



ISSN (E): 2277-7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2022; SP-11(9): 439-443  
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[www.thepharmajournal.com](http://www.thepharmajournal.com)  
Received: 22-07-2022  
Accepted: 24-08-2022

**Nikita Laishram**  
M.Sc. (Agri.), Department of Soil  
Science, Assam Agricultural  
University (AAU), Jorhat,  
Assam, India

**Dr. Rajen Baruah**  
Professor, Department of Soil  
Science, Assam Agricultural  
University (AAU), Jorhat,  
Assam, India

**Dr. Amrita Phukan**  
RA, Department of Soil Science,  
Assam Agricultural University  
(AAU), Jorhat, Assam, India

**Antara Paul**  
Junior Research Fellow,  
Department of Soil Science,  
Assam Agricultural University  
(AAU), Jorhat, Assam, India

**Deepika Charingia**  
Junior Research Fellow,  
Department of Soil Science,  
Assam Agricultural University  
(AAU), Jorhat, Assam, India

**Corresponding Author:**  
**Nikita Laishram**  
M.Sc. (Agri.), Department of Soil  
Science, Assam Agricultural  
University (AAU), Jorhat,  
Assam, India

## Evaluation of cyanobacteria as a bioresource potential

**Nikita Laishram, Dr. Rajen Baruah, Dr. Amrita Phukan, Antara Paul and Deepika Charingia**

### Abstract

A total of 77 cyanobacterial cultures were isolated from 55 numbers of soil samples collected from Jorhat and Golaghat districts of Assam using BGIIo specific liquid media from which 30 cultures thus showed pure colonies. 10 efficient cultures based on enzyme and PGP activities were selected for pot experiment to study its efficiency in growth and yield attributing characters in rice crop variety Luit (Ahu). Results indicated that all the inoculated treatments showed better growth and yield as compared to the uninoculated control which was evident from the data obtained on number of tillers, number of panicles, length of panicles, grain yield (g/ hill), straw yield (g) and dry weight of root (g). Grain yield and straw yield of BGA-41 ranged as 27.67 g/ pot and 12.64 g/ pot respectively. Therefore BGA-41 which was identified as *Anabaena constricta* by molecular characterization (using CTAB method) was regarded superior to other cyanobacterial cultures which was at par with the recommended fertilizer dose treatment.

**Keywords:** Cyanobacterial, PGP, BGIIo, luit, *Anabaena constricta*

### 1. Introduction

Cyanobacteria in rice cultivation directly correlates with their ability to fix nitrogen and proved positive effects for plants and soil and thus, benefits the ecology as a whole. Cyanobacteria are the group of photosynthetic organisms which can easily survive on bare minimum requirement of light, carbon dioxide (CO<sub>2</sub>) and water (Woese *et al.*, 1978) [10]. They can contribute to about 20-30 kg/ha nitrogen as well as the organic matter in the soil (Issa *et al.*, 2014) [1]. Purwani *et al.* (2021) [6] suggested that by exploiting modern green technologies which includes the use of plant growth promoting rhizobacteria (PGPR) like cyanobacteria, the dependence on chemical inputs could thus be reduced while maintaining an ecologically sound environment with ensured soil fertility and crop production.

In field condition, nitrogen is one of the major limiting factor for plant growth for which deficiency of this element is met by fertilizers (Malik *et al.*, 2001) [3]. The paddy field ecosystem provides an ideal ecosystem for the growth of various cyanobacteria with respect to their requirements for light, water, high temperature and nutrient availability which could be the major reason for more abundance of cyanobacteria growth in paddy soils (Kondo and Yasuda 2003) [2]. The oxygen evolving photoautotrophic prokaryotic cyanobacteria are known to cohabit with rice. Commonly occurring species in Indian rice fields include *Anabaena*, *Calothrix*, *Cylindrospermum*, *Gleotrichia*, *Rivularia*, *Scytonema* etc.

