# EFFECT OF CYANOBACTERIA INOCULATION ON WHEAT PRODUCTIVITY AND GRAIN QUALITY EI-Kassas, A. R.\* and E. M. Gaafar \*\*

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## ABSTRACT

At present, a great interest in establishing novel associations between higher plants and a variety of N<sub>2</sub>-fixing microorganisms has entered the scientific scene arising from the prospects and the possibilities of their potentially application. In this paper, data presented is obtained during the co-cultivation of local cyanobacteria strains previously isolated from the Egyptian rice soils and wheat. Results revealed that cyanobacteria inoculation (SBI) exhibited an economical view that it can save about 30 % of the mineral nitrogen amounts required for wheat crop production. This trend was noticed when SBI inoculation was applied at the rate of 3 kg ha<sup>-1</sup> combined with 80 kg N ha<sup>-1</sup> which recorded a grain yield not significantly different from that obtained by 120 kg N ha<sup>-1</sup> the recommended nitrogen dose in wheat cultivation. Cyanobacteria inoculated to wheat crop have also improved both wheat yield components and wheat grains quality.

Keywords: Cyanobacteria, wheat, quality, grains.

#### INTRODUCTION

The modern day intensive crop cultivation requires the use of much nitrogen fertilizers. However, fertilizers are short supply and expensive in developing countries such as Egypt. 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 seeds or soil application and designed to improve soil fertility and plant growth.

The application of N<sub>2</sub>-fixing cyanobacteria as biofertilizers in the cultivation of wet-land rice has beneficial effect on growth and yield (Swaminathan, 1982; and Mule *et al.*, 1999). Reports on the effect of cyanobacteria on growth of other crops other than rice are, however, scarce (Nanda *et al.*, 1991; Gantar *et al.*, 1995 and Abd El Rasoul *et al.*, 2003).

On the other respect, many microorganisms bear extra-cellular sheaths of considerable thickness external to their outer membrane, providing a protective and favorable microenvironment. These sheaths are usually composed mainly of polysaccharides. In plant-microbe associations, polysaccharide, regardless of whether it is plant or microbial origin, may enable a close contact to take place between the two partners that required for their symbiotic or parasitic relationships. N<sub>2</sub>-fixing cyanobacteria are capable of forming symbiotic associations with various plants and fungi (Bergman *et al.*, 1992). There is also a great deal of interest in creating novel associations between agronimically important

plants, particularly cereals, and N<sub>2</sub>-fixing microorganisms including cyanobacteria (Spiller *et al.*, 1993). The heterocystous cyanobacterium *Nostoc sp.* is usual among characterized cyanobacteria in its ability to form tight associations with wheat roots and to penetrate both root epidermis and cortical intracellular space (Gantar *et al.*, 1991 a&b). The N<sub>2</sub> fixed with *Nostoc* sp. in association with wheat is taken up by the plant and supports its growth (Gantar *et al.*, 1995).

The present work is conducted to study the effect of cyanobacteria as biofertilizer on wheat productivity and wheat grain quality.

#### MATERIALS AND METHODS

A field experiment was carried out in a private farm at Kashish, Shibin EL-Kanter, Qualubiya Governorate during the winter season of 2002-2003. The soil of the experimental plots was clayey with pH 7.55, organic matter 2.19 % (Walkley and Black, 1934), total nitrogen 0.20 % (Jackson, 1976) and total P 0.02% (Olsen *et al.*, 1954).

The field was prepared by ploughing and puddling. It was then divided into 21 plots ( $3.5 \text{ m} \times 3.5 \text{ m}$  each) representing 7 treatments with three replicates in randomized block design. The treatments consisted of control (no nitrogen), full recommended dose (RD) of the dried cyanobacteria soil based inoculum SBI (10 kg ha<sup>-1</sup>), urea nitrogen at 120 kg Nha<sup>-1</sup>, 5 kg SBI ha<sup>-1</sup> + 60 kg Nha<sup>-1</sup>, 3 kg SBI ha<sup>-1</sup> + 80 kg Nha<sup>-1</sup>, 6 kg SBI ha<sup>-1</sup> + 40 kg Nha<sup>-1</sup> and 10 kg ha<sup>-1</sup> SBI + 120 kg Nha<sup>-1</sup>.

Wheat grains cultivar Giza 168 were sowing on December 20, 2001 and were harvested on May 15, 2002. Uniform application of superphosphate (15 %  $P_2 O_5$ ) at a rate of 100 kg ha<sup>-1</sup> was done as basal to each plot. Cyanobacteria inoculation was executed 7 days after sowing of wheat seeds. Urea nitrogen treatments were applied in two equal doses 10 days after sowing and 35 days later. Irrigation was carried out at sowing time and then at every 20 days intervals.

