Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants

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ABSTRACT

In this research cyanobacteria (Blue-Green Algae, BGA) were isolated, identified, multiplied and used as an inoculum in pot rice experiment. The pH, moisture and algal population were measured in four seasons. The highest and lowest pH (6.7, 6.2), moisture of soil (43%, 34%) and algal population (12, 20 Colony-Forming Units/50 ml on A and B medium and 4, 5 Colony-Forming Units/50 ml on A and B medium) were recorded in spring and winter, respectively. The only heterocystous cyanobacteria were found in soil samples identified as *Anabaena* with four species (*A. spiroides*, *A. variabilis*, *A. torulosa* and *A. osillarioides*). The germination of rice seeds treated with cyanobacteria was faster than control. The result of pot experiment were: increase of 53% in plant height; 66% in roots length; 58% in fresh leaf and stem weight; 80% in fresh root weight; 125% in dry leaf and stem weight; 150% in dry root weight; 20% in soil moisture; 28% in soil porosity and a decrease of 9.8% in soil bulk density and 4.8% in soil particle density. There were significant differences ($P < 0.05$) in pot treated with BGA as compared with control.

Keywords: cyanobacteria; inoculation; rice growth

Cyanobacteria (Blue-Green Algae) are one of the major components of the nitrogen fixing biomass in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers (Malik et al. 2001). The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water. These problems can be tackled by use of biofertilizers (Choudhury and Kennedy 2005, Rai 2006). Biofertilizers, more commonly known as microbial inoculants, include bacteria (Azotobacter), algae (Blue-green algae) and mycorrhizal fungi; they are natural, beneficial and ecological, and they provide nutrients for the plants and maintain soil structure (Board 2004). Over the past six decades, reports have been published on the use of cyanobacterial inoculants (algalization) to enhance biological N_2 fixation in wetland rice fields. Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (Song et al. 2005). The acts of these algae include: (1) Increase in soil pores with having filamentous structure and production of adhesive substances. (2) Excretion of growth-promoting substances such as hormones (auxin, gibberellin), vitamins, amino acids (Roger and Reynaud 1982, Rodriguez et al. 2006). (3) Increase in water-holding capacity through their jelly structure (Roger and Reynaud 1982). (4) Increase in soil biomass after their death and decomposition. (5) Decrease in soil salinity. (6) Preventing weeds growth. (7) Increase in soil phosphate by excretion of organic acids (Wilson 2006). Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no cost (Mishra and Pabbi 2004). The paddy field ecosystem provides a favorable environment for the growth of cyanobacteria with respect to their requirements for light, water, high temperature and nutrient availability. This could be the reason for more abundant cyanobacteria growth in paddy soils than in upland soils (Roger and Reynaud 1982, Kondo and Yasuda 2003). The abundance of cyanobacteria in rice fields has been reported in numerous papers since Fritsch's accounts (Fritsch 1907a, b). Culture studies were introduced by

Bannerji (1935) and the importance of blue-green algal nitrogen fixation in helping to maintain fertility of rice fields was first recognized by De (1939). Thereafter Watanabe and Konishi (1951), Venkataraman (1972) and Roger and Reynaud (1982) studied further on this base. Beneficial effects of cyanobacterial inoculation were also reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce (Thajuddin and Subramanian 2005). In this study we started research in this case since, unfortunately, with attention to rice production in more than eighteen provinces there is no record in Iran country. The aim of this research is pointing out the role of cyanobacteria as a biofertilizer in rice plants in Iran.

MATERIAL AND METHODS

Soil samples were collected from the depth of 0–10 cm on a paddy field in Azbaram village (6 km to the East of Siahkal in the Gillan province of Iran) in the second month of each season and pH and moisture of soil were measured in four seasons of year (Hayes 1981).

