Advances in Bioresearch
Adv. Biores., Vol 11 (5) September 2020: 85-96

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.11.5.8596

Advances in Bioresearch

ORIGINAL ARTICLE

Growth augmentation of rice seedlings exposed to electricity adapted variant strains of *Anabaena variabilis* GITAM RGP

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ABSTRACT
The present study was designed to evaluate the growth promoting potential of Anabaena variabilis GITAM RGP and its electricity adapted variant on two varieties of rice (HRK– 47 and HRK -127). The effect of algal treatment on the rice crop was monitored with respect to seed germination, plant growth and availability of trace metals. Physicochemical parameters of soil were also analyzed before and after growth of plants. The results indicates significant ($p \leq 0.05$) increase in the seed germination rate, plant root length, shoot length, fresh weight, dry weight and availability of trace metals. Cyanobacteria treated rice plants also exhibited significantly ($p \leq 0.05$) higher uptake of trace metals from soil which suggests a beneficial impact of the algal strains on the plant growth. Findings of this study could help in establishment of algalization with improved varieties of cyanobacteria for increased crop yield at the same time reducing chemical fertilizers application in fields.
Keywords: *Anabaena variabilis GITAM RGP · Cyanobacteria · Algalization · Crop yield*

Received 02.05.2020 Revised 18.06.2020 Accepted 05.08.2020

How to cite this article:
Gaurav Pant and Vishal Khandelwal. Growth augmentation of rice seedlings exposed to electricity adapted variant strains of *Anabaena variabilis* GITAM RGP. Adv. Biores., Vol 11 (5) September 2020: 85-96

INTRODUCTION

Rice (*Oryza sativa*) is a major staple crop with the second highest production worldwide [1]. Developing countries produce nearly 95 % of world's rice, of which 90 % is contributed by Asia [2]. Asian agriculture however faces deficiency of nitrogen and other micro and macronutrients and this is especially common in rice soils due to excessive losses [3]. This necessitates application of nitrogen supplementation in the rice cultivation lands [4].

Cyanobacteria are nitrogen fixers [5] can improve the productivity of a field by increasing organic matter, reducing oxidizable matter and maintaining oxygen level in soil [6]. Rice fields are known to harbor cyanobacteria as the major nitrogen fixing organisms. Cyanobacteria growing in the submerged rice fields generally have an optimum supply of light, water, nutrients and temperature [7, 8) and synthesize several extracellular growth promoting substances *viz*, vitamins and growth regulators [9, 10]. The growth promoting potential of cyanobacteria is directly related to its nitrogen fixation ability and other positive impacts on plants and soil. The process of adding living algal cells to the crop field is commonly called algalization and known to promote the absorption of micro and macro nutrients from the rhizosphere [11]. An increase of 25-30 kg nitrogen/ha and 25 % increase in calcium levels have been reported while using cyanobacteria without any other organic fertilizer [12]. Improvement in the rate of seed germination, growth of rice plants and the quality of the seeds with 15–20 % increase in grain yield has also been reported [13].

Use of improved varieties of diazotrophic cyanobacteria could significantly increase the growth and production of the rice. *Anabaena variabilis* is a free living, filamentous heterocyst-forming cyanobacterium which is one of the important biofertilizers in rice fields [14, 15]. Heterocysts are specialized cells within the cyanobacterial filament where nitrogen fixation takes place [16]. The number of heterocysts is directly proportional to nitrogen fixation capability of the algal filament [17]. Earlier

work showed that minimum electric current (MEC) for a definite time period has an ability to enhance the frequency and size of the heterocysts [18]. An increased number of heterocysts could significantly increase the nitrogen fixation ability of the cyanobacteria. Therefore, the present study was designed to investigate the growth promoting potential of electricity adapted strains of *Anabaena variabilis* GITAM RGP on rice plant in pot culture carried out under greenhouse conditions. Cyanobacteria when present in the field, enhance the nutrient uptake, especially of the trace metals by the rice plants [19]. To test whether the variant of *Anabaena variabilis* also causes this enhanced uptake the micro and macro nutrients uptake studies from soil were conducted.

MATERIAL AND METHODS

Collection of cyanobacterial sample

Cyanobacterial sample was collected aseptically from Visakhapatnam Steel Plant effluent, Visakhapatnam, AP, India (17°38'04.97"N - 83°10'25.47"E) and brought to the Laboratory, Department of Microbiology, GIS, GITAM University for further processing.

