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Research Article

Biotechnology of Nitrogen-Fixing by Cyanobacteria in Wheat Plants

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Abstract:

A field experiment was conducted to study the effect of three isolates of Cyanobacteria individually ((*Nostoc punctiforme* PQ870057, *Nostoc parmelioides* PQ864768 and *Anabaena cylindrica* PQ870058)) and a mixture of them with different ratios of nitrogen concentration (Zero, 20, 40, and 60 kg N/fed). This experiment was conducted in clay soil located in the center of Tanta, Gharbia Governorate. Cyanobacteria isolates showed. A significant increase in plant height, spike length, spike dry weight, number of spikes, grain yield, straw yield, and biological yield. The findings in unequivocally demonstrated that nitrogen fertilization had a major impact on the weight of grains. As a result, the wheat plant's all wheat wield and its components increased by up to 60 kg N/fed nitrogen fertilizer. The application of nitrogen increases all wheat wield and its components and enhances growth. Regarding the Cyanobacteria effect, notable variations found between the employed treatments. It was established by the data Cyanobacteria mixture produced the highest all wheat wield and its components value.

1. Introduction

Recently, the advantages of Cyanobacteria in soil as biofertilizer have received a lot of attention. The quantity of phytohermon and fixed nitrogen produced by these Cyanobacteria may be sufficient to meet the nitrogen a requirement of wheat plants near dements. In this regard, it is important to remember that the release of chemicals that encourage growth while Cyanobacteria are growing.

Cyanobacteria, are photoautotrophic prokaryotes that resemble Gram-negative bacteria in appearance yet carry out oxygenic photosynthesis like higher plants. Fungi and may impede infiltration and make the soil unwettable, according to Schulten (1985), El-Zawawy (2021), and Abou Tahoun et al. (2020). These microbes enhance the organic matter in the soil, where it eventually dies and decomposes.

Nilsson et al. (2002) reported on the novel relationships, such as those between Anabaena and wheat, and examined the impact of numerous Cyanobacterial isolates were associated with wheat, particularly in generating fake linkages under laboratory settings. The soil surface layer maintained by algae enhances soil aggregates, increases infiltration, and reduces runoff. After dying, Cyanobacteria create surface humus and serve as solvents on a variety of soil minerals, supplying components that plants can use (El-Zawawy, 2016). According to research conducted by (Asghari et al., 2022), Cyanobacteria inoculation of the soil improved the behavior of soil water, particularly in coarse-textured soils. As a result, this technique might be used effectively in agricultural areas to regulate drainage and irrigation.

On this basis, the cost of wheat production will be decreased by reducing the amount of nitrogen fertilizer by inoculation of wheat fields with Cyanobacteria inoculants, in addition to protect the environment from pollutions. The purpose of this study is to assess three Cyanobacteria strains' **(*Nostoc punctiforme* PQ870057, *Nostoc parmelioides* PQ864768 and *Anabaena cylindrica* PQ870058)** capacity to form associations with wheat plants

2. Materials and Methods

2.1. Source of Cyanobacterial isolates

It was obtained from the isolation, purification and identification programs for Cyanobacteria isolates for the master's thesis of the student Nouran Al-Sayed Al-Semelawy, Department of Agricultural Botany, Faculty of Agriculture, Tanta University. Cyanobacteria namely **(*Nostoc punctiforme* PQ870057, *Nostoc parmelioides* PQ864768 and *Anabaena cylindrica PQ870058*)**.

2.2. Wheat grain

Wheat grains (*Triticum aestivum* L.) cultivar (Sakha 95) was kindly obtained from wheat Research Institute, Agricultural, Research Center (ARC), Giza. Egypt.

2.3. Nitrogen fertilizer

Urea (46.5% N.) was used in this investigation. It was supplied by Research Center (ARC), Giza. Egypt.

2.4. Media used

Different media were used for isolation, cultivation and maintenance of blue-green algae (Cyanobacteria). The media composition as follows:

Modified Watanabe medium (Watanabe et al., 1951)

This medium was modified by El– El-Nawawy et al. (1958), its composition as follow (g /L): 0.30g K_2HPO_4, 0.20g Mg $SO_4.7H_2O$, 0.20g K_2SO_4, 0.10g $CaCO_3$, 2.00g Glucose, 0.20 ml. $FeCl_3$ 1% (Freshly prepared), 1.00 ml. Micro-nutrient solution, Distilled water 1 L, Composition micro-nutrient solution 2.8g H_3BO_3, 0.22g Zn $SO_4.7H_2O$, 0.08 $CuSO_4.5H_2O$, 1.80g $MnCl_2$, 0.02g Molybdic acid up to 1 L.

