



Original Research Article

Cyanobacterial Consortium in the Improvement of Maize Crop

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ABSTRACT

Keywords

Cyanobacteria,
Zea mays,
Germination,
Growth,
Nitrogenase

The effect of cyanobacterial isolates on growth performance and yield status of *Zea mays* was studied. Fifteen cyanobacterial isolates were used individually and in combination to study the effect of their consortium on the growth performance and grain yield of Maize. The growth performance was measured in association with cyanobacterial isolates individually and in combination. The use of combination of all these cyanobacterial isolates in consortium with Maize crop showed significant increase in percentage of germination, shoot and root lengths, total nitrogen content, Chl-a, Nitrogenase activity and grain yield. The results reveal that these cyanobacterial isolates could bring about positive results in the improvement of Maize crop and might prove to be an effective biofertilizers.

Introduction

Cyanobacteria are gram negative oxygenic photo synthesizers commonly found in fresh water, marine water and soil. They are considered as an important group of microorganisms capable of fixing atmospheric nitrogen. They have a unique potential to contribute to productivity in a variety of agricultural and ecological situations. Many cyanobacteria fix nitrogen under aerobic conditions in specialized cells called heterocyst which comprise 5-10% of cells in a filament (Gantar, 2000). Non-heterocystous cyanobacteria are also able to promote plant growth and can also be used as bio fertilizer.

Besides fixing atmospheric nitrogen, cyanobacteria play a major role in reducing

soil erosion because of ability to secrete polysaccharides that bind soil (Nayak and Prassana, 2007). They also control soil run off and increase soil organic matter content and in producing certain substances which enhance the growth of plants (Ordog, 1999).

Due to this important characteristic of nitrogen fixation, the utility of cyanobacteria in agriculture to enhance production is beyond doubt.

Cyanobacteria evolved very early in the history of life, and share some of the characteristics of gliding bacteria on one hand and those of higher plants on the other. Cyanobacteria can photosynthesize and fix nitrogen, and these abilities, together with

great adaptability to various soil types, make them ubiquitous. Cyanobacteria also have a unique potential to contribute to productivity in a variety of agricultural and ecological situations. Cyanobacteria have been reported from a wide range of soils, thriving both on and below the surface. They are often also characteristic features of other types of sub-aerial environment and many intermittently wet ones such as rice fields. Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no cost. Nitrogen fixation in cyanobacteria is brought about by a high molecular weight, oxygen labile, metalloprotein enzyme known as Nitrogenase. Nitrogenase reduces molecular nitrogen to ammonia in presence of hydrogen.

Many studies have been reported on the use of dried cyanobacteria to inoculate soils as a means of aiding fertility, and the effect of adding cyanobacteria to soil on rice yield was first studied in the 1950s in Japan. The term 'algalization' is now applied to the use of a defined mixture of cyanobacterial species to inoculate soil, and research on algalization is going on in all major rice producing countries. The average of the results from all these studies has shown an increase in grain yield of 15-20% in field experiments. It has been suggested that the cyanobacteria introduced as a result of algalization can establish themselves permanently if inoculation is done consecutively for 3-4 cropping seasons. The basic method of mass production involves a mixture of nitrogen fixing cyanobacteria in shallow trays or polythene lined pits filled with water kept in open air, using clean, sieved farm soil as a carrier material. To each pit 10 kg soil and 250 g single super phosphate is added and water is filled up to a height of 12–15 cm. Starter culture, a mixture of *Anabaena*, *Nostoc*, *Aulosira* and

Tolypothrix, is inoculated in each multiplication unit. Malathion (5–10 ml per tank) or carbofuran (3% granules, 20 g per tank) is also added to prevent insect breeding. In hot summer months, the cyanobacteria form a thick mat over the surface after 10–12 days of growth in open sun. The contents are allowed to dry and the dried flakes are collected, packed and used to inoculate rice fields. The basic advantage of this technology is that farmers after getting the soil based starter culture can produce the biofertilizers on their own with minimum additional inputs. An inoculum of 10–12 kg is considered sufficient to inoculate one hectare of paddy field 3-4 days after transplantation (Upasana Mishra and Sunil Pabhi, 2004).

In India, considerable progress has been made in the development of cyanobacteria based biofertilizers technology. It has also been demonstrated that this 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. It is important to obtain a much more detailed understanding of cyanobacterial population dynamics over the whole annual cycle in agriculture systems. Extensive field studies aimed at developing region specific high quality inoculums are also needed. Understanding the biology of drought resistant cyanobacteria may be useful in terms of extending this approach to dry crops.