Isolation of cyanobacteria. The dilutions of water and soil 10^{-2} and 10^{-3} were prepared. Two nitrogen-free media, Tretykova (A Medium, Table 1) and modified Benecke's solutions (B Medium, Table 1) (Kaushik 1987) were prepared and solidified with 15 g agar. 1 ml from 10^{-2} and 10^{-3} dilutions was added to plates. Then plates were incubated at 26–30°C and 12/12 h photoperiod for 30–35 days in incubator set (Akhtarian, ISH 632/1, Tehran, Iran) (Kaushik 1987). For purification, identification and multiplication of colonies, a part of colony was removed by a loop and transferred to a new plate. After three weeks, colonies were transferred to liquid media, shaken at 100 rpm (Schiefer and Caldwell 1982), at 26–30°C and 12/12 h photoperiod in a shaker (Akhtarian, ISH 632/1, Tehran, Iran) for 14 days (Venkataraman 1972).

Table 1. Tretykova (A medium) and modified Benecke's solution (B medium)

Materials	A medium (g/l)	B medium (g/l)
K_2HPO_4	0.2	0.2
$MgSO_4.7H_2O$	0.2	0.2
$CaCl_2$	0.05	0.1
$NaHCO_3$	0.2	–
Agar	15	15
$FeCl_3$ (1%)	–	2 drops
KNO_3	–	0.2

Seed germination. One hundred rice seeds were soaked with water and 0.1 g wet cyanobacteria in three containers separately. The same experiment was carried out as control without algae. After 20 days, seedlings height and roots length were measured.

Pot culture. The rice seeds were soaked in water for 20 days. Then 5 seedlings with the height of 2 cm were transferred to pots. One week before and one week after transferring the seedlings, 1 g of mixed wet algal inoculum was added to the soil. After three weeks, height of plant, roots length, fresh and dry weight (Meloni et al. 2004) were measured. Moisture (Hayes 1981), bulk density, particle density and porosity of soil (Blake and Hartage 1986) were also recorded.

Statistical analysis. An analysis was performed with independent Samples t-test and significant differences were surveyed at 0.05 level.

RESULTS AND DISCUSSION

Moisture, pH and algal population were calculated and the results that are given in Table 2 show that there is a positive correlation between moisture and algal population because maximum and minimum moisture (43 and 34%) and algal population (12 CFU per 50 ml on A medium and 20 CFU per 50 ml on B medium and 4 CFU per 50 ml on A medium, 5 CFU per 50 ml on B medium) were observed in spring and winter, respectively. There was no difference in pH in different seasons, so moisture was the major reason for the increase in algal population in spring. The results obtained in the first part of this work showed that the spring season is suitable for isolation of cyanobacteria, because the highest cyanobacteria population was recorded in spring season compared to other seasons (Figure 1). Therefore, we can select spring to have sufficiently high biomass of cyanobacteria to produce inoculum. The only genus of heterocystous cyanobacteria was found in soil samples identified as *Anabaena* with four species: *A. spiroides*, *A. variabilis*, *A. torulosa* and *A. osillarioides* (Figure 2). Species of *Anabaena* were identified by the key of Desikachary (Desikachary 1959). In seeds soaked with water and cyanobacteria, germination was observed after 2 days and seedlings height of 7 cm and roots length of 3 cm were recorded; in control, however, germination was

Table 2. The pH and moisture of soil and population of cyanobacteria in four seasons of the year

Seasons	Moisture (%)	pH	Colony-Forming Units/ 50 ml on A medium[a]	Colony-Forming Units/ 50 ml on B medium[b]
Spring	43	6.7	12	20
Summer	39	6.6	10	15
Autumn	36	6.3	7	10
Winter	34	6.2	4	5

[a]A medium = Tretykova; [b]B medium = modified Benecke's solution

observed after 5 days and seedlings height of 4 cm and roots length of 0.5 cm were recorded after 20 days (Table 3, Figure 3). Venkataraman and Neelakantan (1967) showed that the production of growth substances and vitamins by the algae may be partly responsible for the greater plant

