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EFFECT OF CYANOBACTERIA INOCULATION ON MAIZE YIELD AND GRAIN TECHNOLOGY CHARACTERS

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ABSTRACT

Two field experiments were carried out at a private farm at Sakkara, EL-Badrasheen, Giza, Governorate, Egypt, during two successive Summer seasons of 2004-2005 to study the influence of cyanobacteria inoculation in different rates(0,25, 50 and 100% of the recommended dose,i.e.,10 kg dried cyanobacteria inoculum fed^{-1}) individually or in presence and/ or absence of different nitrogen levels (25, 50 and 100% of the recommended dose, (i.e.),100 kg N fed^{-1}) on maize yield and 100-grain weight, some maize grain technology characters (protein and oil yields fed^{-1}, protein, carbohydrates and ash percentages) as well as the maize mineral contents. Results revealed that cyanobacteria inoculation enhanced all maize tested parameters. The highest maize yield values were due to 100% N + 100% cyanobacteria and they were significantly higher than the other tested treatments received the other different combination of cyanobacteria and nitrogen. Cyanobacteria inoculation at the rate of 100% N + 100% cyanobacteria the highest oil yield fed^{-1}. The treatment of 50% cyanobacteria combined with100% N recorded the highest percentages of protein, oil and carbohydrate, while, the highest percentage of ash was due to 25% cyanobacteria combined with50% N. Due to both maize grains hulls and germ percentages, the highest ones were recorded by the treatment received 25% cyanobacteria combined with 50% N, while, maize grain endosperm gave its highest percentage due to the treatment of 25% cyanobacteria combined with 100 % N. As for maize grain mineral content, As for cyanobacteria inoculation had positively affected the maize grains mineral content ,i.e., Mg, Na, Zn, Fe, Ca and K. In conclusion, the use of cyanobacteria inoculation technology in cereal crop production such as maize may lead to reduce the amount of mineral nitrogen required for maize production as well as it ensures good yield quality and safe the environment contaminations resulted from the extensive use of the costly and hazard the so called mineral nitrogen fertilizer.

INTRODUCTION

The period since the 1950s has seen exciting advances in understanding biological nitrogen fixation (BNF). Progress in application of BNF technology to agriculture has been slower, but there have been important innovation. While much of the basic BNF research in the last 30 years has been focused on nodulated legumes and rhizobia, there have been relatively rapid advances in knowledge of other N_2- fixing systems. This includes the actinomycetes that form nodules on some non-leguminous shrubs and trees, free - living N_2- fixers associated with cereals and the cyanobacteria. The latter are widely distributed in nature and form prominent autotrophic microbial populations of wetland soils. Reducing the amount of the organic matter, in turn, affect soil aggregates stability. Considering the very low efficiency of applied nitrogenous fertilizer in crop cultivation, may lead to extensive and undue use of chemical fertilizers may lead to serious environmental problems, some of which are accumulation of NO_3 and NO_2 to hazardous levels in the underground water and plant tissues. So, for the production of healthy food, it may be necessary to find out and exploit potential alternative sources of plant nutrients to sustaining soil fertility such as biofertilizers with the minimum addition of chemical fertilizers. Biofertilizers are safe from the environmental point of view, cheaper and at the same time satisfy the nutrient demands of crop plants (Badawy *et al.,* 1996). One of the most promising biofertilizer is cyanobacteria, either as free-living microorganisms or as symbionts with the water *Azolla* fern. Cyanobacteria as biofertilizer utilization in rice fields is common and promising (Venkataraman and Tilak, 1990). Recent researchers have shown that cyanobacteria also help to reduce soil alkalinity and this opened up possibilities for bioreclamation of such inhospital environment. Very recent reports by Thajuddin and Subramanian (2005) showed that cyanobacteria have beneficial effects on a number of other crops rather than rice such as barely, oats, tomato, radish, cotton, sugar cane, maize, chilli and lettuce. They also added that cyanobacteria have received worldwide attention for their possible use in mariculture, food, feed, fuel, fertilizer, and colorant, production of various secondary metabolites including vitamins, toxins, enzymes and pollution abatement. Jagannath *et al.* (2002) found that cyanobacteria inoculation enhanced the overall growth parameters of chickpea. It enhanced all morphological and biochemical characters such as proteins, carbohydrates, total nitrogen uptake, net grain and biomass yield and grain quality of chickpea. Salem (1999) found that cyanobacteria inoculated to soybean can be successfully overcoming the adverse effect resulted from the saline stress condition. Abd El- Rasoul *et al*. (2004) indicated that inoculation with cyanobacteria to wheat, exhibited an economical view that it can save about 50% of mineral nitrogen amounts required for wheat production. They also showed that this treatment has improved the technological characteristics of wheat grains, i.e., carbohydrate, protein and flour extract percentages. El- Gaml (2006) reported that maize inoculation with a mixture of cyanobacteria strains significantly enhanced maize grain yield, NPK uptake by grains and stover, soil organic matter, reduced both soil reaction and soil electrical conductivity, and increased soil particle size aggregates. These benefits achieved due to cyanobacteria inoculation, are in turn increased the nutrients availability to the cultivated plants that ensure high yield and grain quality. Mussa *et al.* (2002) for example revealed that cyanobacteria liberate extra cellular or organic compounds and photosynthetic O_2 during their growth and contribute biomass, which in turn enhanced the yield of the cultivated crop and improved its grain quality. In a cumulative review, Roger and Kulasooriya (1980) reported that besides increasing soil nitrogen fertility, cyanobacteria have been said to benefit rice plants by producing growth-promoting substances. More direct evidence for hormonal effects has come primarily from treatments of rice seedlings with cyanobacterial culture or their extracts. Presoaking of rice seeds in cyanobacteria cultures or extracts has decreased losses from sulphate – reducing processes and this has been attributed to the enhancement of germination and a faster seedlings growth due to cyanobacterial exudates. Aref and Al- Kassas (2006) reported that cyanobacteria inoculated to maize in combination with 50 % N led to enhance maize grains quality in terms of increasing their protein, oil, carbohydrates and ash per cent.

