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Cyanobacterial Biofertilizer Improved Growth of Wheat

By

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Summary

ABD-ALLA M. H., MAHMOUD A.-L. E. & ISSA A. A. 1994. Cyanobacterial biofertilizer improved growth of wheat. – *Phyton* (Horn, Austria) 34 (1): 11-18. English with German summary.

The effect of commercial inoculant of cyanobacteria on wheat cv. Sakha 69 grown in pot experiments using sterilized or non-sterilized clay and/or sand soils was examined. Treatments were control (water), live cyanobacteria, killed cyanobacteria, live cyanobacteria plus K, P and S, killed cyanobacteria plus K, P and S and K, P and S only. Live inoculant and live inoculant plus K, P and S significantly increased dry weight, total nitrogen, and pigment contents of wheat plants over control and other treatments. The increase in growth parameters could be attributed to the substantial increases of N_2 -fixation due to nitrogenase activity of the cyanobacteria. The promotive effect of cyanobacterial inoculant, especially on growth, hold promise for use of such inoculants to enhance the nitrogen status of irrigated plantation crops.

Zusammenfassung

ABD-ALLA M. H., MAHMOUD A.-L. E. & ISSA A. A. 1994. Cyanobakterien als Biodünger verbessern das Wachstum von Weizen. – *Phyton* (Horn, Austria) 34 (1): 11-18. Englisch mit deutscher Zusammenfassung.

Der Effekt eines käuflichen Biodüngers aus Cyanobakterien auf Weizen cv. Sakha 69 wurde untersucht. Die Pflanzen wurden in sterilisiertem und nicht-sterilisiertem Ton und/oder sandiger Erde kultiviert. Folgende Behandlungsarten wurden durchgeführt: Kontrolle (Wasser), lebende und abgetötete Cyanobakterien, lebende und abgetötete Cyanobakterien mit K, P und S und K, P und S allein. Lebende Cyanobakterien und solche mit K, P und S steigerten Trockengewicht, Gesamt-N- und

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Pigmentgehalt der Weizenpflanzen signifikant gegenüber der Kontrolle und den anderen Behandlungen. Die Steigerung der Wachstumsparameter könnte auf eine erhebliche Zunahme der N₂-Fixierung entsprechend der Nitrogenaseaktivität der Cyanobakterien zurückgeführt werden. Der fördernde Einfluß der Cyanobakterien, speziell auf das Wachstum, ist erfolgversprechend für eine Steigerung des Stickstoffgehaltes bewässerter Getreidefelder.

Introduction

The modern day intensive crop cultivation requires the use of nitrogen fertilizers. However, fertilizers are in short supply and expensive in developing countries. Therefore, it is important to explore the possibility of supplementing nitrogen fertilizers with biofertilizers of microbial origin. Microbial processes are fast and consume relatively less energy than industrial processes.

Microbial inoculants are carrier-based preparations containing beneficial microorganisms in a viable state intended for seed or soil application and designed to improve soil fertility and help plant growth.

The application of diazotrophic cyanobacteria as biofertilizers in the cultivation of wet-land rice has a beneficial effect on the growth and yield (RINAUDO & al. 1971, VENKATARAMAN 1979, SWAMINATHAN 1982, WATANABE & ROGER 1984, GRANT & al. 1986). Reports on the effect of cyanobacteria on growth of other crops than rice are, however, scarce (HENRICKSSON 1971, WITTY 1974, PACHPANDE 1990, NANDA & al. 1991).

The objective of this study was to determine the cyanobacterial potential to promote growth of wheat in sand and clay soils.

Table 1
Some characteristics of the soil used

Soil type	Texture analysis			(o/o w/w) coarse sand	pH	Chemical analysis			
	clay	silt	fine sand			C	N	K	P
						(mg/g soil)			
Choarse sand	3	4	21	70	6.9	2.7	0.1	0.1	0.018
Clay	45	25	18	7	7.2	8.3	0.5	0.3	0.032

Material and Methods

Plant culture

Surface sterilized wheat cv. Sakha 69 grains were planted in plastic pots filled with 2kg of autoclaved and/or non-autoclaved sand and clay soils. The properties of the two soil types used in this investigation are shown in table 1. Ten grains were planted per pot and at emergence the number of plants was reduced to three per

pot. According to the type of soil, pots were divided into four groups. Group 1 autoclaved clay soil, group 2 non-autoclaved clay soil, group 3 autoclaved sand soil and group 4 non autoclaved sand soil. Each plant soil-combination was a separate completely-randomized experiment consisting of ten replications of 6 treatments. The treatments were:

1. Application of live cyanobacteria (a commercially available soil inoculant, cyanobacteria, was obtained from Microbiology Department, Ministry of Agriculture, Cairo, Egypt) according to recommendation of Ministry of Agriculture 200 g/feddan or 480 g/ha).
2. Application of killed cyanobacteria (autoclaved for 30 min).
3. Application of cyanobacteria (live) as well as 100 kg P/ha, 50 kg K/ha and 50 kg S/ha as Ca (PO₃), KCl and Ca SO₄ respectively.
4. Application of cyanobacteria (dead) plus P, K and S at the above rates.
5. Application of P, K and S alone at the above rate.
6. control (without treatments).