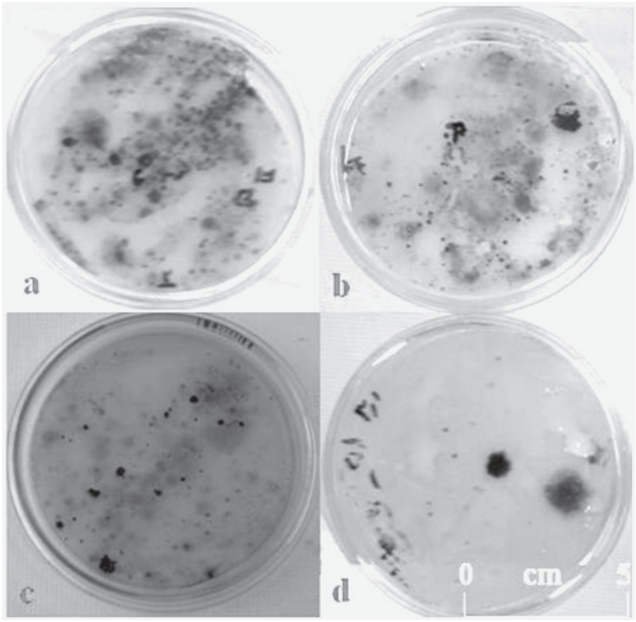

Figure 1. Population of cyanobacteria in: (a) spring, (b) summer, (c) autumn, (d) winter

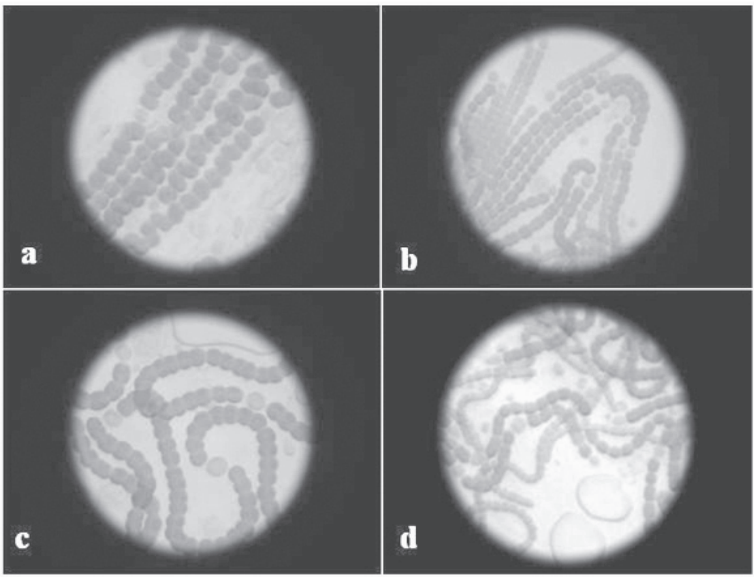

Figure 2. (a) *A. spiroides*, (b) *A. osillarioides*, (c) *A. torulosa*, (d) *A. variabilis*

Table 3. Effect of cyanobacteria on germination of rice seeds

Sample	Control	Treatment
Seedlings height (cm)	4	7
Root length (cm)	0.5	3
Period of time germination (days)	20	10
Germination (%)	42	90

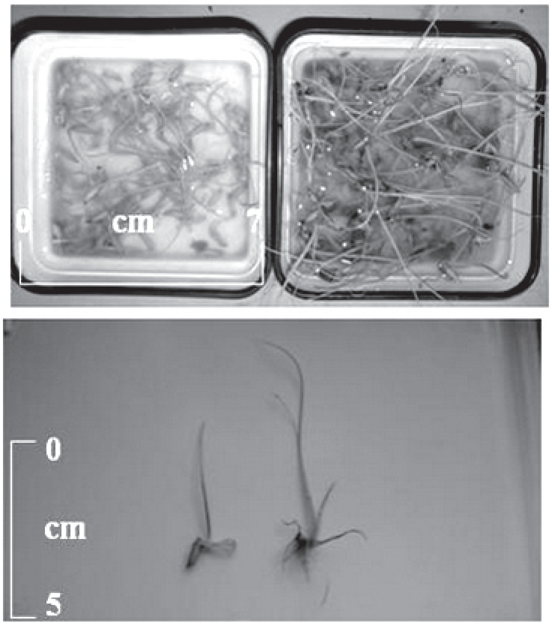

Figure 3. Rice seeds soaked with water (left), rice seeds soaked with water and cyanobacteria (right)