At harvest wheat yield components such as straw and grain yield (tons ha<sup>-1</sup>), 1000-grain weight (g), plant height (cm), number of grains spike<sup>-1</sup>, total nitrogen percent in both grain and straw, total nitrogen uptake (kg Nha<sup>-1</sup>)

and harvest index in percent  $\frac{kg \, grain \, yield}{kg \, grain \, yield + kg \, straw \, yeild} \times 100$  (Yanni,

1991) were determined. Wheat grain quality such as protein % (A. A.O.A.C., 1960), carbohydrate % (Dubois *et al.*, 1965), gluten % (A. A. C. C., 1983), wheat flour extract % (A. A. O. A. C., 1985) and ash % (A. A. O.A.C., 1980) were estimated. All obtained data were then subjected to the statistical analysis according to Gomez and Gomez, (1984).

#### RESULTS

Data in Table (1) indicates the effect of cyanobacteria inoculation and / or urea-N fertilization each either alone at the recommended dose or combined together with different levels on the growth and the yield components of wheat crop cultivar Giza 168.

Results revealed that all the tested treatment increased significantly straw and grain yield over the control treatment except for the grain yield due to the 10 kg SBI ha<sup>-1</sup>treatment. The highest grain and straw yields were attained by the use of 120 kg Nha<sup>-1</sup> treatment. The corresponding yield amounts were 9.02 and 9.85 tons ha<sup>-1</sup>, respectively. However, the highest grain yield was not significantly different from those of 8.34 and 8.95 tha<sup>-1</sup> due to both 3 kg SBI + 80 kg Nha<sup>-1</sup> and 10 kg SBI + 120 kg Nha<sup>-1</sup>, respectively.

Both treatments were also not significantly different from each other. The inoculation with 10 kg SBI (cyanobacteria inoculum) alone slightly raised the grain yield over the control treatment (Table 1).

Owing the straw yield, the highest yield of 9.85 tons ha<sup>-1</sup> (120 kg Nha<sup>-1</sup>) was not significantly different from those of 9.00 and 9.75 tons ha<sup>-1</sup> due to the treatment of 3 kg SBI + 80 kg Nha<sup>-1</sup> and 10 kg SBI + 120 kg Nha<sup>-1</sup>, respectively. However, these two straw yield amounts were not significantly different from each others.

1000-grain weight due to the use of 10 kg SBI ha<sup>-1</sup> (48.58 g) was significantly higher than those recorded by the other treatments. However, no definite trend could be observed in response to the tested treatment.

The plant height of wheat plants exhibited significant increases over the control treatment (80.8 cm) except for 10 kg SBI treatment (86.50 cm). The highest plant height measurement (95.50 cm) was recorded in the treatment of 10 kg SBI + 120 kg Nha<sup>-1</sup>. This high plant height value was significantly different from both control, 10 kg SBI and 6 kg SBI ha<sup>-1</sup> +40 kg N ha<sup>-1</sup>.

The number of grains spike<sup>-1</sup> recorded the highest value of 60 grains spike<sup>-1</sup> by 10 kg SBI + 120 kg Nha<sup>-1</sup> treatment. This high number of grain spike<sup>-1</sup> was not significantly different from those of 58, 55 and 53 grains spike<sup>-1</sup> due to 120 kg N, 5 kg SBI + 60 kg N and 3 kg SBI + 80 kg Nha<sup>-1</sup>, respectively.

Harvest index percentage fluctuated within a relatively narrow range indicating no definite trend for the treatments effects. However, the highest harvest index percentage (48.10) was due to 3 kg SBI ha<sup>-1</sup> + 80 kg Nha<sup>-1</sup> treatment. Also, no significant differences were noted amongst all nitrogen and SBI treatments either each alone or combined together.

The highest nitrogen percentages of 0.28 and 1.50 for both straw and grains were due to 120 kg Nha<sup>-1</sup> treatment. However these two high N percentages were not significantly higher than those recorded by the other treatments except for the control incase of straw and 10 kg SBI ha<sup>-1</sup> treatments incase of grains.

Due to the total nitrogen uptake amounts by wheat crop, it was observed that all treatment exceeded significantly the N-uptake amount of the control treatment (50.32 kg Nha<sup>-1</sup>). The highest total N-uptake by wheat crop of 162.88 kg Nha<sup>-1</sup> (120 kg-N ha<sup>-1</sup>) was not significantly higher than those of 149.52, 147.73 and 122.62 kg Nha<sup>-1</sup> recorded by 10 kg SBI ha<sup>-1</sup> + 120 kg Nha<sup>-1</sup>, 3 kg SBI ha<sup>-1</sup> + 80 kg Nha<sup>-1</sup> and 5kg SBI ha<sup>-1</sup> + 60 kg Nha<sup>-1</sup>, respectively.