2.5. Methods

2.5.1. Preparation of standard Cyanobacteria inoculum for laboratory studies

The inoculum of the Cyanobacteria identified strains were prepared by inoculating 500 ml Erlenmeyer flasks each containing 200 ml of Modified Watanabe liquid medium with a loopfull of 21 days old culture of each Cyanobacteria strains. Inoculated flasks were incubated at 28-30ºC under continuous illumination (2500 lux) for 21 days El-Zawawy (2016).

2.5.2. Plating technique of Cyanobacteria enumeration

N-free culture medium Watanabe medium (Allen and Stanier, 1968) was used for culturing N_2-fixing Cyanobacteria plates. The medium was autoclaved with Bacto agar at 15 Ibs/m^2 for 15 minutes cooled down to 45 ºC, and 30 ml poured into sterile Petri dish. The plates were dried for 2 days before utilization. The soil samples were diluted with distilled water to make 1:10 dilution. This first dilution was stirred for 30 minutes using a magnetic stirrer. Ten-fold dilutions were then made with distilled water. One-ml aliquots of the 10-2 to 10-6 dilution were poured into the surface of the agar medium and spread with an alcohol sterilized triangular glass rod while the plates were being rotated on a rotator. Triplicate plates were used for each soil dilution. The plates remained upright for 2-days to allow the agar to absorb the water before being incubated in inverted position at room temperate for 3 weeks. Incident light of 2500 lux provided by 20-watt cool-white fluorescent bulbs. Counting of the algal was done using a dissociating microscope and the numbers of algal colonies were expressed as colony formed per unit/g soil (CFU).

2.5.3. Total nitrogen determination

Total nitrogen in the Cyanobacteria strains were determined by using the micro-kjeldahl method according to Jackson (1973). Results were expressed as mg nitrogen/100 ml culture.

2.6. Filed experiment

A field experiment was conducted to study the effect of biofertilizers as inoculants prepared as broth cultures from the most efficient isolates namely ***Nostoc punctiforme*** **PQ870057,** ***Nostoc parmelioides*** **PQ864768 and** ***Anabaena cylindrica*** **PQ870058** and mixture (1:1:1) of both of them. This experiment was carried out in a clay soil located at Gharbia Governorate Tanta center, Defra. Mechanical and chemical analyses of the soil experiment are presented in Table (1). Wheat cultivar Sakha 95 was used. The plot size was 4x5m². the soil was thoroughly mixed uniformly with phosphate and potassium fertilizers at rates of 100 and 50 kg fed^{-1} in the form of superphosphates (15 % P_2O_5) and potassium sulfate (48 % K_2O), respectively, while nitrogen added in two split doses, the first ⅔ N dose was added prior to wheat sowing. The second (⅓ N) dose was added after 30 days from sowing. wheat grains mixed with Cyanobacteria strain (1.5 x 107cfu).

2.7. Wheat Yield and its Components

Plant height (cm), spike number, spike dry weight (g), grain dry weight (g), 1000 grain weight (g) and nitrogen, phosphorus as well as potassium content of wheat grains were determined.

Table 1. Mechanical and chemical analyses of the soil experiment.

Characteristics	Clay soil
Sand	30
Silt	28
Clay	42
Chemical analysis meq-L	
Ca++	6.1
Mg^{++}	4.0
Na^+	9.0
K^+	2.0
CO_3^{--}	0.0
HCO_3^-	4.1
Cl^-	9.0
SO_4^{--}	8.0
E.C.	2.3 ds/m
P^H	7.7

2.8. Statistical analysis

A totally randomized design was employed, with three copies. Data gathered using the procedures outlined by Steel and Torrie (1980) were subjected to an ANOVA. The mean differences were compared using the least significant difference (LSD) at 5%.

3. Results and Discussion

Cyanobacteria isolates showed that there were gradual increases in the Cyanobacteria fixed-nitrogen (mg N/100 ml-culture) and dry weight g/100 ml culture along with the experiment proceeding, where the highest Cyanobacteria fixed-nitrogen were recorded with all isolates at the end incubation period. The efficiency of Cyanobacteria isolates to fix the atmospheric nitrogen is one of the most important parameters used for selection of Cyanobacteria isolates for preparing Cyanobacteria inoculants. Results in Table (2) indicated that all isolates were varied in their total fixed-nitrogen (mg N/100 ml-culture) in all isolates. This data aligns well with the findings of El-Zawawy (2021).

Table 2. Mean amounts of (mass production) and (fixed nitrogen), during 30 days growth period of Cyanobacteria isolates.

