

EFFECT OF CHEMICAL AND BIOFERTILIZERS ON NITROGEN STATUS AND AMINO ACIDS IN RICE GRAINS

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Abstract

The application of blue green algae and *Azospirillum* Significantly increased the total nitrogen content of rice grains . the highest increase (7.01%) over the control was observed in the treatment which received the bioinocula of imported algae strain and *Azospirillum*.

The application of 20 and 40 kg N/fed. of urea led to highly significant increase in the total nitrogen content of rice grains. The highest increase (58.11%) over the control was observed in the treatment having 40Kg N/fed.

The amino acids content of rice grains were also affected by bioinoculation and chemical fertilization. The inoculation with alga and *Azospirillum* and fertilization with 20 kg N/fed., Showed high amounts of both essential and nonessential amino acids in the grains.

INTRODUCTION

Blue green algae are one of the first microorganisms which had been known to fix atmospheric nitrogen in flooded rice soils since the work of De (1939).

The importance of blue green algae in biological nitrogen fixation was early

emphasized by many investigators, (Kuda and Yamaguchi 1956; Watanabe 1956; Watanabe and Yamamoto 1971; and Roger and Kulasooriya 1980). They discussed the effect of blue green algae on nitrogen content of rice plants in different types of soils. However, increasing the costs of chemical nitrogen fertilizers had drawn the attention to the biological nitrogen sources as nitrogen is mostly a limiting factor for crop production.

The role of *Azospirillum* spp. in nitrogen fixation was also studied. These organisms are nitrogen fixing bacteria which live on or inside plant roots (Yoshida 1973; Dobereiner 1976; Neria 1977; Smith *et al.* 1977; Monib *et al.* 1982; Line *et al.* 1983 and Okon 1982). The capacity of this group of microorganisms to fix atmospheric nitrogen and produce growth promoting substances had been studied by many investigators (Eid *et al.*, 1986, and Mahgoub *et al.* 1990).

Present study was carried out to reveal the effect of some biofertilizers of different species of blue green algae with or without *Azospirillum* on nitrogen content and amino acids of rice grains as compared to chemical nitrogen fertilizer i.e. urea

MATERIALS AND METHODS

A field experiment was carried out at Sakha experimental station, Kafr El-Shikh governorate in clay loam soil, during the season of 1987 to compare the effect of inoculation of rice plants with two species of blue green algae and *Azospirillum brasilense* No. 40 as compared to the effect of chemical nitrogen fertilizer (Urea 46.5%N) on the total nitrogen content and amino acids of rice grains. This experiment was performed in 84 plots (2.8 m x 5m) corresponding to 21 treatments with 4 replicates.

Inoculants

1 - Blue green algae : two strains of blue green algae were used:

- a. Local strains of *Nostoc muscorum* (BGA1).
- b. Imported strains of *Nostoc* sp. from International Rice Res. Instit. (IRRI), Manila, Philippines (BGA2).

2 - *Azospirillum*

The strain of *Azospirillum brasilense* No. 40 was used.

Growth media:

The following medium described by Watanabe (1951) and as modified by El-Nawawy *et al.*, (1958) was used (g/L) for growing blue green algae; K_2HPO_4 , 0.3; $MgSO_4$, 0.2; K_2SO_4 , 0.2 and micronutrients solution 1 ml/L. The composition of micronutrients solution (g/l) was H_3BO_4 2.8; $MnCl_2$ 1.8; and molybdic acid 0.02 while the pH was adjusted at 7.5.

Preparation of algal inoculant

Algal inoculant was prepared according to the method of Venkatraman (1979). The inoculant was sprayed at a rate of 10 kg/fed. after 10 days of transplanting of rice seedlings.

Preparation of azospirillum inoculum

Azospirillum inoculum was prepared according to the method of Okon (1982).

Chemical fertilizers : (Urea 46.5% N)

To promote the growth of rice seedling, very low amounts of urea were added to the nursery. Nitrogen fertilizer in the field was added as urea nitrogen at rates of 20 and 40kg N/fed. at two equal doses during rice growth. The first dose was added before tillering stage and the second dose was added one month later.

Chemical determinations

Total nitrogen content in rice grains was determined according to A.O.A.C. (1980) by the micro - Kjeldahal method.

Amino acids contents in rice grains were determined qualitatively and quantitatively using thin layer chromatography technique (Keresi and Chlamers 1984)

Statistical analysis

Was carried out according to Sendecor and Cochran (1980)

RESULTS AND DISCUSSION

Table 1. Effect of blue-green algae and /or Azospirillum in the presence of urea-N on the total nitrogen content of rice grains.

Urea - N kg/fed.	Control	BGS (1)	BGS (2)	Azospirillum	BGS (1) + Azospirillum	BGS (2) + Azospirillum	BG(1) + BGA(2) + Azospirillum	Mean
0	5.002	4.25	4.528	4.455	4.740	5.942	5.356	4.921
20	6.397	6.139	5.907	5.303	5.998	6.391	7.80	6.230
40	7.909	6.723	7.134	6.813	7.044	7.780	9.406	7.601
Mean	6.436	5.762	5.856	5.523	6.060	6.704	7.410	6.250
L.S.D.		0.05		0.01				
(A)		1.1686		1.0303				
(B)		0.7650		--				
(C)								

BGA (1) Nostoc muscorum.