Rice (*Oryza sativa*) is the major food crop of nearly half of the world's population. The USDA (United States Department of Agriculture) estimates a production of 503.17 million metric tons of the World Rice Production by 2020/2021. i.e., an increase in 1.36% in rice production around the globe. In India, area of rice production estimated was 43.78 million hectares with a production of 118.43 million tonnes in the year 2019-2020 as per the source given by Directorate of Economics and Statistics (2020). In Assam condition, it is the main staple food occupying a coverage of 2.54 m ha of total 3.3 m ha cropped area (Pathak and Sharma., 2008) [5]. Thus, rice contributes 96% of the total food grain production of the state grown throughout the year with 3 distinct seasons winter (Sali), autumn (Ahu) and summer (Boro). Cyanobacteria in rice cultivation directly correlates with their ability to fix nitrogen and proved positive effects for plants and soil and thus, benefits the ecology as a whole.

### 2. Materials and Method

#### 2.1 Crop

The test crop used was paddy (*Oryza sativa*) with an Ahu variety Luit, a 100-120 days variety.

Firstly the seeds were soaked in water for about a week. When most of the seeds were germinated they were transferred to a tray with puddled soil for seedling growth. After 15 days the seedlings were transplanted to the pot containing 4 kg of sieved soil when the seedlings reached atleast a height of 2 cm. Transplanting was done @ 3 plants/pot.

## 2.2 Potting mixture

Finely ground soil @ 4 kg per pot with each pot filled with compost in all pots except the absolute control. A total 36 pots were arranged.

## 2.3 Fertilizer application

Recommended fertilizer for Transplanted normal Ahu rice (Autumn rice) is 40: 20: 20 (88kg N kg/ha, 125 kg P<sub>2</sub>O<sub>5</sub> kg/ha, 32 kg K<sub>2</sub>O kg/ha) as per guidelines given in Package of practices for selected crops of Assam.

## 2.4 Treatment combinations

**Table 1:** A total of 12 treatments which were selected

Treatments	Treatment combination
T <sub>1</sub>	Absolute control, No NPK
T <sub>2</sub>	Standard NPK (40:20:20)
T <sub>3</sub>	50% N with CB1+full P+K
T <sub>4</sub>	50% N with CB2+full P+K
T <sub>5</sub>	50% N with CB3+full P+K
T <sub>6</sub>	50% N with CB4+full P+K
T <sub>7</sub>	50% N with CB5+full P+K
T <sub>8</sub>	50% N with CB6+full P+K
T <sub>9</sub>	50% N with CB7+full P+K
T <sub>10</sub>	50% N with CB8+full P+K
T <sub>11</sub>	50% N with CB9+full P+K
T <sub>12</sub>	50% N with CB10+full P+K

## 2.5 Replication

Altogether, 12 treatments were replicated thrice.

## 2.6 Cyanobacterial inoculation

250 ml of cyanobacterial inoculate were mixed in each of the treatment pots except for the absolute control in the puddled pot. The inoculum was given at 7 days after transplanting.

## 2.7 Crop duration

The paddy crops were allowed to grow maturity and thereafter harvested.

## 2.8 Observation

Crops along with the roots were harvested and adhering soil particles were removed by gentle washing and following observations were recorded.

### 2.8.1 Rice yield (g/ pot)

Weight of the fresh grains were recorded right after harvesting to the nearest gm.

### 2.8.2 Straw yield (g/ pot)

Shoots were separated from the roots and dried at 60 °C in the oven for 24 hours and dry weight was recorded.

### 2.8.3 Yield characters

#### 2.8.3.1 Number of tillers

Number of tillers for each pot were recorded.

#### 2.8.3.2 Number of panicles

Number of panicle for each pot were recorded.

#### 2.8.3.3 Length of panicles

Length of the panicle for each pot were recorded to the nearest cm.