The experiment was performed in a wire proof greenhouse maintained at $28 \pm 4^\circ\text{C}$ under natural day light in October–November. Plants were irrigated with water when the soil began to dry, samples were harvested at 45 days after planting.

Analyses

Nitrogenase activity of soil was determined from the conversion of acetylene to ethylene. For this purpose we removed the surface layer of the soil from each pot (100g). The soil was put into a closed system (556 ml mannitol bottles) and incubated under air containing 10% acetylene. After 2 h, gas samples of (10 ml) were taken with syringe and put into another closed system containing 2 ml of oxidant solution (80 ml of 0.05 M NaIO₄, 10 ml of 0.005 M KMnO₄, adjust PH to 7.5 with KOH, dilute to 100 ml). The closed system was agitated vigorously on a rotatory shaker at 300 r.p.m. for 90 min at room temperature. One-fourth ml of 4 M NaAsO₂ and one-fourth ml of H₂SO₄ were added, mixing to destroy excess oxidant. One ml of Nash reagent was added (150 g of ammonium acetate, 3 ml of acetic acid and 2 ml of acetyl acetone diluted to 1 litre) and the absorbance at 412 nm is determined after 60 min. Standards containing known amounts of ethylene were carried through the analysis at the same time as the samples (LARUE & KURZ 1973). According to authors the method is as efficient as gas chromatography.

Plants were dried at 80°C for 48h and dry mass was determined. Nitrogen content of plant tissues was determined by the Kjeldahl method (BLACK & al. 1965). The photosynthetic pigments, chlorophyll a, chlorophyll b and carotenoids, were determined using the spectrophotometric method recommended by METZNER & al. 1965.

A known fresh weight of plant leaves was homogenized in 85% (v/v) aqueous acetone for 5 minutes at 14,000 r.p.m. (homogenizer Mechanika precyzyna type 3.02-Poland). After centrifugation, the supernatant, which contained the pigments was taken and diluted by 85% aqueous acetone to the suitable concentration for spectrophotometric measurements using a spectrophotometer (SPEKOL spectro colorimeter VEB Carl Zeiss JENA Type MK 616, Art A. 609).

The extinction was measured against a blank of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm taking into consideration the dilution,

Table 2

Effect of cyanobacterial inoculation on nitrogenase activity of soil and growth of wheat plants grown in autoclaved or non-autoclaved clay soil. Each value represents the mean of five replicates *).

Treatment	Nitro- genase activity (n mol C ₂ H ₄ /g soil. h)	Dry wt (g/plant)	Total nitrogen (mg/plant)	Pigments (mg/plant)		
				Chloro- phyll a	Chloro- phyll b	Carote- noids
Autoclaved soil						
Control	3.00 j	0.25 c	2.29 h	0.55 f	0.14 j	0.11 i
Live inoculum	18.15 b	0.39 a	7.10 b	1.15 b	0.71 b	0.56 b
Dead inoculum	9.10 f	0.22 d	4.05 e	0.70 e	0.32 f	0.32 e
Live inoculum	12.20 d	0.33 b	6.65 c	0.92 c	0.54 d	0.59 b
+						
K, P and S						
Dead inoculum	6.25 h	0.19 e	2.95 g	0.32 g	0.24 h	0.28 g
+						
K, p and S						
K, p and S	5.00 i	0.14 f	2.92 g	0.17 h	0.23 i	0.22 h
Non-autoclaved soil						
Control	5.00 i	0.20 e	2.88 g	0.58 f	0.21 k	0.11 i
Live inoculum	22.10 s	0.34 b	7.35 a	1.58 a	1.73 a	0.62 a
Dead inoculum	10.10 e	0.20 e	4.82 d	0.92 c	0.49 e	0.39 d
Live inoculum	14.50 c	0.33 b	7.05 b	1.08 b	0.68 c	0.44 c
+						
K, P and S						
Dead inoculum	9.00 f	0.22 d	3.55 f	0.83 d	0.27 g	0.30 f
+						
k, P and S						
K, P and S	7.10 g	0.20 de	1.39 i	0.32 g	0.23 i	0.27 g