The role of cyanobacteria as biofertilizer has largely been reviewed by Dola Bhowmik et al. (2010), Nayak and Prassana (2007) Ordog (1999), Haroun and Hussein (2003), Lakshmi and Annamalai (2008), Gallab and Salem (2001), Venkataraman (1972), Singh

(1961) and Relwani and Surahmanyam (1963), etc.

The role of N₂ – fixing cyanobacteria in maintenance of rice fields has been well documented all over world. In India the beneficial effects of cyanobacterial consortium with rice varieties have been demonstrated in a number of field locations (Gayatri and Anand, 2002; Goyal and Venkataraman, 1971; Manoj Kumar et al., 2013, etc.). An additional benefit of cyanobacterial consortium with crops is their capacity to secrete bioreactive substances such as auxins, gibberelins, cytokinins, vitamins, polypeptides, aminoacids, etc. which promote plant growth and development. The plant growth promotory effect on cow pea (*Vigna unguiculata*) using coir pith aqueous extract formulation of cyanobacterium *Phormidium* has been investigated by Pitchai Palaniappan et al. (2010).

Plant growth promoting substances produced by cyanobacterial consortium with crops have largely been reviewed by Karthikeyan et al. (2007), Prasanna et al. (2008), Fatima and Venkataraman (1999) etc. Effect of exopolysaccharides (EPS) produced by a consortium of cyanobacteria of three crops, wheat, rice and maize have been studied by Manu Arora et al. (2010).

The role of cyanobacterial consortium in the improvement of Maize crop has not been studied. Therefore the present study has been undertaken to evaluate the impact of heterocystous and non-heterocystous cyanobacterial consortium in the improvement of Maize crop (*Zea mays*) individually and in combination.

Materials and Methods

The present work was carried out in the Microbiology research laboratory, Dept. of

Biotechnology, College of Commerce, Patna from January 2014 to December 2014.

The Cyanobacterial flora were isolated from shallow water body in agricultural field and identified by relevant monographs (Desikachary, 1959; Tilden, 1910; 1937; Hegewald, 1976). The cyanobacterial samples were maintained in pure culture in BG11 medium in a growth chamber under 12/12hL/D cycle at 25±2°C and 1500 lux light intensity using fluorescent lamps. A consortium of fifteen cyanobacterial isolates viz., *Oscillatoria nigra*, *O. princeps*, *O. curviceps*, *Schizothrix vaginata*, *Lyngbya gracilis*, *Phormidium dimorphum*, *Calothrix clavata*, *Aulosora prolifica*, *Stigonema dendroideum*, *Nostoc muscorum*, *Nostoc calcicola*, *Anabaena oryzae*, *Scytonema varium*, *Gloeocapsa calcarea* and *Tolypothrix tenuis* was prepared by introducing equiproportional inocula (1ml each) in 400ml nutrient broth (pH 7.5) contained in 500ml flask.

Seeds of Maize (*Zea mays*) were initially surface sterilized with 0.1% mercuric chloride for five minutes and then washed with sterile distilled water. The seeds were then soaked separately in 16 days grown cyanobacterial cultures, in individual culture of fifteen isolates and in prepared consortium consisting of equiproportional inocula of each isolate. The seeds of these three species were then sown in petriplates containing moist filter paper. The seeds were found to germinate on the third day and after three days the percentage of germination was recorded.

A total of 51 Petri (15x3 for treatment with individual culture; 1x3 for treatment with cyanobacterial consortium and 1x3 for untreated seeds, considered as control) was used. Seeds of maize were surface sterilized with 0.1% HgCl₂. Twenty five seeds of each crop per plate were used for different

treatment. Response of different crops under various treatments was assessed as % seed germination

One week after germination the seedlings of were transferred to flasks containing BG11±N medium inoculated with fifteen isolates of cyanobacteria. The seedlings of all the three varieties were also transferred separately to flasks containing pure culture of cyanobacterial isolates. The seedlings were introduced into a disposable fin tip of the automatic pipette and with the help of a small hole in a thin plastic sheet; the tip with the seedling was inserted into the flask. One week after transfer, the growth of the seedlings in terms of root and shoot length were measured and the growth, Nitrogenase activity and total nitrogen content of the free living and associated cyanobacteria were determined.

Growth was determined by measuring the chlorophyll-a content. by the method suggested by Mackinney (1941). Nitrogenase activity was assayed in cells of free- living cyanobacterial cultures and cultures associated with the roots plants by the acetylene reduction technique of Stewart et al. (1967).