This work is designed to study the effect of cyanobacteria inoculated to maize variety single hybrid 10 cultivated in clayey soil under different nitrogen levels on maize yield and yield components, grain technology characters.

MATERIALS AND METHODS

Two field experiments were carried out at a private farm in Sakkara, El-Badrasheen, Giza, Governorate, Egypt, during two successive summer seasons of 2004-2005 to study the influence of cyanobacteria inoculation in different rates (Zero, 25, 50 and 100% of the recommended dose, i.e., 10 kg dried cyanobacteria inoculum fed^{-1} individually or in presence and/ or absence of different nitrogen levels (25, 50 and 100% of the recommended dose, i.e., 100 kg N fed^{-1}) on maize yield, 100-grain, some maize grain technology characters (protein and oil yields kg fed^{-1}, protein, oil, carbohydrates and ash percentages) as well as the mineral contents of maize grains. The soil used was clayey in texture, having pH 7.55, total N 0.20% (Jackson, 1973), total P 0.02% (Olsen *et al*., 1954) and organic matter 2.19% (Walkley and Black, 1934).

Prior to maize grains cultivation the uniform principal practices recommended by the Ministry of Agriculture and Land Reclamation were completed. Phosphate and potassium fertilizers were added as superphosphate at the rates of 100 kg fed^{-1} (15.5% P_2O_5) and 50 kg fed^{-1} as potassium sulphate (48% K_2O).

The experimental area was divided into plots of 3 x 3.5 m. Grains of *Zea maize* variety single hybrid 10 were inoculated with cyanobacteria inoculum (15 days after sowing) , which is composed of a mixture of individual strains namely, *Nostoc muscorum, Nostoc calcicola, Anabaena oryzae and Clyndrospermum muscicola.* This inoculum kindly supplied by Agric. Microbiol. Dept. Soils, Water & Environ. Res., Inst., Agric. Res. Center, Giza, Egypt. Maize grains were then drilled in rows 30 cm apart. Nitrogen fertilizer was applied in 3 levels, (i.e.), 25, 50 and 100% of the recommended dose (100 kg N fed^{-1}) in the form of urea (46% N). These nitrogen levels were added in two split equal doses, (i.e.), 20 days after sowing and 50 days later.

The experimental design was in a split plot design with 12 treatments in three replications. Nitrogen fertilization levels of 25, 50, and 100 % N represent the main plot, while the rates of dried cyanobacteria inoculum (0, 25, 50, and 100 %) represent the sub plots. The experiment comprises the following treatments:

1- Zero cyanobacteria inoculum (CSBI) + 25%N dose
2- Zero CSBI + 50%N dose
3- Zero CSBI + 100%N dose
4- 25% CSBI + 25%N dose
5- 25% CSBI + 50%N dose
6- 25% CSBI + 100%N dose
7- 50 % CSBI + 25%N dose

8- 50 % CSBI + 50% N dose
9- 50 % CSBI + 100% N dose
10- 100 % CSBI + 25% N dose
11- 100 % CSBI + 50% N dose
12- 100 % CSBI + 100% N dose

Analytical procedures:

At harvest, maize yield and 100-grain weight were recorded. Maize grains quality such as protein, oil, ash, hulls, germ and endosperm % and maize grains mineral content were determined according to the methods outlined in A. A. O. A. C. (1980). While, carbohydrates % in grains were determined as described by Dubios *et al*. (1965).