Isolation and identification of cyanobacterial sample

For isolation and purification the techniques used were similar to those described earlier by Newton and Herman, 1979 [20]. Identification of the isolated cyanobacteria was based on morphological characters, microscopic studies, pigment production and 16S rRNA sequencing [21]. Identification on the basis of genomic study was performed by isolation of DNA from a 25 days - old pure culture using an ultraclean plant DNA isolation kit (MoBio Inc, USA). Amplification of 16S rRNA gene were carried out using universal primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3') using Quanta-Bio (Biotron, USA) thermal cycler. Montage PCR Clean up kit (Millipore) was used for removal of unincorporated PCR primers and dNTPs. The PCR amplified 16S rRNA genes were sequenced by Big Dye terminator cycle sequencing kit (Applied Biosystems, USA) by using M13 forward and reverse primers. The sequenced products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied Biosystems, USA). Isolated sequence of cyanobacterial strain was rearranged and compared with those available in the NCBI databases. After identification, the sequence was submitted to NCBI GenBank. Finally a phylogenetic tree was constructed with Clustal W and Tree view 2 software.

Electric shock treatment method

Design of electric chamber and electric shock treatment method on cyanobacterial cells were same as earlier reported by Pant et al. 2012 [22]. An electrophoresis unit with perforated diaphragm was used as electric chamber for the study. This diaphragm separates stage within the chamber and allows electric current and broth medium to circulate. Both anode and cathode terminals were joined to electrophoresis power supply unit. Cyanobacterial cell suspension, 5% (OD: 0.15 at 560 nm) were transferred in centric position of the stage after filling the chamber with 142.5 ml of autoclaved BG 11^- broth medium. Desired amperes of current and time were set by using the setting knobs for current and time on electrophoresis power supply unit. Mass production of purified algal culture was carried out by inoculating the culture in sterile BG 11^- medium. The culture was incubated under photon flux density (300 $\mu molm^{-2} s^{-1}$) at a temperature of 25 °C ± 1 for 15 days and then used for further investigation [23]. Electricity treated cyanobacterial cells were studied for the change in morphology under Motic images plus 2.0 ML microscope at 100 X magnification. The sample was transferred into a drop of water on a clean glass slide and observed under high power magnification for post electric treatment effect on vegetative cell and heterocyst dimensions.

Collection of seeds

Two varieties of rice seeds (HRK – 47 and HRK – 127) were provided by Uttaranchal Seeds & Tarai Development Corporation Ltd., Pantnagar, Uttarakhand, India.

Seed germination study

Two hundred rice seeds of HRK – 47 and HRK – 127 varieties were surface sterilized for 5 minutes with 5 % sodium hypochloride solution (NaOCl) then thoroughly rinsed with distilled water. For soaking twenty seeds were spread uniformly in each 15 cm Petri dish lined with Whatman No.1 filter paper. Three sets of Petri dishes were prepared for both the varieties of seeds (each set contained 3 Petri plates). In first and second set 5 ml of the control and test cyanobacterial cell suspension (prepared by dissolution of the 0.1 g fresh centrifuged cells) was added respectively. While third setup was maintained with water alone and used as experimental control. Finally all the three sets were kept for 16:8 hours, light:dark conditions under photon flux density of 300 $\mu molm^{-2} s^{-1}$ having relative humidity of 70-80 % and a temperature of 25/20 ± 2 °C, day/night. After 10 days, seedlings height, root length and percentage of germination were measured [24].

Pot culture study

To evaluate the potential of cyanobacterial strains (control and test) on both the varieties of rice (HRK – 47 and HRK - 127), pot culture experiments were designed. Soil pots (12" size) having 10 kg of (autoclaved) soil/pot (soil sample was taken from rice field of Anakapalle, Dist-Visakhapatnam, Andhra Pradesh., India - 17°41'00.11"N – 83°01'44.98"E) were employed in the study and irrigated to maintain 60 % of water holding capacity (WHC). Three sets of pots were prepared for both the varieties of seeds (each set contain 3 pots). 5 seedlings per pot of both the varieties of rice were transplanted after 10 days of seed germination. In first and second set control and test cyanobacterial cell suspensions were added respectively. Log phase cyanobacterial suspensions (12-15 days old) of control and test strains were prepared by suspending the pellet (obtained from high speed centrifugation at 10,000 rpm min^{-1} for 10 minutes) in sterile water followed by two steps of washing and finally added at the rate of 5 µg chlorophyll g^{-1} per pot, similar to process described earlier by Prasanna *et al*. [25]. While third set of pots was maintained with water alone and used as experimental control. All the three sets were kept for 40 days at 16:8 hours, light: dark conditions under photon flux density of 300 µmolm $^{-2}$ s $^{-1}$ and maintained with 60 % WHC at 25/20 ± 2 °C, day/night temperature.

After 40 days, impact of cyanobacterial inoculants on plant growth was evaluated in terms of different parameters including plant height, root length, weight of fresh and dry leaves and stem and weight of fresh and dry roots. The root and shoot length of plants from all the pots were measured after 40 days of growth. Fresh weight of leaves and roots were weighed with electric weighing balance (Precisa, 300-9321/M AG, Switzerland). Plant parts were dried at 70ºC for 48-72 h and weighed for obtaining dry weight. Soil parameters were measured before and after the growth of rice plants. Soil samples were collected from 0-20 cm depth from each pot. Determination of soil pH was carried out as described by Imoro et al. 2012 [26]. Soil sample of 10 gm was mixed with 25 ml of deionized water finally pH of suspension was measured by digital pH meter (Systronics - 335). Moisture percentage in soil samples were determined by moisture analyzer (OHAUS MB - 45) as per the operating manual. Particle density and porosity of soil was measured as described by Zhu *et al.* [27].