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Results in (Table 2) revealed that application of both nitrogen fertilizer and cyanobacteria inoculum had improved the wheat grain quality in comparison to the control treatments. The use of 3 kg SBI ha<sup>-1</sup> + 80 kg Nha<sup>-1</sup> recorded the highest percentages of 12.48 (protein), 82.00 (carbohydrate), 15.65 (gluten), 74.12 (flour extraction) and 98.72 (ash). These percentages were not significantly higher than those obtained by the use of 120 kg Nha<sup>-1</sup> the full nitrogen recommended dose.

#### DISCUSSION

These results are in agreement with those described by Abd-Alla et al. (1994) who attributed the increase in wheat growth parameters to the substantial increases of N2 fixation in soil due to nitrogenase activity of the cyanobacteria inoculation. Consequently, this could explain that the reduction of the recommended nitrogen dose required for wheat cultivation, can be compensated by the use of cyanobacteria, *i.e* this reduction was either it 1/2 (60 kg Nha<sup>-1</sup>) or 2/3 (80 kg Nha<sup>-1</sup>) the nitrogen recommended dose. They also added that inoculation of wheat with cyanobacteria either alive or killed led to a significant increase in dry- matter accumulation over control treatments. El-Mancy et al. (1997) revealed that cyanobacteria inoculation to rice (SBI) increased significantly both rice grain and straw yields to the extent of 2.07 and 17.06 % over the control, respectively. The combination of SBI inoculation with N and P chemical fertilizers can lead to saving chemical N fertilizer (about 50 %) improving rice grain quality, NPK uptake and N and P recovery, reducing the bad effects of the high doses from chemical fertilizers and consequently increasing the possibility for producing high and good rice yield.

Inoculation of rice fields with cyanobacteria (SBI) might help to regenerate quickly and improve the soil structure. SBI are known to excrete extracellularly a number of compounds like polysaccharides, peptides, lipids...etc. during their growth in soil particles and hold /glue them together in the form of micro-aggregates (Mandal *et al.* 1999). This soil improvement was due to cyanobacteria inoculation being reflected on soil fertility and consequently on cultivated crop. Gantar (2000) emphasized the cyanobacteria-wheat association and stated that when wheat seedlings are co-cultivated with *Nostoc sp.* in hydro-ponics, the cyanobacteria colonizes the endorhizosphere at low frequency. He suggested that mild association of the roots dramatically increased the number of cyanobacteria within the root tissues. The cyanobacteria penetrated the roots in the form of motile filaments (hormogonia), at once inside, they divided and transformed into a seriate packages, which showed nitrogenase activity. Thus, co-cultivation of wheat with cyanobacteria could partially meet the wheat nitrogen needs.

Amarit *et al.* (1987) indicated that cyanobacteria applied to rice increased grain and straw yields, N-uptake, plant height, number of panicles, 1000-grain weight and grain carbohydrate and protein contents. These increases were owed to  $N_2$  and organic carbon added to soil by cyanobacteria inoculation as well as the decrease of the bad effect of intensive use of chemical nitrogen fertilizer. El-Kassas (2002) stated that *Azospirillum* inoculated wheat plants combined with 75 % of the

recommended nitrogen increased significantly the wheat grain quality such as protein; carbohydrate; dry gluten and wheat flour extract percentage. He explained that  $N_2$  fixed by *Azospirillum* was the direct reason for these increases.

Generally, cyanobacteria fertilizers are a promising alternative to avoid soil pollution caused by agrochemicals and recover the nutrient content and structure lost after as they bring to soil combined nitrogen (some of them are  $N_2$ - fixers and secrete exo-ploysaccharide that improve soil structure and bio-active substances that enhance the plant growth.