In order to measure Nitrogenase activity approximately 1g of air-dried cyanobacterial isolates, obtained from free living cultures and cultures associated with the roots of maize plants was dispensed into 25ml glass vials. Sterilized distilled water was added to give a total volume of 7.5ml. The vials were sealed with screw caps fitted with silicon rubber septa.

Acetylene generated from calcium carbide was injected into the vials, giving a gas atmosphere in the vials of air plus 10% acetylene. All samples were set up in duplicate sets of three each for light and dark incubations; the latter for assessing

dark nitrogen fixation. After incubation for specific duration in light or dark according to the requirement of the experiment, 100µl of gas phase was withdrawn using a gas-tight Hamilton syringe and analyzed for ethylene on AIMIL- Nucon 5765 model gas chromatograph with FID detector fitted with Porapak- T SS column (80–100 mesh; carrier gas nitrogen, 30ml/min; column temperature, 100°C; injector temperature, 100°C; detector temperature, 120°C). Acetylene reduction activity was expressed as n mol ethylene /g air –dried material/h (Umbreit et al., 1972).

The total nitrogen content of free living cyanobacterial cultures and the cultures associated with roots of maize plants were estimated according to modified micro-Kjeldahl method (Nesslerisation) of Umbreit et al. (1972). Total nitrogen in cyanobacterial culture filtrate was carried out in two phases:

Digestion phase: A suitable amount (about 50ml) of culture filtrate was taken in Kjeldahl flask and to this added 50ml of digestion reagent. Digestion reagent was prepared by dissolving 134gK₂SO₄ in 650ml of distilled water and 200ml of concentrated H₂SO₄. 2g HgO was dissolved in 6NH₂SO₄. This solution was then added to test solution. The solution was diluted to 1 liter and stored at temperature above 14°C to prevent crystallization.

Distillation phase: Kjeldahl flask was then placed in its proper position in distillation apparatus and turned on heat. 0.5ml of phenolphthalein indicator followed by sodium hydroxide and sodium thiosulphate was added till pH reach just above 8.3. After distillation 200ml of distillate was collected in 50ml boric acid. On completion of distillation the flask was removed and put off heat to avoid back suction. The concentration of ammonia was measured by

nesslerization. The distillate was then titrated with 0.02N H₂SO₄ till the indicator turned a pale lavender color. The total nitrogen (organic nitrogen) was calculated by following formula:

$$\text{Total Nitrogen} = (A-B) \times 280/\text{ml of sample}$$

Where A= ml of 0.02N H₂SO₄ used with sample and B= ml of 0.02N H₂SO₄ used with blank.

The experiments were also conducted in earthen pots (25x30cm) in replicates of five with 5kg of sterilized field soil of pH 6.8. The experiments were conducted with following cyanobacterial consortium:

1. Control i.e. without association of cyanobacteria
2. With separate association of cyanobacterial isolates
3. With combined association of all the 15 cyanobacterial isolates.

For studying the effect of consortium the roots of Maize seedlings were immersed overnight in cyanobacterial culture suspension to effect formation of consortium with the root system separately before transplantation into pots. The shoot length of the plant was measured in cm. at two different stages:

- (a) After two months, and
- (b) After three months i.e. when the ears started emerging.

The yield was measured in terms of gm of grain/plant in pot and the results were analyzed statistically by one way analysis of variance (ANOVA). The results obtained have been presented in Tables 1, 2, 3 and 4.

Result and Discussion

The seeds of Maize (*Zea mays*) kept on petriplates containing moist filter paper were found to germinate after three days and the germination percentage was determined. In control room, seeds of *Zea mays* showed only 45% of germination. The seeds exhibited 88% germination when soaked in *Oscillatoria curviceps*, *O. nigra*, and *O. princeps* (Table 1). The germination percentage was found to be low (55% to 70%) when seeds of *Zea mays* were soaked separately in *Schizothrix vaginata*, *Lyagbya gracilis*, *phormidium dimorphum* and *Calothrix clavata*. Seeds when soaked in *Gloeocapsa calcarea* showed 70% germination. Seeds when soaked in heterocystous cyanobacteria exhibited 70 to 90% germination. Seeds when soaked in *Aulosira prolifica*, *Stigonema dendroideum*, *Nostoc muscurum* and *Tolypothrix tenuis* exhibited 80–90% germination and in *Nostoc calcicola* and *Anabaena oryzae* 90% germination. The seeds when soaked in a consortium of fifteen cyanobacterial isolates exhibited 100% germination (Table 1)

