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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 N_2-Fixierung entsprechend der Nitrogengenaseaktivität 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) clay	silt	fine sand	coarse sand	pH	Chemical analysis 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 obained 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 $28 \pm 4°$ 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 actylene 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 $NaAsO_2$ and one-fourth ml of H_2SO_4 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 Kjeldahal 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 preczyina 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 spectrocolorimeter (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	Nitrogenase activity (n mol C_2H_4/g soil. h)	Dry wt (g/plant)	Total nitrogen (mg/plant)	Pigments (mg/plant)		
				Chlorophyll a	Chlorophyll b	Carotenoids
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 + K, P and S	12.20 d	0.33 b	6.65 c	0.92 c	0.54 d	0.59 b
Dead inoculum + K, p and S	6.25 h	0.19 e	2.95 g	0.32 g	0.24 h	0.28 g
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 + K, P and S	14.50 c	0.33 b	7.05 b	1.08 b	0.68 c	0.44 c
Dead inoculum + k, P and S	9.00 f	0.22 d	3.55 f	0.83 d	0.27 g	0.30 f
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:

Chlorophyll a = 10.3 E_{663} – 0.918 E_{644} = mg/ml

Chlorophyll b = 19.7 E_{644} – 3.87 E_{663} = mg/ml

Carotenoids=4.2 $E_{452.5}$–(0.0264 Chlorophyll a + 0.4260 Chlorophyll b) = 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	Nitrogenase activity (n mol C_2H_4/g soil. h)	Dry wt (g/plant)	Total nitrogen (mg/plant)	Pigments (mg/plant)		
				Chlorophyll a	Chlorophyll b	Carotenoids
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 + K, P and S	12.30 a	0.22 c	5.24 d	1.61 d	0.47 d	0.62 c
Dead inoculum + K, P and S	5.30 c	0.19 c	4.01 h	1.52 f	0.49 de	0.54 g
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 + K, P and S	13.15 a	0.19 c	5.46 c	1.70 c	0.53 c	0.63 c
Dead inoculum + K, P and S	7.15 b	0.19 c	4.42 g	1.52 f	0.49 d	0.56 f
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

Table 4. Count of algae in the surface 1 cm of soil after cyanobacterial inoculation

Species	Clay soil												Sand soil											
	autaclaved						non-autaclaved						autaclaved						non-autalcaved					
	Control	Live	Dead	live + KPS	Dead + KPS	KPS	Control	Live	Dead	Live + KPS	Dead + KPS	KPS	Control	Live	Dead	Live + KPS	Dead + KPS	KPS	Control	Live	Dead	live + KPS	Dead + KPS	KPS
* *Anabaena oscillaroides*	–	110	–	70	10	–	–	110	20	170	–	–	–	30	–	60	–	–	10	30	–	40	–	–
Anacystis montana	30	100	–	130	30	–	50	130	10	160	–	–	–	20	–	10	–	–	10	50	–	50	–	–
Caltharix wambaereasis	–	110	10	110	90	–	10	–	–	110	–	–	10	–	30	50	70	30	–	–	10	–	60	–
Dermocarpa sp.	–	130	20	50	70	–	30	110	–	50	10	40	–	10	–	70	–	–	60	140	–	160	–	40
Fischerella thermalis	–	150	50	40	20	–	–	110	10	60	–	–	–	50	–	90	–	–	–	–	–	–	–	–
F. musicola	–	100	–	90	–	10	–	50	–	80	30	–	–	30	–	30	–	–	–	–	–	–	–	–
Glococapsa Turgida	70	120	–	110	–	–	50	70	–	70	–	30	10	40	50	110	90	30	20	70	–	90	–	–
G. punctata	40	160	–	–	–	–	20	30	30	110	–	–	–	60	–	130	–	–	40	90	30	110	130	10
* *Glocotrichia sp.*	–	110	–	130	–	60	–	20	–	60	–	–	40	40	–	70	–	70	–	20	–	30	–	–
Lyngbya Lutae	60	120	–	120	–	–	–	190	–	220	–	10	–	20	–	40	–	–	–	80	–	80	–	–
* *Nodularia spumigena*	–	170	–	170	10	–	–	120	50	170	90	–	–	10	–	10	–	–	–	30	–	60	–	–
* *Nostoc microscopicum*	–	50	–	220	50	30	–	90	–	110	–	–	–	–	–	–	–	–	–	60	–	110	–	–
* *N. verrocasum*	–	60	–	130	–	–	30	200	–	90	–	–	–	30	–	30	–	–	40	40	10	50	40	30
* *N. linckia*	–	110	–	150	30	–	–	300	40	230	110	20	–	60	–	100	–	–	20	40	20	30	10	–
* *N. muscorum*	50	170	60	70	20	–	–	310	–	270	–	–	–	–	–	–	–	20	–	20	–	10	–	–
Oscillatoria limosa	110	130	160	90	90	50	70	160	90	300	150	80	10	80	70	150	100	10	–	10	–	–	–	–
O. splendida	100	70	110	150	20	10	40	110	–	290	–	70	30	40	110	110	110	80	40	20	40	160	60	10
Phormidium retzii	70	60	110	50	20	–	50	80	110	160	140	30	20	60	80	70	90	70	30	80	30	40	30	10
Ph. molle	30	30	50	30	10	–	20	50	60	130	110	50	10	20	40	50	50	140	20	10	60	30	10	10
Diatoms	200	170	220	360	200	150	400	460	310	360	280	300	300	270	310	380	320	300	560	610	530	570	420	660
Number of species	10	20	9	19	14	5	11	19	11	20	8	9	8	17	7	18	7	9	11	17	8	16	8	6
Total no. of organisms	760	2230	790	2270	670	300	770	2700	730	3200	920	630	430	870	690	1550	830	750	850	1400	840	1620	760	770
% of N_2-fixer organisms *	6.6	35.0	7.6	41.4	11.9	20	3.9	42.6	19.2	34.4	2.71	3.2	9.3	19.5	0.0	17.4	0.0	12.0	17	117.1	3.6	20.4	6.6	3.9

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 cartenoids are presented in Table 2 and 3. Results revealed that incoulating plants with live or killed cyanobacteria either alone or with K, P and S increased chloroplylls 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 *Westiellopis prolifica,* an N_2-fixing cyanobacterium, promoted germination and their subsequent growth and develepment. 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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