Study of metal content in rice plants and soil

Plant parts were fractionated into leaves, stems and roots. Gently samples were washed with deionised water and then dried at 70°C for 48 hours. One gram of dried powder of plant parts were added to 10 ml of concentrated HNO_3 (ultrapure 65 %) and incubated at room temperature for overnight. After incubation sample were heated for 4 hours at 120°C and then to 140°C until only 1 ml of liquid remained. After cooling, liquid was filtered and diluted up to 50 ml with deionized water. Chemical analysis were carried out from the same extract for measurement of Cu, Fe, Mn and Zn while available nutrients and heavy metals were detected in soil by ammonium bicarbonate – diethylene triamine penta acetic acid (AB – DTPA) method using flame atomic absorption spectrometry (Varian – AA240) [28, 29].

Statistical analysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD) of three replicates. Results were analyzed statically and accomplished by using Microsoft Excel 2007 (Roselle, IL, USA). Level of significance was evaluated by t-test at $^{**}P \leq 0.05$.

RESULTS

Effect of different current levels and time on the survival of *A. variabilis* GITAM RGP [Accession Number details – (JX134587) 1 sequence (20th Aug 2012) Authors: Pant, G & Prasuna, R. G.] and development of electricity adapted improved variant strain of *A. variabilis* GITAM RGP are similar as described by Pant *et al.* [30].

Post electric treatment effect on cell dimensions and heterocysts

Significant effects were observed on length and breadth of vegetative cells and heterocysts after exposure to electricity (Table 1). Electricity adapted strains (improved strain) showed 19.64 % and 21.35 % increase in the average length and breadth of the vegetative cells as compared to the vegetative cells of the wild strain. Similar effect was also observed with the heterocysts showing 28.18 % and 22.63 % increase in the length and breadth as compared to the wild strain. Heterocyst frequency of wild and improved strains were measured on X, XII and XIV day after electric exposure (Table 2). Heterocyst ferquency was found to increase by 139.79 % in improved strain as compared to the wild strain on the 14th day of incubation.

Table 1 Cell dimensions (in µm) of isolated *A. variabilis* (Wild strain) and there electricity adapted improved strain

Strains	Cell types			
	Vegetative cell		Heterocyst	
	Length	Breadth	Length	Breadth
***A. variabilis* (Wild strain)**	34.97 ±0.41	34.70 ±1.35	46.94± 1.41	47.01± 0.89
Electricity adapted strain (Improved strain)	41.84 ±0.29	42.11 ±1.53	60.17 ±1.13	57.65 ±0.83

Here values are expressed as mean ± standard deviation (n = 20)

Table 2 Heterocyst frequency after X, XII and XIV days of treatment

Days of treatment	Cyanobacterial Cells	No. of Heterocyst	No. of veg. cells between two heterocyst	Percentage of heterocyst
X	**Wild**	17.66±1.52	23.66±2.08	4.29±0.27
	Improved	9.0±1.0	10.33±1.52	9.81±1.39
XII	**Wild**	23.0±10.81	22.3±6.11	4.5±1.07
	Improved	10.66±3.21	10.0±1.0	10.06±1.01
XIV	**Wild**	16.33±2.51	21.33±3.5	4.85±1.26
	Improved	13.0±2.0	10.33±1.52	11.63±0.89

Values are expressed as mean ± standard deviation (*n* = 10).

Seed Germination study

Germination of (HRK – 47 and HRK - 127) rice seeds were recorded after 10 days of soaking with water and both (control and test) cyanobacterial suspensions (Fig. 1). Interesting results were recorded which illustrate an enhancement of 54 % and 63 % on germination of HRK – 47 and HRK -127 rice seeds respectively on soaking in test cyanobacterial cell suspension as compared with water (Table 3A and B). A comparison between soaking in test cyanobacterial cell suspension and in control strain cell suspension showed an increase of 13 % and 19 % on germination of HRK – 47 and HRK – 127 rice seeds respectively. The biofertilizer potential of the test cyanobacterial strain was also exhibited in increasing seedlings height by 68.38 % and 78.37 % for HRK – 47 and HRK – 127 rice seeds respectively when compared with water, and an increase of 29.13 % and 39.08 % when compared with control cells. Similarly the root growth was also improved on soaking seeds in cyanobacterial suspension as compared to water.