#### 2.8.3.4 Root weight (on dry weight basis)

Roots were separated from the plant and dried at 60 °C in the oven for one day and dry weight was recorded.

## 3. Results and Discussion

### 3.1 Pot culture evaluation

A total of 10 efficient cyanobacterial cultures were selected on the basis of enzyme activities and PGPR activities out of the 30 cultures isolated for pot culture evaluation on rice crop (*Oryza sativa*), variety Luit (Ahu) at Biofertilizer unit of Soil Science Department during February of 2021. The 10 efficient cultures were identified as follows by molecular characterization using CTAB method:

BGA-02 (*Nostoc* sp.); BGA-12 (*Wollea ambigua*); BGA-38 (*Aliinostoc tiwarrii*); BGA-40 (*Anabaena constricta*); BGA-41 (*Anabaena constricta*); BGA-49 (*Anabaena constricta*); BGA-60 (*Nostoc* sp.); BGA-64 (*Anabaena constricta*); BGA-76 (*Nostoc* sp.); BGA-77 (*Anabaena constricta*).

Altogether 12 treatments were replicated thrice (Table 1). The crop was grown for 130 days till maturity. After that crop was harvested and yield and yield attributing characters were recorded.

#### 3.1.1 Weight of fresh grain

The paddy crop inoculated with cyanobacteria significantly affected the weight of fresh grains. Results (Table 1) showed highest fresh weight of grains with cultures of BGA-41 (27.67 g/pot) closely followed by BGA-38 (25.48 g/pot) and lowest fresh weight of grain was recorded in BGA-12 (16.31 g/pot) as compared to the control. The RD treatment (T<sub>2</sub>) and BGA-41 inoculated treatment (T<sub>7</sub>) showed almost equivalent effect. As shown in Figure 1 highest per cent increase over the control in grain yield was shown by recommended dose treatment (131.80%) i.e., T<sub>2</sub> but no significant difference was observed with BGA-41 inoculated treatment (T<sub>7</sub>) which showed a relative per cent increase over the control of 126.83% which means that they were at par followed by BGA-38 (108.85%) and lowest percent increase over the control was observed in BGA-12 (33.66%).

#### 3.1.2 Straw weight (on dry weight basis)

Results (Table 1) revealed that among the cultures the straw yield was found highest in BGA-41 (12.64 g/pot) followed by BGA-38 (12.51 g/pot). The BGA-41 inoculated treatment (T<sub>7</sub>) and BGA-38 inoculated treatment (T<sub>5</sub>) were at par with the recommended dose treatment (T<sub>2</sub>). The highest percent increase over the control among the inoculated cultures was seen in BGA-41 (127.22%), followed by BGA-38 (124.88%). Lowest straw yield was recorded with BGA-02 (6.25 g/pot) but higher than the uninoculated control (5.56 g/pot).

#### 3.1.3 Yield Attributing Parameters

##### 3.1.3.1 Number of tillers

Table 2 showed that the paddy crop inoculated with cyanobacterial cultures significantly affected the tiller number. The increase in tiller number between the treatments were significantly higher than the uninoculated control. The

results revealed culture BGA-41 gave almost equivalent tiller number and was statistically at par with the recommended fertilizer doses which recorded the highest tiller number

(16.67) among the cyanobacterial cultures. The increase in number of tillers was also observed in