All obtained data for both tested seasons were tabulated and subjected to the combined statistical analysis as described by Gomez and Gomez (1984).

RESULTS

Grain yield and 100-grains weight

Data in Table (1) indicate the effect of cyanobacteria inoculation in different rates (0, 25, 50 and 100% of the recommended dose (RD) in presence or absence of different levels of nitrogen fertilizer (25, 50, and 100% RD) on maize yield, maize 100-grain weight and protein, oil yields fed^{-1}.

Due to nitrogen effect, results reveal that the use of 100% N dose recorded the highest mean grain yield value of 32.864 ardab fed^{-1}. This mean value was significantly higher than those recorded by any of 25 and 50% N dose treatment.

For cyanobacteria inoculation (CSBI), data reveal that 100% RD CSBI gave significantly the highest mean grain yield value of 35.399 ardab fed^{-1} compared to the other utilized CSBI of 25 and 100% Rd rates.

For the interaction effect of nitrogen fertilization (A) x cyanobacteria inoculation (B), the treatment of 100% N x 100% CSBI gave significantly the highest grain yield of 37.434 ardab fed^{-1} compared to the other tested interaction treatments.

Owing to 100-grains weight, nitrogen fertilization at the level of 50% recorded significantly the highest mean value of 41.34g compared to the other mean value of 38.15 and 37.31 g for 100 and 25%N levels, respectively.

In case of the cyanobacteria inoculation effect, the use of 50% CSBI rate gained the highest mean value for 100-grain weight of 40.64 g. This mean value was significantly higher than 39.64 g (25% CSBI) while was not significantly different from that of 40.31 g recorded by the use of 100% CSBI rate.

However, all the treatments received cyanobacteria inoculation were significantly higher than that of control without cyanobacteria (38.87 g).

For the effect of interaction (A x B) on 100-grain weight, data revealed that 100% N x 100% CSBI gave the highest 100-grain weight of

44.30, which was not significantly different from the other values recorded by the other interactions except for all A x B interactions treatments of control (no CSBI) x any of 25, 50 and 100 % N, 25% N x 25% CSBI, % N, 100 % N x 25% CSBI, % N, 25% N x 50% CSBI, % N, 100 % N x 50 % CSBI, % N, 25% N x 100% CSBI, and % N, 100 % N x 100 % CSBI (Table 1).

Protein and oil yield

Protein and oil yield fed^{-1} obtained due to the inoculation with different rates of cyanobacteria (CSBI) in the presence and/or absence of different levels of nitrogen are indicated in Table (1).

Nitrogen fertilization at the rate of 100% N recorded significantly the highest mean values of both protein and oil yields fed^{-1}. The corresponding mean values were 463.77 and 241.10 kg fed^{-1} compared to the other obtained mean values corresponded to 25 and 50% N levels.

On the other hand, cyanobacteria inoculation at the rate of 100% gave significantly the highest mean values of 675.48 and 266.62 kg fed^{-1} for protein and oil yields, respectively, compared to control, 25 and 50% CSBI, respectively.

Due the interaction A x B, the treatment 100% N x 100% (CSBI) gave significantly the highest protein (675.0 kg fed^{-1}) and 280.9 kg fed^{-1} for oil yield compared to the other values recoded by the other treated interactions treatments (Table 1).

Effect of cyanobacteria inoculation on maize protein, oil, carbohydrates and ash percentages of maize grains

Protein and oil percentages of maize grains

Table (2) revealed that the mean protein percentage recorded due to nitrogen fertilization was 11.04 (25%N). This high mean protein % was significantly different from that of 10.45% recorded by 50% N treatment and was not for 10.08% recorded by 100%N treatment.

For cyanobacteria inoculation, the highest protein % of 13.63 was due to 100%N treatment. This high protein % was significantly higher than 10.76% (50% CSBI), while it was insignificantly different from that of 12.2% due to 25% CSBI treatment. However all inoculated treatments gave significantly protein % higher than uninoculated treatment (control).

In respect to the interaction effect between nitrogen fertilization (A) and cyanobacteria inoculation (B) on protein %, data in Table (2) showed no significant interaction response. However, the highest protein % of 14.26 was due to the interaction of 100% N x 50% (CSBI) treatment.

