2006) Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant Pantoea agglomerans R-42 isolated from soybean rhizosphere. Biores Technol 97:204–210.

7. Zaki N, Gomaa AM, Galal A, Farrag AA (2009) The associative impact of certain diazotrophs and farmyard manure on two rice varieties grown in a newly cultivated land. Res J Agric Biol Sci 5:185–190.
8. Tejera N, Luch C, Martinez-Toledo MV, Gonzalez-Lopez J (2005) Isolation and characterization of Azotobacter and Azospirillum strains from the sugarcane rhizosphere. Plant Soil 270:223– 232.
9. .Choudhury ATMA, Kennedy IR (2004) Prospects *and potentials* for systems of biological nitrogen fixation in sustainable rice produc- tion. Biol Fertil Soils 39:219–227.
10. Kannan T, Ponmurugan P (2010) Response of paddy (Oryza sativa L.) varieties to Azospirillum brasilense inoculation. J Phytol 2:8–13.
11. Mohammed,S.S., Osman1,A.G., Mohammed, A.M., Abdalla, A.S., Sherif, A.M. and Rugheim, A.M.E. (2012). Effects of organic and microbial fertilization on wheat growth and yield. *International Research Journal of Agricultural Science and Soil Science.* Vol. 2(4) : 149-154.
12. Sendecor C.W. and Cochran W.C., (1987). Statistical Methods, 7th ED. Ames, I.A. The University of Iowa State Press. PP 221-222.
13. Kavimandan S.K., (1986). Root Nodule Bacteria to Improve Yield of Wheat. Plant and Soil 86: (1), 141-144.
14. Alev Sezen, Murat Ozdal, Kubra Koc and Omer Faruk Algue (2016). Isolation and Charactrization of Plant Growth Promotig Rhizobacteria (PGPR) and Their Effects on Improving Growth of Wheat.Journal of Applied Biological Science 10 (1).41 – 46.