Table 3 Germination and seedling growth of rice seeds (HRK 47 and HRK 127) with water, control and test cell suspension after 10 days

(A) HRK – 47 seed

Sample	Water[1]	Control[2] Cells	Test[3] Cells
Germination (%)	61	83	94
Height (cm)	8.16 ± 0.26	10.64 ± 0.33	13.74 ± 0.42
Root length (cm)	2.58 ± 0.29	4.15 ± 0.23	4.97 ± 0.29

[1, 2 & 3] Plant seeds soaked in water, control cell suspension and test cell suspension

*Values are expressed as mean ± standard deviation (*n* = 3). Data was found significant at P ≤ 0.05.

(B) HRK – 127 seed

Sample	Water[1]	Control[2] Cells	Test[3] Cells
Germination (%)	57	78	93
Height (cm)	7.86 ± 0.36	10.08 ± 0.31	14.02 ± 0.19
Root length (cm)	2.64 ± 0.15	4.27 ± 0.33	5.04 ± 0.59

[1, 2 & 3] Plant seeds soaked in water, control cell suspension and test cell suspension

*Values are expressed as mean ± standard deviation (*n* = 3). Data was found significant at P ≤ 0.05.

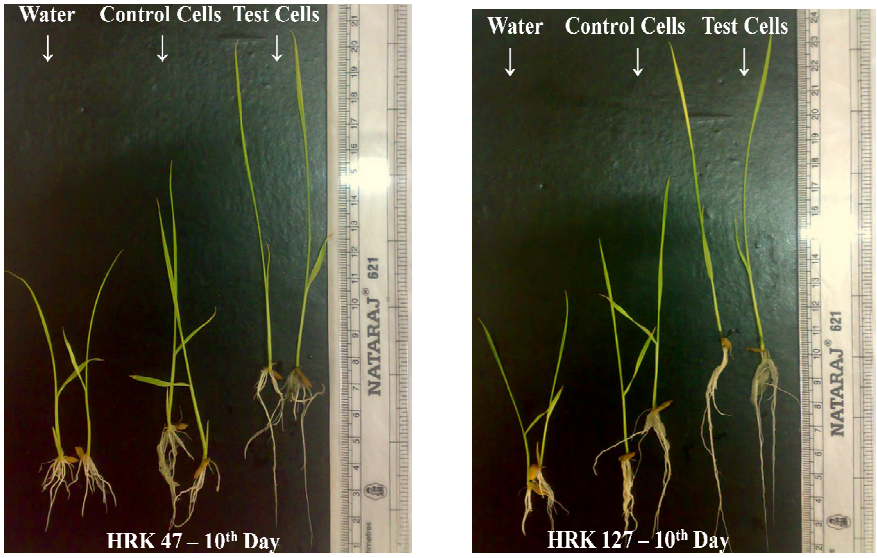

Fig. 1 Seedlings of rice varieties (HRK-47 and HRK-127) after 10 days of germination, with and without cyanobacterial suspension.

Pot culture study

Growth of rice plants with test strain suspension showed significant increase when compared to plants grown with water and control cell suspension (Fig. 2). The parameters were recorded after 40 days of growth (Table 4). In HRK-47 rice plants grown with test strain suspension, highest increase of 151.61 % in fresh weight of roots, followed by 99.9 % in fresh weight of leaves and 98 % in root length, least increase was observed in dry weight of root (87.87 %) when compared to plants grown with water. In the case of HRK-127 highest increase of 132.99 %, 110.8 %, and 104.55 % was observed with root length, leaves fresh weight and root fresh weight respectively in test strain treated plants compared to those grown with water. A higher growth was also obtained with test strain suspension used during plant growth as compared to the control strain suspension with highest increase in fresh weight of root (93.33 %) observed in HRK – 47 rice plants. The other rice variety HRK- 127 showed the highest increase of 86.95 % in fresh weight of leaves and a minimum of 48.57 % in dry weight of leaves.

Table 4 Effect of water, control cells and test cells on rice plants (HRK-47 and HRK-127) growth after 40 days

(A) HRK – 47 seeds

Sample	Water[1]	Control[2] Cells	Test[3] Cells
Plant height (cm)	52.38 ± 2.18	71.62 ± 1.88	102.76 ± 1.00
Roots length (cm)	7.24 ± 0.786	9.96 ± 0.594	14.31 ± 0.808
Weight of fresh leaf and stem (g)	4.180 ± 0.024	5.897 ± 0.024	8.359 ± 0.028
Weight of fresh root (g)	0.558 ± 0.034	0.726 ± 0.022	1.404 ± 0.03
Weight of dry leaf and stem (g)	0.292 ± 0.001	0.367 ± 0.002	0.559 ± 0.003
Weight of dry root (g)	0.066 ± 0.002	0.089 ± 0.081	0.124 ± 0.088

[1, 2, & 3] Indicate the presence or absence of cyanobacterial strains in the pot culture of HRK-47 rice plants after 40 days. [1] Plants were grown with tap water, [2] Plant grown with control cell suspension, [3] Plant grown with test strain suspension. *Values are expressed as mean ± standard deviation (n = 3). Data was found significant at $P \leq 0.05$.