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تأثير التلقيح بالسيانو يكتريا على انتاجية محصول القمح وصفات الحبوب
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أجريت تجربة حقلية بمزرعة خاصة بقرية كاشيش محافظة القليوبية وذلك فى موسم٢٠٠٢/ ٢٠٠٣ وذلك لدراسة أثر التلقيح بالسيانوبكتريا الجافة منفردة أو بمصاحبة جزء أو كل النيتروجين المعدنى الموصى به على انتاج محصول القمح صنف جيزة ١٦٨ ومحلول الميكروبات الفعالة كسماد حيوى يوفر جزء من السماد النيتروجينى اللازم لعملية انتاج القمح من حيث مكونات المحصول من الحبوب والقش وكذا محتواه النيتروجينى وكذلك الصفات التكنولوجية للمحصول مثل النسبة المئوية لكل من البروتين والجلوتين الجاف والكربو هيدرات ونسبة استخلاص دقيق القمح وكانت أهم النتائج كما يلى:-

- آ- حققت المعاملة ٣ كجم سيانوبكتريا جافة/هكتار + ٨٠ كجم نيتروجين/هكتار أعلى محصول لكل من الحبوب والقش والذي لم يختلف معنويا عن المحصول الناتج من استخدام ١٢٠ كجم نيتروجين/هكتار (المعدل الموصى به).
- ٢- حققت المعاملة ١٢٠ كُجم نيتروجين/هكتار أعلى محتوى نيتروجينى لمحصول القمح والذى لم يختلف معنويا عـن ذلك المتحصلعايه مـن المعاملـة ٣ كجـم سـيانوبكتريا جافـة/هكتار + ٨٠ كجـم نيتروجين/هكتار.
  - ٣- أدى التلقيح بالسيانوبكتريا الى تحسن كل من مكونات محصول القمح وكذا صفاتة التكنولوجية.
- ٤- أوضحتا الدراسة أن التلقيح بالسيانوبكتريا لـه عائدا اقتصاديا حيث أمكن توفير حوالى ٣٠% من كمية السماد النتروجيني الكيميائي الموصى به.
- من هذه النتائج يتضح أيضا أن استبدال جزء من السماد النيتروجيني بالتلقيح بالسيانوبكتريا الجافة يقلل
   من التلوث البيئي الناتج من الاستخدام المكثف للسماد النيتروجيني من أجل زيادة الانتاج.

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Treatments	Yields (tons ha <sup>-1</sup> )		1000- grain weight	No. of grains	Plant Height	Nitrogen %		nitrogen Uptake	Harve-st index
	Straw	Grains	(g)	spike-1	(cm)	Straw	Grains	(kg Nha <sup>-1</sup> )	%
Control	6.30	5.00	46.33	43	80.80	0.14	0.83	50.32	44.31
10 kg SBI ha <sup>.1</sup>	7.00	6.00	48.58	47	86.50	0.15	1.10	76.50	46.15
120 kg-N ha <sup>-1</sup>	9.85	9.02	43.59	58	90.50	0.28	1.50	162.88	47.80
5kg SBI ha <sup>-1</sup> + 60 kg-N ha <sup>-1</sup>	8.20	7.40	42.67	55	93.20	0.25	1.38	122.62	47.43
3kg SBI ha <sup>-1</sup> + 80 kg-N ha <sup>-1</sup>	9.00	8.34	41.59	53	95.20	0.27	1.48	147.73	48.10
6kg SBI ha <sup>.1</sup> + 40 kg-N ha <sup>.1</sup>	8.00	6.60	40.41	45	87.15	0.22	1.35	106.70	45.21
10kg SBI ha <sup>-1</sup> + 120 kg-N ha <sup>-1</sup>	9.75	8.95	43.12	60	95.50	0.23	1.42	149.52	47.86
L . S . D . < 0.05	2.01	1.30	2.24	10.59	6.14	0.11	0.53	27.38	4.34

## Table (1):Effect of cyanobacteria inoculation (SBI) and nitrogen fertilization on wheat yields components.

# Table (2) Effect of cyanobacteria inoculation (SBI) and nitrogen fertilization on wheat grain quality

Treatment	Protein %	Carbohydrate %	Gluten %	Flour Extraction %	Ash %
Control	4.95	64.32	7.51	65.95	98.47
10 kg SBI ha <sup>-1</sup>	6.09	68.25	8.36	71.24	98.42
120 kg N ha <sup>-1</sup>	5.55	66.78	12.14	73.37	98.57
5 kg SBI ha <sup>-1</sup> + 60 kg N ha <sup>-1</sup>	8.37	70.32	12.48	72.10	98.51
3 kg SBI ha <sup>-1</sup> + 80 kg N ha <sup>-1</sup>	12.48	82.00	15.65	74.12	98.72
6 kg SBI ha <sup>-1</sup> + 40 kg N ha <sup>-1</sup>	9.81	78.17	13.74	73.60	98.50
10 kg SBI ha <sup>-1</sup> + 120 kg N ha <sup>-1</sup>	6.51	66.78	11.68	72.10	98.48
L. S . D. < 0.05	0.99	3.48	1.30	1.56	0.13