**Table 1:** Effect of cyanobacterial inoculation on the grain yield of paddy (g/pot) and straw yield of paddy (g/pot)

Treatment	Grain yield (g/ pot)	Per cent increase over the control	Straw yield (g/ pot)	Per cent increase over the control
T <sub>1</sub> : Absolute Control	12.20	-	5.56	-
T <sub>2</sub> : Recommended dose	28.28	131.80	12.78	129.79
T <sub>3</sub> : 50% N+BGA-02+P+K	20.06	64.43	6.25	12.49
T <sub>4</sub> : 50% N+BGA-12+P+K	16.31	33.66	8.05	44.76
T <sub>5</sub> : 50% N+BGA-38+P+K	25.48	108.85	12.51	106.90
T <sub>6</sub> : 50% N+BGA-40+P+K	17.79	40.33	6.46	16.18
T <sub>7</sub> : 50% N+BGA-41+P+K	27.67	126.83	12.64	127.22
T <sub>8</sub> : 50% N+BGA-49+P+K	17.40	42.65	7.56	35.95
T <sub>9</sub> : 50% N+BGA-60+P+K	20.33	70.27	9.46	70.11
T <sub>10</sub> : 50% N+BGA-64+P+K	24.11	97.60	10.59	90.31
T <sub>11</sub> : 50% N+BGA-76+P+K	22.15	81.56	8.18	46.97
T <sub>12</sub> : 50% N+BGA-77+P+K	19.98	63.80	8.26	48.48
S.ED ( $\pm$ )		1.79		1.44
C.D		3.71		2.99

Each pot contain 4 kg of unsterile soil

All the pot received compost @ 5 t/ ha except the absolute control

BGA-38 (14.67), BGA-64 (14.33), BGA-77 (14.00) which were at par. The lowest among the cultures was recorded in BGA-12 (11.00). Results also showed that there was no significant difference between BGA-60 and BGA-76.

### 3.1.3.2 Length of the panicles

Data from Table 2 showed that highest panicle length was observed in the treatment receiving BGA-41 (18.47 cm) which significantly differed from all other cyanobacterial treatments and was at par with the recommended dose treatment (T<sub>2</sub>) followed by BGA-38 (16.35 cm) and BGA-64 (14.97 cm). However, BGA-64 was significantly at par with BGA-60.

### 3.1.3.3 Number of panicles

Results (Table 2) revealed that among the cultures the highest

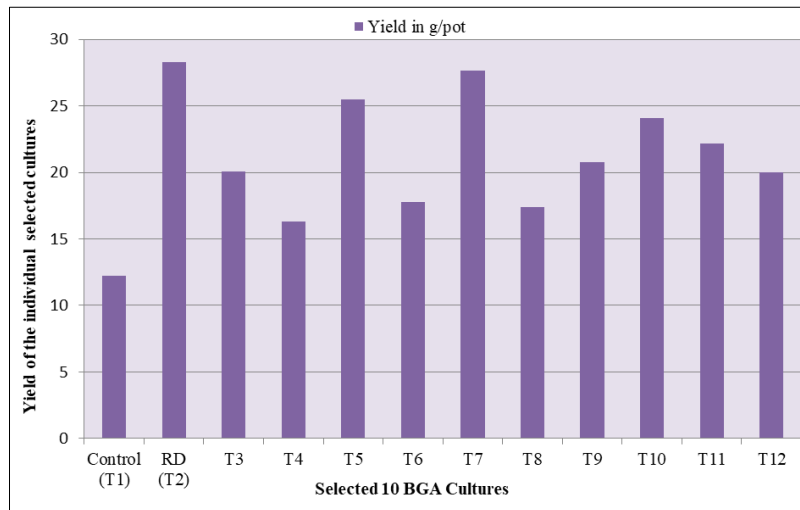
number of panicle was observed in the treatment inoculated with BGA-41 (13.67) followed by BGA-64 (12.67) and lowest number of panicles was showed by BGA-12 (8.00). BGA-64 and BGA-38 were significantly at par. There was no significant variation of BGA-41 which was at par with the recommended dose.