(B) HRK – 127 seeds

Sample	Water[1]	Control[2] Cells	Test[3] Cells
Plant height (cm)	34.56 ± 2.02	49.94 ± 1.793	67.76 ± 1.364
Roots length (cm)	5.94 ± 0.808	8.3 ± 0.406	13.84 ± 0.32
Weight of fresh leaf and stem (g)	2.916 ± 0.016	3.288 ± 0.019	6.147 ± 0.015
Weight of fresh root (g)	0.395 ± 0.019	0.486 ± 0.02	0.808 ± 0.031
Weight of dry leaf and stem (g)	0.279 ± 0.002	0.315 ± 0.003	0.468 ± 0.003
Weight of dry root (g)	0.038 ± 0.041	0.047 ± 0.003	0.071 ± 0.001

[1, 2, & 3] Indicate the presence or absence of cyanobacterial strains in the pot culture of HRK-127 rice plants after 40 days. [1] Plants were grown with tap water, [2] Plant grown with control cell suspension, [3] Plant grown with test strain suspension. *Values are expressed as mean ± standard deviation (*n* = 3). Data was found significant at P ≤ 0.05.

Fig. 2 Effect of cyanobacterial cells (control and test) on growth of rice plants (HRK – 47 and HRK - 127) after 40 days.

Physicochemical parameters of soil

Soil parameters such as pH, moisture content etc. were measured before and after the growth of seedlings with various treatments. Addition of test cyanobacterial suspension to the soil brought about an increase in moisture content and soil porosity as compared to control cell suspension. There was an increase in moisture and porosity of soil in the presence of plant and test cyanobacterial strain (16 % and 38.09 % respectively for HRK – 47 grown soil, and 18.28 % and 30.43 % respectively for HRK - 127 grown soil) as compared to that of control plant without cyanobacteria. Soil with plant and test cyanobacterial strain suspension also showed 4.84 % and 6.93 % decrease in pH for HRK – 47 and HRK - 127 grown soils respectively when compared to soils with the plants and control cells (Table 5). A reduction in bulk density and electrical conductivity by 7.33 % and 6.66 % respectively for HRK – 47 grown soil and 3.44 % and 7.14 % for HRK – 127 grown rice soil were observed.

Table 5 Physicochemical parameters of soil before and after growth of rice plant

Parameter	Seed variety	Initial Soil[1]	Control[2]	Wild[3] strain	Improved[4] strain
pH	HRK 47	7.07 ± 0.060	6.97 ± 0.101	6.81 ± 0.234	6.48 ± 0.496
	HRK 127		6.91 ± 0.026	6.63 ± 0.035	6.17 ± 0.025
Moisture (%)	HRK 47	24.82 ± .035	25.88 ± .025	31.74 ± .035	36.82 ± .122
	HRK 127		25.96 ±.030	32.26 ±.025	38.16 ±.036
Bulk density (g/ml)	HRK 47	1.69 ± 0.015	1.59 ± 0.030	1.50 ± 0.026	1.39 ± 0.015
	HRK 127		1.53 ± 0.045	1.45 ± 0.035	1.40 ± 0.010
Porosity (%)	HRK 47	11 ± 1.000	16 ± 1.000	21 ± 1.000	29 ± 1.000
	HRK 127		19 ± 1.000	23 ± 1.527	30 ± 1.000
EC (ds m^{-1})	HRK 47	0.21 ± 0.015	0.17 ± 0.001	0.15 ± 0.001	0.14 ± 0.001
	HRK 127		0.17 ± 0.001	0.14 ± 0.001	0.13 ± 0.002

Physicochemical parameters of soil before and after (40 days) growth of rice plants (HRK – 47 and HRK -127). [1]Initial soil parameteres before plant growth, [2]Soil parameters after growth of rice plants with water, [3]Soil parameters after growth of rice plants with control cells, [4]Soil parameters after growth of rice plants with test cells. *Significant difference at 0.05 level (P ≤ 0.05)

Metal concentrations in soil exhibit reduction in copper and iron levels by 19.48 % and 3.9 % for HRK – 47 rice soil and 23.23 % and 25 % for manganese and zinc levels respectively when soil inoculated with test strain suspension were compared with that inoculated with control strain suspension. In case of HRK -127 rice soil, presence of test strian suspension showed 10.76 % and 3.55 % reduced level for copper and iron while 19.9 % and 20.45 % for manganese and zinc level when compared with control cell suspension as shown in figure 3.