Cyanobacteria isolates	*Anabaena cylindrica* Q870058					*Nostoc punctiforme* PQ870057					*Nostoc parmelioides* PQ864768				
	Culture age (days)														
	7	11	15	22	30	7	11	15	22	30	7	11	15	22	30
Dry weight, g/100 ml culture	0.070	0.110	0.172	0.183	0.206	0.080	0.160	0.176	0.182	0.200	0.082	0.162	0.180	0.198	0.225
Fixed-N_2 mg/100 ml culture	2.66	4.67	5.12	6.34	7.62	6.12	6.95	8.21	9.77	13.88	8.40	11.64	15.36	16.23	16.86

Plant parameters

1. Plant height (cm)

The information presented in Table 3 indicates that the height of plants increased significantly as a result of adding nitrogen. It was determined that different types of Cyanobacteria significantly affected plant height and plant height values were higher when a mixture of Cyanobacteria was used. This data aligns well with the findings of Abou Tahoun et al. (2020) and El-Zawawy (2019).

Table 3. Effect of inoculation with Cyanobacteria inoculants and nitrogen fertilization on plant height (cm) of wheat plants.

Fertilization Kg N/fed / Inoculation	No. Nitrogen, Control	20 Kg N/fed	40 Kg N/fed	60 Kg N/fed
No. inoculation Control	77.12	79.00	97.23	99.85
Anabaena cylindrica PQ870058	80.11	84.00	99.00	100.18
Nostoc punctiforme PQ870057	85.12	87.12	101.00	102.97
Nostoc parmelioides PQ864768	90.00	90.87	101.98	1003.11
Mixture	98.88	99.11	103.24	104.15

LSD, 0.05 = 0.65

2. Spike length (cm)

The results in Table (4) showed that adding different rates of nitrogen to the Cyanobacteria isolates a significant effect on spike length. The best strain was *Nostoc parmelioides* PQ864768, while the mixture of Cyanobacteria was better than the other (Ghazal et al., 2018; Abou Tahoun et al., 2020; Sadvakasova et al., 2022).

Table 4. Effect of inoculation with Cyanobacteria inoculants and nitrogen fertilization on spike length of wheat plants.

Fertilization Kg N/fed / Inoculation	No. Nitrogen, Control	20 Kg N/fed	40 Kg N/fed	60 Kg N/fed
No. inoculation Control	7.1	8.3	9.1	10.1
Anabaena cylindrica PQ870058	9.7	10.2	11.5	12.3
Nostoc punctiforme PQ870057	10.6	11.8	13.5	15.2
Nostoc parmelioides PQ864768	11.7	13.6	14.9	17.3
Mixture	15.7	16.1	17.8	18.21

LSD, 0.05 = 0.35

3. Dry weight of spike

Table (5) displays the data collected on the dry weight of ears affected by nitrogen levels and Cyanobacteria strains. The highest dry weight values were recorded with a mixture of Cyanobacteria and 60 nitrogen units. Compare 0% N to the lowest dry weight values. It is also clear that the physiological effect of the plant's higher response to nitrogen is due to its ability to increase photosynthesis activity. Regarding the effect of Cyanobacteria. It was found that adding a mixture Cyanobacteria more effective in enhancing the dry weight of spike (Ghazal et al., 2011; Abou Tahoun et al., 2020; Sadvakasova et al., 2022).

Table 5. Effect of inoculation with Cyanobacteria inoculants and nitrogen fertilization on dry weight of spike/plant wheat plant.

Fertilization Kg N/fed / Inoculation	No. Nitrogen, control	20 Kg N/fed	40 Kg N/fed	60 Kg N/fed
No. inoculation Control	1.80	2.87	3.69	3.84
Anabaena cylindrica PQ870058	1.93	3.40	4.05	4.17
Nostoc punctiforme PQ870057	2.60	3.99	4.11	5.47
Nostoc parmelioides PQ864768	2.99	4.08	5.45	5.54
Mixture	3.09	4.39	5.54	5.78

LSD, 0.05 =0.51

4. Number of spikes/m^2

The results in Table (6) showed that increasing the level of nitrogen fertilizers has a significant impact on the number of ears. The information also showed that different Cyanobacteria strains had significant effect. In *Nostoc parmelioides* PQ864768 outperformed the alternative. On the other hand, using mixture of Cyanobacteria performed better than the other (Ghazal et al., 2011; Ghazal et al., 2018; Abou Tahoun et al., 2020; Sadvakasova et al., 2022; Aboseif, et al., 2023).

Table 6. Effect of inoculation Cyanobacteria inoculants nitrogen fertilization on number of spikes/m2 of wheat plant.