### 3.1.3.4 Root weight

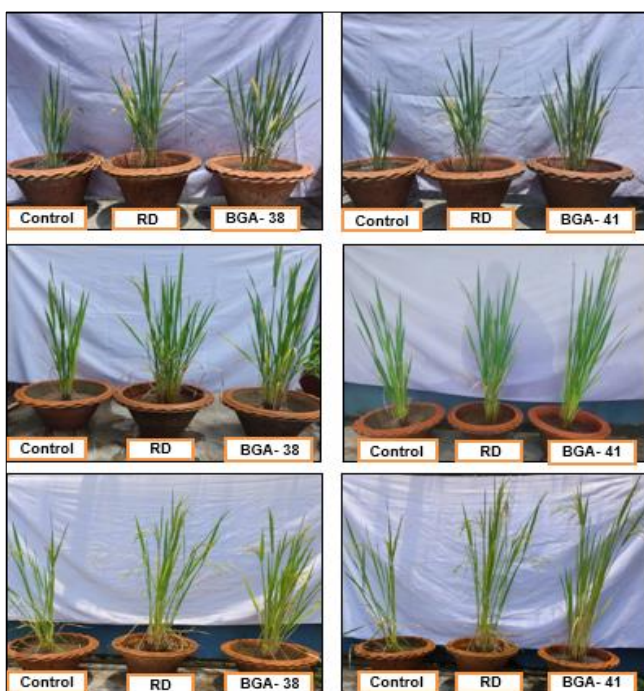
Results (Table 2) also showed that among the cultures BGA-41 showed the highest root weight (6.38 g) followed by BGA-38 (6.29 g). BGA-41 and BGA-38 were found to be significantly at par with the recommended fertilizer dose treatment and the lowest weight of dry root was found in BGA-12 (3.73 g). BGA-76, BGA-49 and BGA-12 were found to be at par to one another.

**Table 2:** Effect of cyanobacterial inoculation on number of tillers, number of panicles, length of panicles and root dry weight

Treatment	No. of Tillers	Length of panicle (cm)	No. of panicles	Dry wt. of the root (g)
T <sub>1</sub> : Absolute Control	8.00	12.02	6.00	3.06
T <sub>2</sub> : RD	17.33	19.13	14.33	6.42
T <sub>3</sub> : 50% N+BGA-02+P+K	12.33	13.37	9.67	4.64
T <sub>4</sub> : 50% N+BGA-12+P+K	11.00	13.41	8.00	3.73
T <sub>5</sub> : 50% N+BGA-38+P+K	14.67	16.35	12.33	6.29
T <sub>6</sub> : 50% N+BGA-40+P+K	12.00	13.62	10.00	4.81
T <sub>7</sub> : 50% N+BGA-41+P+K	16.67	18.47	13.67	6.38
T <sub>8</sub> : 50% N+BGA-49+P+K	12.67	14.60	9.00	4.10
T <sub>9</sub> : 50% N+BGA-60+P+K	13.33	14.80	9.33	4.57
T <sub>10</sub> : 50% N+BGA-64+P+K	14.33	14.97	12.67	5.10
T <sub>11</sub> : 50% N+BGA-76+P+K	13.33	14.17	11.33	4.20
T <sub>12</sub> : 50% N+BGA-77+P+K	14.00	14.23	11.67	5.31
S.E.D ( $\pm$ )	1.73	1.33	1.43	0.82
C.D <sub>0.05</sub>	3.59	2.76	2.96	1.69



**Fig 1:** Yield (g/ pot) as affected by the cyanobacterial cultures



**Plate 1:** Growth of rice seedling at 16 DAT, 95 DAT and 115 DAT



**Plate 2:** Root growth of paddy T<sub>1</sub> (Control), T<sub>2</sub> (RD) and T<sub>7</sub> (BGA-41)

Thus, interestingly from the above findings it was observed that BGA-41 identified as *Anabaena constricta* showed superiority over other inoculated cyanobacterial cultures on enhancing yield as well as yield attributing characters in rice followed by BGA-38 (*Allinostoc tiwari*) and BGA-64 (*Anabaena constricta*) which was evident from the data recorded i.e., weight of grain, straw weight, number of tillers, number of panicles, length of panicle and root weight (on dry weight basis). The above result further showed that in almost all the cases the yield attributing character of BGA-41 inoculated treatments were at par with recommended dose treatment.