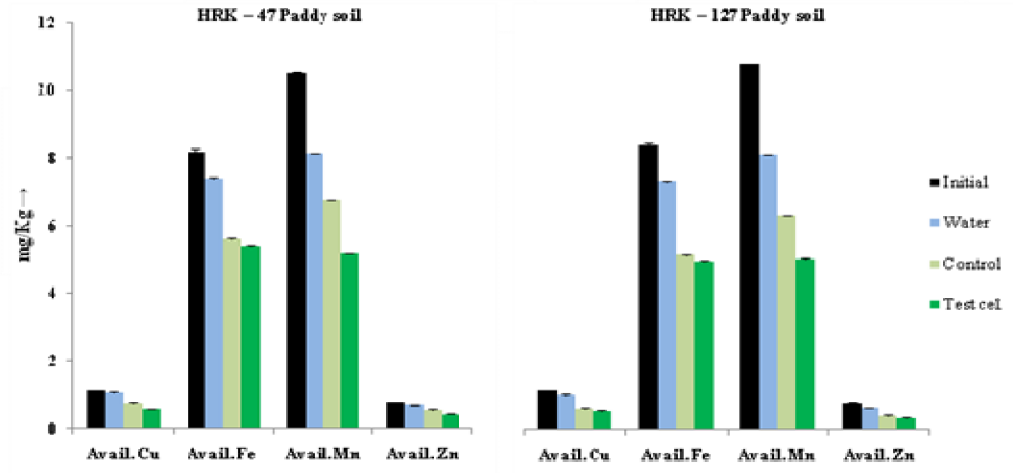

Fig. 3 Metal concentration (Cu, Fe, Mn and Zn) in soil before and after growth of rice plants with and without cyanobacterial cells (control and test) after 40 days

Metal studies in rice plant

Metal concentration in plant leaves and roots demonstrated improved level when cyanobacterial cells were present in soil. There was a significant increase of 28.78 % and 38.65 % of copper and iron levels in leaves of HRK – 47 rice plant while 21.34 % and 21.74 % increase in manganese and zinc concentration respectively when plant grown with test strain cell suspension were compared with those of control (Table 6A). In addition metal concentration in roots also exhibit enhanced concentration as shown in table 6B. Metal concentration studies in HRK – 127 rice plants with test cells also demonstrates elevated levels as shown in table 6C and D. An enhanced concentration of 57.64 %, 49.39 %, 34.76 % and 39.79 % of copper, iron, manganese and zinc were found in rice leaves in presence of test *A.variabilis* cells.

Table 6 Metal concentration (mg/kg) in plant parts with water, control cells and test cells

(A) Leaves of HRK – 47 rice plant

Metal Concentration	Control[1]	Wild[2] strain	Improved[3] strain
Avail. Cu	1.833 ± 0.022	3.700 ± 0.143	4.165 ± 0.113
Avail. Fe	4.949 ± 0.046	9.818 ± 0.024	13.617 ± 0.022
Avail. Mn	7.599 ± 0.013	11.429 ± 0.038	13.868 ± 0.022
Avail. Zn	2.765 ± 0.126	3.794 ± 0.165	4.619 ± 0.064

Metal concentration in [1] HRK-47 plant leaves grown with tap water, [2] HRK-47 plant leaves grown with control cell supension and [3] HRK-47 plant leaves grown with test cell suspension. *Significant difference at 0.05 level (P ≤ 0.05).

B) Roots of HRK – 47 rice plant

Metal Concentration	Control[1]	Wild[2] strain	Improved[3] strain
Avail. Cu	6.661 ± 0.103	10.478 ± 0.113	13.899 ± 0.060
Avail. Fe	11.641 ± 0.044	14.845 ± 0.054	17.935 ± 0.038
Avail. Mn	9.834 ± 0.037	12.572 ± 0.023	15.716 ± 0.199
Avail. Zn	3.930 ± 0.047	5.809 ± 0.055	8.057 ± 0.061

Metal concentration in [1]HRK-47 plant roots grown with tap water, [2] HRK-47 plant roots grown with control cell supension and [3] HRK-47 plant roots grown with test cell suspension. *Significant difference at 0.05 level (P ≤ 0.05).

(C) Leaves of HRK – 127 rice plant

Metal Concentration	Control[1]	Wild[2] strain	Improved[3] strain
Avail. Cu	1.986 ± 0.017	2.956 ± 0.052	4.66 ± 0.084
Avail. Fe	3.748 ± 0.057	7.38 ± 0.026	11.023 ± 0.061
Avail. Mn	6.584 ± 0.008	9.944 ± 0.030	13.392 ± 0.014
Avail. Zn	1.779 ± 0.197	2.42 ± 0.064	3.383 ± 0.104

Metal concentration in [1] HRK-127 plant leaves grown with tap water, [2] HRK-127 plant leaves grown with control cell supension and [3] HRK-127 plant leaves grown with test cell suspension. *Significant difference at 0.05 level (P ≤ 0.05).