Owing to oil %, both nitrogen fertilization and the A x B interaction had no significant effect on maize grain oil %. Neverthless, the highest mean oil percentage of 5.36 was due to 50 % N level, while, the highest mean oil percentage of 5.54 was due to 50 % N x 100% CSBI interaction treatment.

On the other hand, cyanobacteria inoculation affected maize oil % significantly. This significant effect was due to all inoculation treatments compared to the control one received no inoculation. However, the highest mean maize grains oil % of 5.36 was due to 100% CSBI treatment followed by 5.34 and 5.17% for 50% and 25% CSBI treatments, respectively (Table 2).

<u>T1-2</u>

Carbohydrates and ash% of maize grains

Maize grains carbohydrate % (Table 2) showed similar trend to that achieved by maize grains oil %, since both nitrogen fertilization and the interaction of A x B had insignificantly affected maize grain carbohydrate %. However, the highest mean carbohydrate% of 62.41 was due to 50 % N treatments followed by 60.09 and 59.87% for 25 and 50%N treatments, respectively. Meanwhile, the highest interaction effect (A x B) resulted in 69.68% carbohydrate due to 25% N x 25% CSBI treatment.

In the view of maize grain ash%, both cyanobacteria inoculation (B) and the interaction effect resulted from the combination between cyanobacteria inoculation and nitrogen fertilization showed no significant effect on maize grain ash %. However, the corresponding highest mean percentage ash % of 1.38and the percentage of 1.49 was due to 25% cyanobacteria and the interaction between 25% N and 25% CSBI treatment.

On the other hand, nitrogen fertilization only had affected significantly maize grain ash%. The highest mean ash% of 1.32 (100% N) was significantly higher than those of 1.27 and 1.22% for 25 and 50 % N

Effect of nitrogen fertilization and cyanobacteria inoculation on maize grain hulls, grem and endosperm%

Data in Table (3) revealed that nitrogen fertilization levels exhibited different influences on maize grain hulls, grem and endosperm percentages. Herein, 25%N gave the highest significant mean hulls % (9.65), while 50 and 100%N recorded the highest mean grem and endosperm percentages, respectively compared to those achieved by the other tested nitrogen levels. The corresponding highest mean percentages were 8.60 and 83.82.

Same fluctuation recorded due to nitrogen levels was observed for cyanobacteria inoculation rates against hulls, germ and endosperm percentages. Since, 100 %, 25% and 50% cyanobacteria inoculum rates recorded the highest corresponding mean percentage of 9.90, 7.90 and 83.75 for maize grain hulls, germ and endosperm, respectively, compared to those obtained due to the other cyanobacteria inoculation treatments.

Due the interaction effect of nitrogen levels (A) x cyanobacteria inoculation rates (B), data revealed that 25% N x 100% cyanobacteria, 50% N x 25% cyanobacteria and 100%N x 50% cyanobacteria interaction gave significantly the highest corresponding percentage of 11.41, 9.47 and 84.24 for maize grain hulls, germ and endosperm, respectively, compared to those recorded by the other interaction treatments.

Data in Table (4) indicate the effect of both nitrogen fertilization and cyanobacteria inoculation each solely or both in combination on mineral content of maize grains. Nitrogen fertilization at the level of 100%, 50%, 50%, 50%,50% and 50% gave the highest mean values of Mg, Na, Zn, Mn, Fe, Ca and K for maize, respectively. The corresponding content mean values were 0.385, 0.863, 0.44, 0.027, 0.136, 0.232 and 5.340 mg/100g.

Due to cyanobacteria inoculation rates, the highest mean values of 0.376(Mg), 0.960(Na), 0.049(Zn), 0.026 (Mn), 0.147(Fe), 0.174(Ca) and 5.747(K) mg/100g were corresponded to cyanobacteria inoculation rates of 100%, 50%, 100%, control, 50%, 100% and 50%.

T3

T4

Owing to the interaction A x B treatments, the highest values of 0.546(Mg), 1.348(Na), 0.055(Zn), 0.034(Mn), 0.169(Fe), 0.298(Ca) and 7.899 (K) mg/100g were due to the interaction of 100%A x 25%B, 100%A x 25%B, 50%A x 100%B, 50%A x 50%B, 25%A x control without cyanobacteria and 50% A x 50%B, respectively.