The initial shoot length and root length before transfer to Erlenmeyer flask was found to be 4.5cm and 3.5cm respectively. One week after transfer into Erlenmeyer flasks, the shoot and root length of *Zea mays* was measured. It was found that the maize seedlings growing in *Oscillatoria curviceps*, *O.nigra*, *O.princeps*, *Aulosira prolifica*, *stigonema dendroideum*, *Nostoc muscurum*, *N.calcicola*, *Anabaena oryzae*, *Scytonema varium* and *Tolypothrix tenuis* showed a significant increases in both shoot length (7.21 to 7.48cm) and root length (6.12 to 6.62cm) in comparison to control experiment where shoot and root length were found to be 5.35cm and 4.51cm respectively. *Schizothrix vaginata*, *Lyngbya gracilis*, *Phormidium dimorphum*, *Calothrix*

clavata and *gloeocapsa calcarea* promoted slightly low seedling growth (shoot length 6.12 to 6.65cm and root length 5.15 to 5.70cm) of *Zea mays*. The seedlings growing in consortium of 15 cyanobacterial isolates showed a maximum increase in the growth of both shoot and root length. The shoot and root growth of seedling in consortium of 15 cyanobacterial isolates were 10.25cm and 9.51cm respectively (Table-2).

After seedling growth measurement, the cyanobacterial isolates associated with the root system was considered to various physiological studies such as growth in terms of Chl-a, Nitrogenase activity, total nitrogen content and ammonia assimilating enzymes.

From the result (Table 3) it is evident that the chl-a content of heterocystous cyanobacteria viz., *Anabaena oryzae*, *Scytonema varium*, *Tolypothrix tenuis*, *Nostoc calcicola*, *N. muscurum*, *Stigonema dendroideum* and *Aulosira prolifica* was maximum in free living condition, in the range of 0.470 to 0.431 μ g/ml and minimum in non heterocystous forms (0.042 to 0.277 μ g/ml). In cyanobacterial filaments associated with root system of *Zea mays*, the chlorophyll-a content was found to be higher in associated *Oscillatoria princeps* (0.965 μ g/ml), followed by *Tolypothrix tenuis* (0.725 μ g/ml), *Anabaena oryzae* (0.723 μ g/ml), *Nostoc calcicola* (0.712 μ g/ml), *N. muscurum* (0.708 μ g/ml), *Stigonema dendroideum* (0.705 μ g/ml), *Aulosira prolifica* (0.703 μ g/ml), *Oscillatoria nigra* (0.615 μ g/ml); *Shizothrix vaginata* (0.581 μ g/ml), *Oscillatoriya curviceps* (0.518 μ g/ml) and *L. gracilis* (0.513 μ g/ml) (Table 3). The maximum chl-a content (2.56 μ g/ml) was observed when roots of maize seedling were in consortium with all the fifteen cyanobacterial isolates selected for present investigation. The

results clearly indicated that the ability of photosynthesis and hence the capacity of maize variety to fix CO₂ was enhanced when their root systems were consorted with a large number of cyanobacteria. The present findings gain support from the work of Manoj Kumar et al. (2013) who also observed a more or less similar results in case of cyanobacterial consortium with wheat crop.

The Nitrogenase content in free living cyanobacteria was maximum in heterocystous cyanobacteria viz., *Aulosira prolifica*, *Stigonema dendroideum*, *Nostoc muscurum*, *N. calcicola*, *Anabaena oryzae*, *Scytonema varium* and *Tolypothrix tenuis* in the range of 0.580 to 0.590 n moles C₂H₂ produced/h/mg.f.wt, and minimum in non-heterocystous forms (0.310 to 0.353 n moles C₂H₂ produced/h/mg.f.wt), viz., *Oscillatoria curviceps*, *O. nigra*, *O. princeps*, *Schizothrix vaginata*, *Lyngbya gracilis*, *Phormidium dimorphum* and *Gloeocapsa calcarea*. A consortium of 15 cyanobacterial isolates in it free living state showed a high nitrogenase level, 0.985 n moles C₂H₂ produced/h/mg. f. wt. All fifteen cyanobacterial isolates when associated with root system of *Zea mays* showed an enhanced Nitrogenase activity. A consortium of 15 cyanobacterial isolates in association with root system of present cereal cultivar showed a significantly highest Nitrogenase activity (1.87 n moles C₂H₂ produced/h/mg. f. wt) (Table 3). The results indicated that non heterocystous cyanobacteria selected in the present investigation also had the capacity to fix atmospheric nitrogen. Enhanced Nitrogenase activity in associated conditions of cyanobacteria might be due to cooperative interaction.