BGA (2) Nostoc sp. imported from Inter. Rice Res. Inst. (IRRI)

A biofertilization treatment.

B Nitrogen fertilization treatment.

C Interaction between bio- and nitrogen fertilization treatments.

The effects of algal and /or *Azospirillum* inoculant as compared with chemical fertilizer on the total nitrogen content of rice grain are presented in Table 1. It is obvious that the use of biofertilizer alone without urea nitrogen didn't cause any increase in total nitrogen content except in the case of the mixed inoculant of any strain of BGA and *Azospirillum*. The highest increase over the control of 7.07% was observed in the treatment which received the bio-inoculum of the imported algal strain combined with *Azospirillum*.

The application of urea N at rates of 20 and 40kg /fed. had led to highly significant effect on the total nitrogen content of rice grains. The highest increase over control of 58.11% was observed in the treatment which received 40kg N/fed. in absence of biofertilizer.

On the other hand, the interaction between mineral fertilizer as urea nitrogen and the used biofertilizer led to a nonsignificant effect on the total nitrogen content of rice grains. It seems that the use of biofertilizer in the presence of chemical nitrogen may cause an adverse action between bio and chemical fertilizer since the urea produces ammonia which may be considered as repression factor in the pathway of nitrogen fixation (Rai *et al.* 1980).

In general, the application of either biofertilizer alone or urea nitrogen alone significantly increased the total nitrogen content of rice grains. This is in agreement with Abou El-Fadl *et al.* (1970) and Goyal *et al.* (1987).

Amino acids content of rice grains was estimated to compare the effect of biofertilizer on both essential and nonessential amino acids in presence of 20 and 40kg N/fed. Samples which contained high percentage of nitrogen content were chosen. The data in Table 2, showed that the essential amino acids such as leucine, lysine, methionine and valine and nonessential amino acids such as aspartic, glutamic, serine, tyrosine and cysteine were found in relatively high amounts as compared with the treatments which received the same inocula and 40 kg N/fed.

The rice protein does not contain enough lysine, threonine and methionine. Therefore for proper protein nutrition, the food should be supplemented with such amino acids for those who consume large amount of rice in their food. This is in agreement with Juliano *et al.* (1964 and 1973)

Table 2. Effect of algal and/or bacterial inoculation on the percentage of amino acids of rice grains.

Nitrogen level	20 kg N/fed.	40 kg N/fed.
	BGA (1) + BGA (2) + Azospirillum	BGA (1) + BGA (2) + Azospirillum
Amino acids (%)		
Essential amino acids		
Arginine	0.52	0.45
Histidine	0.32	0.32
Iso-leucine	0.25	0.30
Leucine	0.30	0.20
Lysine	0.29	0.16
Methionine	0.34	0.20
Phenyl alanine	0.14	0.12
Threonine	0.22	0.22
Tryptophan	0.23	0.25
Valenine	0.84	0.20
Non-essential acids		
Aspartic	0.35	0.39
Glutamic	0.43	0.40
Glycine	0.10	0.21
Proline	0.27	0.30
Seine	0.40	0.22
Tyrosine	0.59	0.15
Cystine	0.52	0.50

REFERENCES

- 1 . Abou - El - Fadl, M.; M.R Hamisaa; A.S. El - Nawawy and M.S. Abdel -Aziz, (1970). Evaluation of the blue green alga *Tolypothrix tenuis* as nitrogen source for rice plants. The Agric., Conf. of Rice. Cair (in arabic).
- 2 . A.O.A.C. (1980). Official methods of analysis of the association of official analytical chemists. 12th ed. Washington, D.C.
- 3 . De P.K. (1939). The role of blue green algae in nitrogen fixation in rice fields . Proc. Soc. (London), 127 (8) 121 - 129.
- 4 . Dobereiner, J. and J.M. Day. (1976). Associative symbiosis in tropical grasses; characterization of microorganisms and dinitrogen fixing sites. In proceeding of the First International Symposium on Nitrogen Fixation. 3-7 June 1974 Pullman, WA. Edited by W. E. Newton and C.G. Nyman. Washington State Univ. Press, Pullman, pp. 518 - 588.
- 5 . Eid, M.A.; A.M. Abdel Shafi; E.H. Bedawi; R.A Mitkess and M.N. Alla El-Din, (1986). The trace for significant relations in the plant N-fixing bacteria association . Proc VI. Conf. Microbiolog , Cairo, Vol. 1 part II paper no . 14.
- 6 . El-Nawawy, A.S., A.S.H. Lotfi and M. Fahmy, (1974) Studies on the ability of some blue -green algae to fix atmospheric nitrogen and their effect on growth and yield of rice plants . Agric. Res. Rev. 36 (308 - 320).
- 7 . Goyal, S.K. (1987) Algal - Biofertilizer-an appraisal. J. Maharshine Agric . Univ. 12 (1) : pp. 11-19.
- 8 . Goyal , S.K., B.M. Sharma and R.S. Gipta, (1984). Algal flora of rice field soils of Jume and Kashmir state. Phykos 23-59.
- 9 . Juliano, B.O.; A.A. Antonion and B.V. Esmara, (1973). Effect of protein content on the distribution and properties of rice protein . Journal of the Science of food and Agric . 24 (3) : 295 - 306.
- 10 . Juliano, B.O., L. Esler Albano and B. Gloria, (1964). Variability in protein content , amalyse content and alkali digestibility of rice varieties in Asia Philip-pine Agric. 48: 234. C. F. Rice Chemistry and technology AACC Monograph Series No. 4 (1972).
- 11 . Kerese , I. and R.A. Chalmers, (1984). Methods of protein analysis . Mc Grow - Hill , New York, London.
- 12 . Kuda, A. and M. Yamaguchi, (1956). Distrbution of nitrogen fixing microorgani-smis in paddy soils in Japan. Tran 6th inter.