DISCUSSION

Obtained results indicated that the use of 100 %N combined with 100% cyanobacteria inoculation gave the highest significant grain yield (37.434 ardab fed^{-1}) and oil yield (280.90kg fed^{-1}), while the use of 50%N combined with 50% cyanobacteria inoculation achieved the highest significant protein yield (679.31 kg fed^{-1})and the highest significant 100-grain weight (44.309). The N_2- fixed by *Nostoc* sp. in association with wheat is taken up by the plant and supports its growth, improving grain yields and grain quality (Gantar *et al.,* 1995). Inoculation with the nitrogen fixing *Azospirillum* to wheat as biofertilizer combined with ½ recommended N dose increased significantly grain and straw yields and NPK- uptake by grains and straw, improved the grain quality (protein, dry gluten and flour extract percentages) compared to the control without inoculation (AL- Kassas, 2002). Inoculation with cyanobacteria combined with $^1/_4$ N dose increased significantly both wheat protein and carbohydrate contents over the control treatment without inoculation and the full nitrogen dose treatments (Gaffar and AL-Kassas, 2005). Such results were confirmed by Kaushik (2000) who reported that cyanobacteria inoculation to maize crop even in presence of high level of nitrogen up to 120 kg N ha^{-1} recorded higher yield compared those receive 50 and 100 kg N ha^{-1} in combination with cyanobacteria inoculation. The author added that the recommended high levels of nitrogen fertilizer complemented with cyanobacteria inoculation resulted in yield significantly higher than that without cyanobacteria inoculation. Such increases in the yield and yield components are due to that cyanobacteria excrete growth promoting substances, which prominently well identified as vitamins (vitamin B_{12}, ascorbic acid folic acid, nicotinic acid and pantothenic acid), auxins (indole acetic acid and 3-methylindol acetic acid, sugars, polysaccharides, peptides and lipo-peptides. There growth promoting substances lead to improve the yield and the technological grain quality such as protein, oil and carbohydrates contents, huls, germ and endosperm percentages.

Cyanobacteria inoculation increased the soil organic matter which in turn enriched the soil microbial biomass and enzyme activity which in turn increased the available macro and micronutrients in the soil to plant. This availability of the nutrients in soil leads to improve the grain quality and increased their contents from such nutrients, i.e., Mg, Ca, Zn, Mn, K, P and Fe (El-Shahat, 2007).

Mandal *et al*. (1999) reported that cyanobacteria inoculation to cereal crops resulted in increasing in solubilizing phosphate hindered in the soil due to its alkalinity and contributes in saving nitrogen in soil because its ability to fix the atmospheric nitrogen. The abundance of these elements in soil due to cyanobacteria inoculation resulted in increasing the yields of cereal crops and enhances its mineral contents as well as their quality. However, the obtained results in this work, may lead to suggest that inoculation with cyanobacteria to maize may be advisable even with high of nitrogen because of their natural growth promoting substance excreted substance which increase the yield and improve its technological quality and mineral contents achieving good nutritional value for maize as animal and human food.

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أثرالتلقيح بالسيانوبكتريا على محصول الذرة والخواص التكنولوجية للحبوب

ابو العلا رجب القصاص
معهد بحوث تكنولوجيا الغذاء - مركز البحوث الزراعية- الجيزة- مصر

اجريت تجربتين حقليتين بمزرعة خاصة بمنطقة سقارة – البدرشين- محافظة الجيزة فى موسمى صيف 2005/2004 وذلك لدراسة أثر التلقيح بالسيانوبكتريا بمعدلات مختلفة منفردة أوبمصاحبة مستويات مختلفة من النيتروجين على محصول الذرة والصفات التكنولوجية للحبوب وقد كانت أهم انتائج ما يلى:-

1- أدى التلقيح بالسيانوبكتريا الى تشجيع نمو الذرة وكذا محصوله وصفاتة التكنولوجية.
2- سجلت أعلى قيم لمكونات محصول الذرة استجابة للمعاملة 100% نيتروجين + 100% سيانوبكتريا.
3- ان التلقيح بالسيانوبكتريا بمعدل 100% + 100% نيتروجين اعطى أعلى محصول للفدان لكل من البروتين والزيت.
4- ان التلقيح بالسيانوبكتريا بمعدل 50% + 100% نيتروجين اعطى أعلى نسب مئوية لكل من البروتين والزيت والكربوهيدرات , بينما تحققت أعلى نسبة مئوية للرماد استجابة للمعاملة 25% سيانوبكتريا + 50% نيتروجين.
5- ان التلقيح بالسيانوبكتريا بمعدل 25% سيانوبكتريا + 50% نيتروجين اعطى أعلى نسب مئوية لكل من الـ germ , hulls بينما النسبة المئوية للاندوسبيرم تحققت مع المعاملة 50% سيانوبكتريا + 50% نيتروجين.
6- أدى التلقيح بالسيانوبكتريا الى زيادة محتوى الحبوب من العناصر المعدنية.