Among 15 cyanobacterial isolates the total nitrogen content was found to be minimum in *Gloeocapsa calcarea* (0.6 μ g/ml. f. at), *O.nigra* (0.7 μ g/ml) and *O. princeps*

(0.8µg/ml) and maximum in heterocystous forms viz., *Anabaena oryzae* (5.5µg/ml), *Scytonema varium* (5.4µg/mg), *Tolypothrix tenuis* (5.3µg/mg), *Aulosira prolific* (2.5µg/mg), *Stigonema dendroideum* (2.7µg/mg), *Nostoc muscurum* (3.2µg/mg) and *N. calcicola* (3.5µg/mg) in free living state. *Oscillatoria curviceps*, *Schizothrix vaginata*, *Lyngbya gracilis*, *Phormidium dimorphum*, *Colothrix clavata* showed mild nitrogen concentration in their free states. All the fifteen isolates showed enhanced total nitrogen content when associate with the root system of maize. A consortium of fifteen cyanobacterial isolates, on association with root system of maize showed a significant increase in their total nitrogen content of 16.0µg/mg. f.-wt.16.0µg/mg.f.wt. (Table 3).

The initial shoot length of *Zea mays* seedlings before association and transplantation into earthen pots and field was observed to be 10.0cm. The shoot length was measured at two different stages, one after two months and the other after three months i.e., at grain stage. The statistical analysis of data showed that there was a significant increase in the shoot length of Maize associated with both heterocystous and non heterocystous cyanobacterial isolates. Heterocystous cyanobacterial isolates caused maximum increment in shoot length 177 to 215cm (Table 4).

The non-heterocystous cyanobacterial isolates, although promoted less increment in shoot length, but the observed value was greater in comparison to those treated with farmyard manure and untreated cultivars. The association with a consortium of fifteen cyanobacterial isolates caused significantly highest increment in shoots length after three months of plantation. This consortium caused an increment of shoot length to 250cm. The statistical analysis reveals that

the use of a consortium of 15 cyanobacterial cultures results in a significant increase in the shoot length of seedling of Maize.

The grain and straw yield of Maize was also recorded and statistically analyzed. Similar to shoot length, the grain and straw yield in pot were found to show a significant increase when associated with cyanobacterial isolates. The grain and straw yield in control plants of Maize was 12.25 g/plant and 18.50 g/plant (Table 4). The statistically data reveals that there was a significantly high yield of grains of Maize when their seedling were treated with cyanobacterial isolates. Maximum grain yields was recorded with heterocystous cyanobacterial isolates in pot condition, 25.25 to 35.00 g/plant.. A consortium of 15 cyanobacteria isolates on the other hand caused a significantly highest yield (45g/plant) (Tabla 4). The statistically data shows that the shoot length and yield of Maize was significant at 95% level.

In the present investigation significant increase in shoot length and root length of Maize was observed in the presence of all the fifteen cyanobacterial isolates. The present findings gain support from the work of Mule et al., (1999) who have reported increase in the dry weight and shoot length of rice seedlings in the presence of *Tolypothrix tenuis* and *Nostoc muscurum*. The higher Nitrogenase was observed only when cyanobacterial isolates were used in combination. The present finding is also in accordance with Manoj Kumar et al. (2013) who have observed increased shoot and root length, and grain yield of wheat (*Triticum aestivum* Linn. NP6).

The use of cyanobacteria as a biofertilizers for rice crops was reported by De (1939), Kannaiyan (1979), Roger and Kulassariya (1980) and Roger (1996).

Table.1 Showing Percentage germination of Maize seeds after three days, soaked in different combinations of cyanobacterial isolates

Treatment	Percentage of germination
	<i>Zea mays</i>
Control room	45
<i>Oscillatoria curviceps</i>	88
<i>O. nigra</i>	88
<i>O. princeps</i>	88
<i>Schizothrix vaginata</i>	55
<i>Lyngbya gracilis</i>	55
<i>Phormidium dimorphum</i>	55
<i>Calothrix clavata</i>	65
<i>Aulosira prolofica</i>	70
<i>Stigonema dendroideum</i>	75
<i>Nostoc muscurum</i>	85
<i>N. calcicola</i>	90
<i>Anabaena oryzae</i>	90
<i>Scytonema varium</i>	90
<i>Gloeocapsa calcarea</i>	70
<i>Tolypothrix tenuis</i>	80
<i>Consortium of 15 isolates</i>	100