- 13 . Lin, W., W. Dokon and R.W.F. Hardy.(1983). Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with *Azospirillum brasilense*. Appl. Environ. Microbiol. 45, 1975 - 1979.
- 14 . Mahgoub, G. M.A., H. Esaad Bedaiwi; M.A. Younis and Y.Y.Sh. El-Sherbieny, (1990). Effect of bacteria inocula and nitrogen fertilizer on maize plants. Annals of Agric. Sci., Moshtohor, 29 No 2.
- 15 . Monib , M., N.A. Hegazi, M. Eid and El-Sayed Shoker (1982) Yield increase of grain sorghum (*sorghum vulgare*) after inoculation with nitrogen fixing *Azospirillum*. The first OAU/STRC inter-African Conf. on Bio-fertilizer, 22-26 March.
- 16 . Okon, Y. (1982). Methods for growing *Azospirillum* in new culture Appl. Environ. Microbiol., 33, 86-88.
- 17 . Rai, A.N., P. Rowell, and D.P. Stewart. (1980). Ammonia assimilation and N₂-ase regulation in the lichen *New phytolo.* 85:656.
- 18 . Roger, p.A. and S.A. Kulasooriya, (1980). Bule -green algae and rice P.13-14. IRRI, Mainilla, Philippines.
- 19 . Smith, R.L., J.H. Bouton, S.C. Sohank and K.H. Ouesenberry, (1977). Yield increase of tropical grain and forage grasses after inoculation with *Spirillum lipoferum* in Florida. In Biological Nitrogen Fixation in farming systems of the tropics. ed. by A. Ayanaba, and P.J. Dart, (19) pp. 807 - 811. John Wiley and Sons tns., New York.
- 20 . Snedecoor, G.W. and W.G. Cochran, (1980). atistical Methods 7th Ed. Iowa State Univ. press Amer. Iowa U.S.A. pp. 233-237.
- 21 . Venkatraman, G.S. (1979). Algal inoculation of rice fields . In Nitrogen and Rice Pub. IRRI Philippines.
- 22 . Watanabe, A. (1951) . Production in culture solution of some amino acids by the atmospheric nitrogen fixing bule green algae Arch. Biochem.Biphys . 34, 50.
- 23 . Watanabe, A. and Y. yamamoto, (1967). Heterotrophic nitrogen fixation by *Anabaena circualins* Nature. London. 27: 237.
- 24 . Yoshida, T. (1981). Fundamentals of rice crop. Science, 267. The International Rice Res. Inst., Los Banos, Phillippines.
- 25 . Yoshida, T. and R.R. Aroajaa, (1973). Nitrogen fixing activity in upland and flooded rice fields. Soil Sxince Soc. American Proc. 37 (1) : 42.

تأثير التسميد الكيماوى والحيوى علي محتوى حبوب الارز من النتروجين الكلى للاحماض الامينية

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أجريت تجربة حقلية في محطة البحوث الزراعية بكفر الشيخ في موسم ١٩٨٧ لمعرفة تأثير التسميد الكيماوى والحيوى علي محتوى حبوب الارز من النتروجين الكلى والاحماض الامينية.

اوضحت النتائج ان تلقيح الارز سواء بالطحالب الخضراء المزرقه او الازوسبيرلا لم يؤدي الي زيادة معنوية في محتوى الحبوب من النتروجين الكلى في حين ان استخدام السماد النتروجينى ادى الي زيادة معنوية وخاصة عن استخدام ٤٠ كجم نتروجين / فدان . وبالنسبة لتأثير هذه المعاملات على محتوى حبوب الارز من الاحماض الامينية فقد اوضحت النتائج ان التسميد الحيوى بخليط من السلالتين المستوردة والمحلية من الطحالب الخضراء المزرقه والازوسبيرلا تسبب في زيادة النسبة المئوية للاحماض الامينية الاساسية والغير اساسية في حبوب الارز عند استخدام معدل ٢٠ كجم سماد نتروجين للفدان .