Fertilization Kg N/fed / Inoculation	No. Nitrogen, Control	20 Kg N/fed	40 Kg N/fed	60 Kg N/fed
No. inoculation Control	270	282	301	320
Anabaena cylindrica PQ870058	282	292	310	330
Nostoc punctiforme PQ870057	291	300	307	365
Nostoc parmelioides PQ864768	300	320	355	382
Mixture	327	330	377	490

LSD, 0.05 =9.32

5. Grain yield(ton/fed):

The findings in Table (7) unequivocally demonstrated that nitrogen fertilization had a major impact on the weight of grains. As a result, the wheat plant's grain weight increased by up to 60% nitrogen fertilizer. The application of nitrogen increases grain weight and enhances growth. Regarding the Cyanobacteria effect, notable variations in grain weight were found between the employed treatments. It was established by the data Cyanobacteria mixture produced the highest grain weight value. These results are in a good harmony with those reported by El-Zawawy (2019), Abou Tahoun et al. (2020), El-Awady et al. (2020), and Tawfiek et al. (2023).

Table 7. Effect of inoculation with Cyanobacteria inoculants and nitrogen fertilization on grain yield (ton/fed) of wheat plant.

Fertilization Kg N/fed / Inoculation	No. Nitrogen, Control	20 Kg N/fed	40 Kg N/fed	60 Kg N/fed
No. inoculation Control	933	1210	1520	1950
Anabaena cylindrica PQ870058	1790	1800	2170	2422
Nostoc punctiforme PQ870057	1990	2200	2410	2620
Nostoc parmelioides PQ864768	2230	2500	3000	3130
Mixture	2550	2850	3110	3150

LSD, 0.05 =8.83

6. Straw yield

The results in Table (8). The nitrogen application improves growth and increase Straw yield. As for Cyanobacteria effect, significant differences were detected among the used treatments in Straw yield. From data listed in Table (8), it became fact that the mixture of Cyanobacteria gave highest value of Straw yield. Many authors found that there were great differences in Straw yield as affected by Cyanobacteria. These results are in a good harmony with those reported by El-Zawawy (2019) and Abou Tahoun et al. (2020).

Table 8. Effect of inoculation with Cyanobacteria inoculants and nitrogen fertilization on Straw yield (ton/fed) of wheat plant.

Fertilization Kg N/fed / Inoculation	No. Nitrogen, Control	20 Kg N/fed	40 Kg N/fed	60 Kg N/fed
No. inoculation Control	3210	3350	3590	4220
Anabaena cylindrica PQ870058	3303	3496	4201	4710
Nostoc punctiforme PQ870057	3477	4123	4689	5110
Nostoc parmelioides PQ864768	4084	4411	5099	5340
Mixture	4340	4960	5210	5420

LSD, 0.05 = 8.81

7. Biological yield

The results in Table (9), clearly showed that nitrogen fertilization significantly affected the biological yield in different Cyanobacteria. Therefore, increasing nitrogen fertilizer levels gradually increased biological yield in wheat plant up to 60%. In contrast, the lowest values of biological yield were obtained when no nitrogen fertilizer was used. As for Cyanobacteria effect, significant differences were detected among the used treatments in biological yield. From data listed in Table (9), it became fact that the mixture of Cyanobacteria gave highest value of biological yield. Many authors found that there were great differences in biological yield as affected by Cyanobacteria. These results are in a good harmony with those reported by El-Zawawy (2019) and Abou Tahoun et al. (2020).

Table 9. Effect of inoculation with Cyanobacteria inoculants and nitrogen fertilization on biological yield (ton/fed) of wheat plant.

Fertilization Kg N/fed / Inoculation	No. Nitrogen, Control	20 Kg N/fed	40 Kg N/fed	60 Kg N/fed
No. inoculation Control	4143	4560	5110	6170
Anabaena cylindrica PQ870058	5093	5296	6371	7132
Nostoc punctiforme PQ870057	5467	6323	7099	7730
Nostoc parmelioides PQ864768	6314	6911	8099	8470
Mixture	6890	7810	8320	8570

LSD, 0.05 = 19.11

4. Conclusion

Our conclusion was that, the study of the effect of three isolates of Cyanobacteria ((Nostoc punctiforme PQ870057, Nostoc parmelioides PQ864768 and Anabaena cylindrica PQ870058)) and nitrogen minerals (Zero, 20, 40 and 60 kg N/fed) of wheat plant showed that a significant differences in all cyanobacteria treatments and the best treatment was a mixture of cyanobacteria with 60 nitrogen unites in all traits.

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