Table.2 Showing effect of Cyanobacterial isolates on the growth of Maize seedlings one week after transfer into Erlenmeyer flasks

Treatment	<i>Zea mays</i>	
	Shoot length ^a X ±SD	Root length ^a X± SD
Control	5.35± 0.02	4.51± 0.03
<i>Oscillatoria curviceps.</i>	7.21 ±0.02	6.12 ±0.01
<i>O. nigra</i>	7.23± 0.03	6.20± 0.04
<i>O. princeps</i>	7.25± 0.02	6.21± 0.01
<i>Schizothrix vaginata</i>	6.55 ±0.03	5.68± 0.02
<i>Lyngbya gracilis</i>	6.00± 0.02	5.11± 0.03
<i>Phormidium dimorphum</i>	6.15± 0.02	5.00 ±0.01
<i>Calothrix clavata</i>	6.48± 0.04	5.51± 0.01
<i>Aulosira prolifica</i>	7.36± 0.04	6.25± 0.02
<i>Stigonema dendroideum</i>	7.38± 0.02	6.08± 0.03
<i>Nostoc muscurum</i>	7.42 ±0.02	6.31± 0.04
<i>N. calcicola</i>	7.48± 0.04	6.36± 0.02
<i>Anabaena oryzae</i>	7.52± 0.03	6.62± 0.04
<i>Scytonema varium</i>	7.48± 0.02	6.60± 0.01
<i>Gloeocapsa calcarea</i>	6.21± 0.03	5.15± 0.01
<i>Tolypothrix tenuis</i>	7.65 ±0.04	6.68± 0.03
<i>Consortium of 15 isolates</i>	10.25 ±0.03 ^b	9.51 ±0.01 ^b

a = average of four replicates; b = P< 0.05 level

Table.3 Showing chlorophyll- a, Nitrogenase and Total nitrogen content in free-living Cyanobacterial cultures and cultures associated with roots of Maize (Chl-a inµg/mL, Nitrogenase in n moles C2H2 produced/hr/mg f.wt, Total nitrogen in µg/mg f.wt)

Treatment	Chlorophyll-a		Nitrogenase		Total Nitrogen	
	Free-living	Associated	Free-living	Associated	Free living	Associated
<i>Oscillatoria curviceps.</i>	0.145	0.355	1.3	1.8	0.415	0.518
<i>O. nigra</i>	0.277	0.352	0.7	1.7	0.417	0.615
<i>O. princeps</i>	0.154	0.353	0.8	1.7	0.418	0.965
<i>Schizothrix vaginata</i>	0.143	0.345	1.2	1.8	0.415	0.581
<i>Lyngbya gracilis</i>	0.146	0.341	1.3	1.6	0.416	0.513
<i>Phormidium dimorphum</i>	0.135	0.345	1.5	1.8	0.423	0.471
<i>Calothrix clavata</i>	0.145	0.342	1.4	1.6	0.419	0.473
<i>Aulosira prolifica</i>	0.431	0.585	2.5	5.3	0.840	0.703
<i>Stigonema dendroidum</i>	0.445	0.590	2.7	5.8	0.847	0.705
<i>Nostoc muscurum</i>	0.456	0.587	3.2	6.5	0.853	0.708
<i>N. calcicola</i>	0.465	0.585	3.5	6.8	0.851	0.712
<i>Anabaena oryzae</i>	0.470	0.584	5.5	7.0	0.870	0.723
<i>Scytonema varium</i>	0.462	0.582	5.4	6.9	0.866	0.721
<i>Gloeocapsa calcarea</i>	0.042	0.310	0.6	1.7	0.420	0.475
<i>Tolypothrix tenuis</i>	0.456	0.588	5.3	6.8	0.861	0.725
Consortium of 15 isolates	0.665	0.985	10.5	16.0	1.87	2.56

Table.4 Showing Shoot length (cm) after addition of cyanobacterial biofertilizers in 2nd month, and 3rd month and grain yield (g/plant) and straw yield of *Zea mays* in Earthen pot