(D) Roots of HRK – 127 rice plant

Metal Concentration	Control[1]	Wild[2] strain	Improved[3] strain
Avail. Cu	5.945 ± 0.041	9.817 ± 0.031	12.905 ± 0.008
Avail. Fe	10.810 ± 0.058	13.516 ± 0.038	16.776 ± 0.037
Avail. Mn	4.926 ± 0.052	6.614 ± 0.021	8.832 ± 0.331
Avail. Zn	2.853 ± 0.130	3.793 ± 0.068	5.583 ± 0.100

Metal concentration in [1]HRK-127 plant roots grown with tap water, [2]HRK-127 plant roots grown with control cell supension and [3]HRK-127 plant roots grown with test cell suspension. *Significant difference at 0.05 level (P ≤ 0.05).

DISCUSSION

Rice is one of the most important staple food crops in the whole world. Cyanobacteria have long been associated with rice fields. The application of living algal cells to the crop fields is known as "algalization technique" has long been advocated as an ecofriendly and efficient method of introducing biofertilizer to the field [11]. Free living heterocystous cyanobacteria, for example *Aulosira sp.*, *Aphanothece*, *Myxosarcina, Oscillatoria, Cylindrospermum* etc. are well known for aerobic phototrophic nitrogen fixation in rice field ecosystems under flooded water conditions [12, 31, 32].

Increasing population and limited area of fertile, arable soil necessitate the development of new techniques to enhance crop productivity. One of the methods employed to achieve higher crop productivity and reduction in yield loss is usage of improved biofertilizer strains. Wild strains can be improved either by genomic mutations or by exposure to abiotic stress factors which includes temperature, salinity or water stress *etc* [33, 14]. Exposure to such stress factors may cause development of stress tolerance mechanism in cyanobacteria, allowing growth and survival in various adverse conditions. For last few eras, *Anabaena* species has been used to understand the types of nitrogenases, pesticide resistance, secondary metabolite production and it has also been one of the most used cyanobacterium for genetic manipulations [34, 35, 36].

Present study was designed to provide an initiation to the use of electric current as an agent for strain improvement in cyanobacteria. Over the classical techniques such as X rays, UV rays, chemicals, abiotic factors etc, the application of electric current is highly effective being easier to control at the same time. On the basis of requirement, stimulatory (under controlled - low intensity) or inhibitory (strong pulses) electric currents can be applied. The use of electric current on plant growth has been evaluated by a few authors long back, in which they reported 30% enhancement in linear growth of Scotia tomato plants with significant increases in metal uptake capacity (K, Ca and P) when plants were grown in presence of electric current. They elucidated role of electric current in internal distribution of growth regulators and stimulation of active ion pumps [37]. Pohl and Todd, 1981 [38] and Pohl, 1977 [39] showed the electric field as a potential factor for enhancing crop productivity. They explained the mechanisms and effectiveness of electric treatment which stimulates seed germination, earlier blossoming and finally growth in *Exacum affine* (Persian violet) under greenhouse conditions. Further, Moon and Chung, 2000 [40] reported 2.8 times enhancement in germination percentage by exposure to electric and magnetic field on tomato seeds.

Earlier studies on the effect of electric current on survival and growth of native (unadapted) *A. variabilis* GITAM RGP cells revealed that at 10 amperes current for 80 minutes was the maximum tolerated exposure time by the strain [18]. On exposure to electric field beyond this time, significant lethal effects

were observed. The microscopic observation showed pigment leakage, cell membrane/wall degradation, and absence of intact cells in the cultures exposed to the higher time periods. This could be brought about by the denaturation of the cell membrane through drastic damage to the proteins or lipids of the membranes. Incubation of these cells did not reveal any growth restoration which might also indicate complete breakdown of the cell machinery including the genome.

To test development of resistance to the electric field, the cyanobacterial samples surviving after the initial exposures, were again used for electric exposures after 15 days interval for growth. The cells initially exposed to 10 amperes current for 80 minutes were used for development of electricity adapted improved variant of *A. variabilis* GITAM RGP. Improved cells showed enhancement in limit of tolerance to electric current as they survived even after subjecting to electric current of 10 amperes upto 6 hours. Such electricity adapted cultures showed the increase in heterocyst frequency, presence of multiple heterocysts and change in cell dimensions. Change in heterocyst frequency as an effect of various environmental factors such as high light intensity, high temperature has been reported in various cyanobacterial species. Adams and Carr, 1981 [41] reported enhancement of heterocyst differentiation rate and cell division in *Anabaena cylindrica* due to controlled high light intensity. Mixed results were obtained by Kannaiyan and Somporn, 1989 [42] who described effect of controlled high temperature on growth of five species of *Azolla-Anabaena* symbiosis (*A. Mexicana* BR-GL, *A.caroliniana* WT-V, *Azolla sp*. ST-SI, *A. filiculoides* BR-H and *A.micro-phylla* BR-GI). *A.caroliniana* WT-V and *A. filiculoides* species showed poor growth in high temperatures, while larger biomass was obtained with *A. microphylla* BR-GI and *Azolla sp*. ST-SI strain. Similarly, the increased frequency of heterocysts observed in *A. variabilis* GITAM RGP electric adapted strain might be a direct effect of any mutation or may be a response to nitrogen starvation. Nitrogen starvation might be caused by alteration in permeability of heterocyst or vegetative cell membranes causing inept assimilation of fixed nitrogen, or due to the faster growth of the organism requiring more fixed nitrogen.