*) Numbers in the same column followed by the same letter(s) are not significantly different at the 5 % level by Duncans multiple range test.

it was possible to determine the concentration of pigment fractions (chlorophyll a, chlorophyll b, and carotenoids) as mg/ml using the following equation:

$$\text{Chlorophyll a} = 10.3 E_{663} - 0.918 E_{644} = \text{mg/ml}$$

$$\text{Chlorophyll b} = 19.7 E_{644} - 3.87 E_{663} = \text{mg/ml}$$

$$\text{Carotenoids} = 4.2 E_{452.5} - (0.0264 \text{ Chlorophyll a} + 0.4260 \text{ Chlorophyll b}) = \text{mg/ml}$$

Finally these pigment fractions were also calculated as mg/plant.

Following harvest, for above-soil biomass, we removed the 1 cm surface layer of soil from each pot. Four cm of this surface material was placed in a petri dish, wetted with deionized water, and kept illuminated with fluorescent lamp. Within 2 weeks genus and species of the algae present were identified.

Table 3

Effect of cyanobacterial inoculation on nitrogenase activity of soil and growth of wheat plants grown in autoclaved or non-autoclaved sand soil. Each value represents the mean of five replicates *).

Treatment	Nitro- genase activity (n mol C ₂ H ₄ /g soil. h)	Dry wt (g/plant)	Total nitrogen (mg/plant)	Pigments (mg/plant)		
				Chloro- phyll a	Chloro- phyll b	Carote- noids
Autoclaved soil						
Control	1.25 de	0.14 de	2.90 k	1.21 i	0.28 g	0.20 j
Live inoculum	14.15 a	0.24 b	5.87 b	2.33 b	0.57 b	0.90 b
Dead inoculum	8.25 b	0.21 c	4.87 f	1.56 e	0.52 c	0.58 e
Live inoculum	12.30 a	0.22 c	5.24 d	1.61 d	0.47 d	0.62 c
+						
K, P and S						
Dead inoculum	5.30 c	0.19 c	4.01 h	1.52 f	0.49 de	0.54 g
+						
K, P and S						
K, P and S	4.20 cd	0.17 cd	3.77 i	1.39 h	0.44 f	0.51 h
Non- autoclaved soil						
Control	2.15 d	0.16 de	3.15 j	1.38 h	0.43 f	0.47 i
Live inoculum	15.30 a	0.29 a	7.05 a	2.38 a	1.68 a	0.99 a
Dead inoculum	9.20 b	0.20 c	5.01 e	1.58 e	0.50 d	0.61 d
Live inoculum	13.15 a	0.19 c	5.46 c	1.70 c	0.53 c	0.63 c
+						
K, P and S						
Dead inoculum	7.15 b	0.19 c	4.42 g	1.52 f	0.49 d	0.56 f
+						
K, P and S						
K, P and S	5.30 c	0.17 cd	3.79 i	1.47 g	0.47 de	0.52 h

*) Numbers in the same column followed by the same letter(s) are not significantly different at the 5 % level by Duncans multiple range test.

Statistical Analysis

Differences in nitrogenase activity, total nitrogen and chlorophylls were tested for statistical significance between treatments, using one-way analysis of variance of means (Pc-State computer program).

Results and Discussion

Inoculation of wheat with cyanobacteria either alive or killed lead to a significant increase in dry-matter accumulation over controls in all four experimens (Table 2 and 3). Application of live inoculum only and live

inoculum plus K, P and S treatments were significantly greater than other treatments. Analysis of variance revealed that dry weight of wheat plants growing in autoclaved soils was significantly less than plants growing in non-autoclaved ones.

The results obtained for total nitrogen per dry mass showed similar trends (Tables 2 and 3). Chlorophyll a, chlorophyll b and carotenoids are presented in Table 2 and 3. Results revealed that inoculating plants with live or killed cyanobacteria either alone or with K, P and S increased chlorophylls and carotenoids.

The significant increase in dry weight, total nitrogen and pigments content of plants inoculated with live or killed inoculum alone or with K, P and S could be attributed to nitrogenase activity (Table 2 and 3) of nitrogen fixing organisms in the surface of the soil (Table 4).

Similar results were obtained by TIEDEMANN & al. 1980, PACHPANDE 1990 and NANDA & al. 1991. These authors reported that soaking seeds of cucumber and pumpkin with extract of *Westiellopsis prolifica*, an N_2 -fixing cyanobacterium, promoted germination and their subsequent growth and development. FOGG & PATTANAIK 1966 reported that ammonium- and amide-nitrogen accounted for most of the total extracellular nitrogen produced by nitrogen fixing cyanobacteria.

The results of this study indicate that application of cyanobacterial inoculant enhanced the growth of irrigated plantation crops.

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