Treatment	Shoot length in 2 nd month	Shoot length in 3 rd month	Grain yield	Straw yield
	X ± SD	X ± SD	X ± SD	X ± SD
Control.	20.25±0.02	150±0.03	12.25±0.03	18.50±0.03
<i>Farmyard manure</i>	22.50±0.01	156±0.04	13.25±0.04	18.25±0.01
<i>O. curviceps</i>	23.55±0.02	158±0.03	14.25±0.03	21.75±0.02
<i>O. nigra</i>	23.50±0.05	158±0.04	14.00±0.02	22.00±0.01
<i>O. princeps</i>	23.25±0.04	158±0.03	14.50±0.01	22.25±0.02
<i>Sch. vaginata</i>	23.15±0.01	157±0.02	14.00±0.06	21.50±0.03
<i>L. gracilis</i>	22.75±0.01	156±0.04	13.50±0.02	21.50±0.03
<i>Ph. dimorphum</i>	22.50±0.02	156±0.05	13.25±0.01	21.15±0.03
<i>Cal. clavata</i>	22.55±0.06	158±0.02	14.00±0.04	21.25±0.04
<i>Aul. prolifica</i>	34.00±0.06 ^b	178±0.06 ^b	25.50±0.04 ^b	37.50±0.03 ^b
<i>Stig. dendroidum</i>	34.25±0.04 ^b	177±0.03 ^b	25.25±0.03 ^b	38.15±0.02 ^b
<i>N. muscurum</i>	35.00±0.03 ^b	180±0.03 ^b	29.25±0.07 ^b	55.25±0.03 ^b
<i>N. calcicola</i>	35.75±0.04 ^b	185±0.04 ^b	32.50±0.06 ^b	58.25±0.04 ^b
<i>Anab. oryzae</i>	36.50	215±0.03 ^b	35.00±0.03 ^b	60.50±0.04 ^b
<i>Scyt. varium</i>	35.25±0.01 ^b	175±0.02 ^b	28.50±0.06 ^b	57.25±0.03 ^b
<i>G. calcarea</i>	23.25±0.02	157±0.04	13.25±0.03	18.50±0.02
<i>Tolyp. tenuis</i>	34.50±0.03 ^b	180±0.02 ^b	26.00±0.04 ^b	40.25±0.01 ^b
Consortium of 15 isolates	42.50±0.06 ^b	250±0.04 ^b	45.00±0.04 ^b	62.25±0.05 ^b

In the present investigation use of heterocystous and non-heterocystous cyanobacterial isolates singly and in combination was found to increase significantly the yield of maize. The study reveals that these cyanobacteria could bring about positive results in the improvement of Maize crops and might prove to be an effective biofertilizers for Maize fields also.

Acknowledgement

The authors are grateful to Dr. Manoj Kumar, Associate Professor, Dept. of biotechnology, College of Commerce, Patna for providing laboratory facilities.

References

- De, P. 1939. The role of Blue-green algae in nitrogen fixation in rice fields. *Proc. Roy. Soc. London*, B127: 121–139.
- Desikachary, T.V. 1959. *Cyanophyta*, ICAR Monograph on Algae, ICAR, New Delhi, 686 Pp.
- Dola Bhowmik, Jaishree Dubey, Sandeep Mehra, 2010. Evaluating Potential of *Spirulina* as Inoculants for Pulses. *Acad. J. Plant Sci.*, 3(4): 161–164.
- Fatima, T., Venkataraman, L.V. 1999. Cyanobacterial and microalgal potential as biochemical. In: cyanobacterial and algal metabolism and environmental biotechnology. Narsa Publishing house, New Delhi. Pp. 92–112.
- Gantar, M. 2000. Mechanical damage of roots provides enhanced colonization of the wheat endorhizosphere by the dinitrogen-fixing cyanobacterium *Nostoc* sp. strain 2S9 B. *Biol. Fertil. Soils*, 32: 250–255.
- Gayatri, V., Anand, N. 2002. Role of cyanobacterial association in the improvement of rice crop, *J. Indian Bot. Soc.*, 81: 41–46.
- Ghallab, A.M., Salem, S.A. 2001. Effect of biofertilizer treatments on growth, chemical composition and productivity of wheat plants grown under different levels of NPK fertilization. *Ann. Agri. Sci. Cairo*, 46: 485–509.
- Goyal, S.K., Venkataraman, G.S. 1971. Response of high yielding rice varieties to alkalization interaction of soil types with algal inoculation, *Phykos*, 10: 32–33.
- Haroun, S.A., Hussein, M.H. 2003. The promotive effect of algal biofertilizers on growth, protein pattern and some metabolic activities of *Lupinus termis* plants grown in siliceous soil. *Asian J. Plant Sci.*, 2(13): 944–951.
- Hegewald, E. 1976. A contribution of algal flora at Jamaica Nova Hedwigia, Vol. 28. Hafner Publishing Co. New York and London, Pp. 45–69.
- Kannaiyan, S. 1979. Blue green algal biofertilizers for rice crop. *Farmers Parliaments*, 14(11): 19–25.
- Karthikeyan, N., Prasanna, R., Lata, Kaushik, B.D. 2007. Evaluating the potential of plant growth promoting cyanobacteria as inoculants for Wheat. *Eur. J. Soil Biol.*, 43: 23–20.
- Lakshmi, P.T.V., Annamalai, A. 2008. The effect of cyanobacterial (blue-green algae) culture filtrate on the biomass and biochemicals of *Withania somnifera* Dunnal. *Asian J. Plant Sci.*, 7(1): 37–43.
- Mackinney, G. 1941. Absorption of light by chlorophyll solutions. *J. Biol. Chem.*, 140: 315–322.
- Manoj Kumar, Baidyanath, K., Anand, M. 2013. Cyanobacterial consortium in the improvement of wheat crop. *Natural Sci. Today*, 16(16): 1–9.
- Manu Arora, Kaushik, A., Nisha Rani, Kaushik, C.P. 2010. Effect of cyanobacterial exopolysaccharides on salt stress alleviation and seed