The data based on the recurrent exposure of electric current to the samples initially given sublethal dose of electricity indicated that the *Anabaena variabilis* can be adapted to the electric field exposure. Present study hypothesized that being an abiotic stress factor, the exposure to electric field might be generating some chemically active species such as the free radicals or reactive oxygen species, leading ultimately to changes in the nucleic acids, proteins or the membranes. However more work needs to be done on this aspect to deduce the exact mechanism of action of the electric field on the cyanobacterium.

Various experiments were performed to ascertain changes in the electricity adapted strain (improved strain) as compared to the wild strain (untreated strain). Wild cells of *A. variabilis* GITAM RGP showed typical growth curve with lag, log and stationary phase, similar pattern of growth has been recorded by others with *Anabaena variabilis* [43, 44]. An increase in the dimensions of the vegetative cells and heterocysts were observed, with an enhancement of the heterocyst frequency in the improved strain. The cell size is a fundamental factor influencing the growth and ecology of algae as it controls the uptake and assimilation of various nutrients. However the higher growth rate of the improved strain suggested that the growth rate is not limited by the nutrient requirements. This increase in cell size could have resulted from an alteration in the membrane structure. In comparison to the wild strain there was an increase in growth observed in electricity adapted strain of *A. variabilis* GITAM RGP.

Present work assessed the potential of the *Anabaena variabilis* GITAM RGP, wild and improved (electric stress adapted) strains as a biofertilizer for rice plants. Various parameters of plants growth such as seed germination and seedlings growth were recorded in the presence and absence of wild and improved cyanobacterial strains in the medium or soil, as the case may be. Two rice varieties were used for the studies namely HRK–47 and HRK-127. The data obtained established the potential of cyanobacterial cells over their absence on enhanced seed germination and growth parameters of rice plants. All the parameters studied, such as the root length, fresh weight of root, fresh weight of leaves, height of seedling were seen to enhance in the presence of algae. The potential of growth enhancement was more with the test or improved strain as compared to the wild cyanobacterial strain. Of the two rice varieties tested, HRK-127 showed a higher growth enhancement than HRK-47. This is interesting as HRK-47 variety initially showed higher seedling germination and plant height, but lower root length than HRK-127. There may be some kind of association formed between the roots of the plants and the cyanobacterium applied. The longer roots of seedlings of HRK-127 may provide for higher interaction or association, achieving better growth enhancement. Present results are in accordance with those obtained by other researchers working with the rice and cyanobacterial systems [45]. There has also been growth enhancement obtained with other plants such as wheat, maize and tomato using cyanobacteria [46, 47, 48]. The data obtained suggest the possible use of the obtained improved strain as a biofertilizer.

In addition present study also assessed the soil characteristics before and after cyanobacterial application and found enhancement of moisture content and soil porosity as compared to control. There was also a reduction in soil pH observed with the cyanobacterial application. This property may be useful for the reclamation of high pH soils for agriculture. The reclamation of non-arable lands to agriculture has also been reported earlier [49]. The concentrations of metal such as copper, iron, manganese and zinc were observed to reduce in soil inoculated with improved strain suspension and wild strain suspension as compared to the control. Whereas the available metal concentration in the leaves and roots of plants grown in the same experiment showed an increase with respect to all the four metals tested. This points to a possible role of cyanobacteria in absorbing the metals from the soil but releasing or transferring it to the rice plants.

CONCLUSION

Rice is an important food crop in the whole world. Due to increasing population and fixed area of fertile soil, new techniques are applied and appreciated which enhances crop productivity with maintenance of field fertility. Large number of studies has reported that long term application of chemical fertilizer has serious adverse effect on environment and also makes the soil permanently infertile. To overcome this problem "algalization technique" is used which is an ecofriendly, efficient biological tool. Free living cyanobacteria are well known for aerobic phototrophic nitrogen fixation in rice field ecosystem with flooded water conduction. The present work established the potential of test algal cells over their absence on enhanced growth parameters of rice plant. In addition algal cells also enhance metal uptake capability by plant which helps in growth and productivity with maintaining soil fertility without any pollution. Our observations also demonstrated a new method to improve this potential of cyanobacterial cells by minimum electric current (MEC) treatment.

ACKNOWLEDGEMENT

The authors are grateful to GLA University, Mathura, U.P. and GITAM University, Visakhapatnam, Andhra Pradesh, India providing the facilities for research.

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