Cyanobacteria are considered as a potent nitrogen fixer which contribute to about 20-30 kg/ha nitrogen as well as the organic matter in the soil (Issa *et al.*, 2014) [1] which exerts beneficial effects on the host plant growth and development by the production of phytohormones (IAA, GA, Cytokinin), suppression of phytopathogens through siderophore, HCN production, activation of phosphate solubilization, ammonia production and most importantly the nitrogenase enzyme activity which is an important property of diazotrophs that proved to enhance crop yield and production.

In our present study, based on all the above parameters discussed 10 efficient samples were selected based on multiple plant growth promoting traits and their effect on the growth and yield attributing parameters on rice crop variety Luit (ahu) via pot culture experiment. The rice crop was grown for 130 days and thereafter harvested. The different growth and yield attributing parameters were recorded such as tiller number, number of panicles, length of panicles (cm), grain wight (g/ hill), straw yield (g/ hill) and dry weight of root (g). All these characters showed remarkable variation between all treatments taken. Among all the treatments, culture BGA-41 recorded significantly highest growth as well as yield attributing characters followed by BGA-38 and BGA-64. BGA-41 inoculated treatment (T<sub>7</sub>) which was at par with recommended dose treatment (T<sub>2</sub>). The results of pot culture experiment showed that BGA-41 (*Anabaena constricta*) recorded highest yield and yield attributing parameters.

Therefore, the present study revealed that some of the cyanobacteria can thus function as an efficient bioinoculant in soil especially with the rice crop. Similar findings were also reported by Saadatnia and Riahi (2009) [7] where the effect of four species of heterocystous cyanobacteria *Anabaena* (*A. spiroides*, *A. variabilis*, *A. torulosa* and *A. osillarioides*) was

observed in a pot culture experiment. The germination of rice seeds treated with cyanobacteria was faster than control. They further reported increase in plant height, root length, fresh root weight and dry root weight with a significant differences ( $p < 0.05$ ) in pot treated with BGA as compared to the control. In another experiment carried out by Mandal *et al.* (2011)<sup>[4]</sup> reported that with the inoculation of cyanobacterial inoculum 1 kg ha<sup>-1</sup> (*Nostoc spongiaeforme* Dh164, *Nostoc commune* Dh 169, *Calothrix marchica* Dh 167 and *Stigonema* Dh 168) along with the application of 20-40% less fertilizer-N than recommended dose revealed a significant higher number of tillers/ hill, panicles/ hill, length of panicle, weight of 1000 grains, yields of grain and straw as compared to control using two HYV varieties of rice namely BR-28 and BR-29. Similarly, Sundara *et al.* (1963)<sup>[8]</sup> and Venkataraman and Goyal (1963)<sup>[9]</sup> reported significant contribution of cyanobacteria in boosting up the yield as well as straw yield in lowland rice field.

#### 4. Conclusion

Therefore from the above study out of 30 isolated cyanobacteria cultures, 10 best cultures based on enzyme and PGP activities were selected for pot experiment to study its efficiency in growth and yield attributing characters in rice crop variety Luit (Ahu).

Results indicated that all the inoculated treatments showed better growth and yield as compared to the uninoculated control which was evident from the data obtained on number of tillers, number of panicles, length of panicles, grain yield (g/ hill), dry weight of shoot (g) and dry weight of root (g). BGA-41 was regarded superior to other cyanobacterial cultures which was at par with the recommended fertilizer dose treatment followed by BGA-38 and BGA-64.

Overall conclusion from our present study revealed that BGA-41 (*Anabaena constricta*) was found as the most efficient culture for algalization effect on rice variety Luit followed by BGA-38 (*Aliinostoc tiwarii*).

However, in future course application of combination effect of cyanobacteria with other plant growth promoting rhizobacteria in field condition with diverse crops should be employed. Interactions of other soil microbial communities could enlighten the behaviour of cyanobacteria.

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