- germination. *J. Environ. Biol.*, 31(5): 701–704.
- Mule, M.Z., Caire, G.Z., Cono, M.S., Palma, R.M., Colombo, 1999. Effects of cyanobacterial inoculation and fertilizers on rice seedlings and post harvest soil structure. *Commun. Soil Sci. Plant Anal.*, 30: 97–107.
- Nayak, S., Prassana, R. 2007. Soil pH and its role in cyanobacterial abundance and diversity in ricefield soils. *App. Ecol. Enviro. Res.*, 5(2): 103–113.
- Ordog, V. 1999. Beneficial effects of microalgae and cyanobacteria in plant /soil systems, with special regard to their auxin and cytokinin like activity. International workshop and training course on microalgal biology and biotechnology, Mosonmagyaróvár, Hungary. Pp. 13–26.
- Pitchai Palaniappan, P., Malliga, K., Manian, S., Sivaramakrishnan S., Madhaiyan, M., Tongmin, S.A. 2010. Plant growth promotory effect on cow pea (*Vigna unguiculata*) using coir pith aqueous extract formulation of *Cyanobacterium Phormidium*, *Am.-Eur. J. Agric. Environ.*, 8(2): 178–184.
- Prasanna, R., Jainita, J., Kaushik, B.D. 2008. Cyanobacteria as potential options for environmental sustainability promises and challenges. *Indian J. Microbiol.*, 48: 89–94.
- Relwani, L.L., Surahmanyana, R. 1963. Role of blue- green algae chemical nutrients and partial soil sterilization on paddy yield. *Curr. Sci.*, 32: 441–443.
- Roger, P.A. 1996 Biology and management of the flood- water ecosystem in rice fields. International Rice Research Institute, Manila, Philippines. Pp. 250.
- Roger, P.A., Kulasooriya, S.A. 1980. Blue-green algae and rice. International Rice Research Institute, Los Bonos, Laguan, Philippines. 112 Pp.
- Sergeeva, E., Liaimer, A., Bergman, B. 2002. Evidence for population of the Phytohormone indole- 3-acetic acid by cyanobacteria, *Planta*, 215: 229–238.
- Singh, R.N. 1961. The role of blue-green algae in nitrogen economy of Indian Agriculture. Indian Council of Agriculture Research, New Delhi, India.
- Stewart, W.D.P., Fitzgerald, G.P., Burris, R.H. 1967. *In situ* studies on nitrogen fixation using the acetylene reduction technique. *Proc. Natl. Acad. Sci. USA*, 58: 2071–2078.
- Tilden, J.E. 1910. The *Myxophyceae* of North America. *I. Bibl. Phycol. Band*, 4: 1–328.
- Tilden, J.E. 1937. The algal and their life relation facsimile of the 1937 edition,
- Umbreit, W.W., Burris, R.H., Stauffer, J.F. 1972. Manometric and biochemical techniques. Burgess Publishing, Minnesota. Pp. 260.
- Upasana Mishra, Sunil Pabhi, 2004. Resonance, - General article, IARI, New Delhi.
- Venkataraman, G.S. 1972. Algal biofertilizers and rice cultivation. New Delhi, India, today and blue-green algae chemical nutrients and partial soil. Tomorrows Printers and Publishers.