yield. This evidences that hormonal effects have come from treatments of rice seedlings with algae. In pot culture, 53% increase in plant height, 66% increase in plant roots length, 69% increase in plant fresh weight, 137.5% increase in plant dry weight, 20% increase in soil moisture, 28% increase in soil porosity, 9.8% decrease in soil bulk density, and 4.8% decrease in soil particle density were determined in comparison of inoculated variant to control. Significant differences were observed in treatments as compared to control (Table 4, Figure 4). Venkataraman and Neelakantan (1967) also observed 89.5% increase in root dry weight of rice inoculated with algae over control after 30 days of rice root inoculation. In addition, Sankaram (1967) surveyed physical properties of the soil as influenced by algal inoculation and Aiyer et al. (1972) surveyed chemical properties of the soil as influenced by algal inoculation and they recorded positive effects of algae in soil. Rice inoculation with heterocystous cyanobacteria isolated from Iran rice field showed that algae have positive effects for rice planted *in vitro*. Besides *in vitro* they modify physical and biological properties of soils in ways which are beneficial to the rice and soil. Because of chemical fertilizers extensively used throughout most of agricultural Asia (Asia is the world's largest user of chemical fertilizers, consuming around 40% of the global total each year) and problems caused by using chemical fertilizers, organic fertilizers, biofertilizers and other microbial products are viable components of a healthy and pleasant ecosystem. In conclusion, cyanobacteria can play a crucial role in soil fertility as nitrogen-fixing microorganisms. In today's world, considerable progress has been made in the development of cyanobacteria-based biofertilizer technology. It was demonstrated that this

Table 4. Effects of cyanobacteria on plant and soil. Analysis was performed with independent Samples *t*-test

Sample	Control	Treatment	Ratio Control/ Treatment (%)
Plant height (cm)	13*	20*	65
Roots length (cm)	3*	5*	60
Weight of fresh leaf and stem (g)	0.17*	0.27*	62
Weight of fresh root (g)	0.26*	0.47*	55
Weight of dry leaf and stem (g)	0.04*	0.09*	44
Weight of dry root (g)	0.06*	0.15*	40
Moisture (%)	25*	30*	83
Bulk density (g/ml)	1.68*	1.53*	109
Particle density (g/ml)	1.95*	1.86*	104
Porosity (%)	14*	18*	77

*significant difference at 0.05 level ($P < 0.05$)

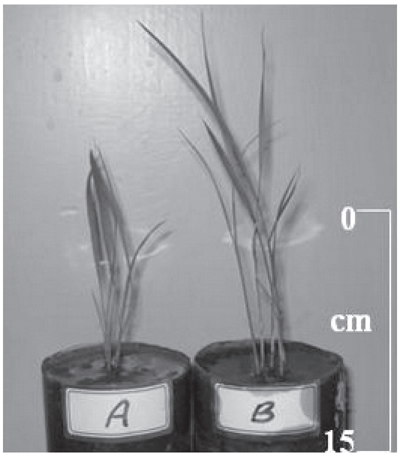

Figure 4. A – control pot (without algae); B – treatment pot (with algae)

technology can be a powerful means of enriching the soil fertility and improving rice crop yields. However, the technology needs to be improved further for better exploitation under sustainable agriculture systems. Extensive field studies aimed at developing region-specific high quality inoculum are also needed.

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Rice yields decline with time, as noted by Ladha et al. [5] in long-term experiments carried out in a rice-rice intensive system under tropical conditions, and this is related by the authors to the decrease of the physiological N use efficiency. Long-term effects of urea and green manure on rice yield and nitrogen balance were also verified in temperate conditions [6] where the nitrogen use efficiency (NUE) was particularly low.Â Moreover, to use the fern as a biofertilizer in conventional farming, not just in organic farming, some resistance to the most popular herbicides is required. In other words, the fern should reach a good growth rate during spring, with a high percentage of nitrogen, and should be quite resistant to the most common herbicides used in the rice areas.