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Role of Potential Cyanobacterial N_2 Fixer on Growth and Photosynthetic Pigments of Basmati Rice

Premsing Shivsing Marag*, Pranita Jaiswal, Archna Suman and Dolly Wattal Dhar

Division of Microbiology, Centre for Conservation and Utilisation of Blue Green Algae, ICAR-Indian agricultural Research Institute, New Delhi-110012, India

**Corresponding author*

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A B S T R A C T

The effect of potential cyanobacterial N_2 fixer (*Anabaena cylindrospermoides*) was studied on growth parameters, root architecture and photosynthetic pigments of basmati rice (variety : PB-1121) grown in pots with soil as rooting medium under controlled conditions of National Phytotron Facility of ICAR-IARI, New Delhi. The treatments used were as T1: 100% N, T2: 75%N, T3: 75%N + N_2 fixer, T4: 50% N, T5: 50% N + N_2 fixer. In general, treatment T3 showed enhanced total plant length, root length, total fresh weight, root and shoot fresh weight as well as dry weight of plants as compared to treatment T1. However, the treatment effect was non-significant for root and shoot dry weight as well as number of leaves. Root architecture also altered with treatments and total root length, surface area and root volume in under treatment T3 were *at par* with the observations under treatment T1. Chlorophyll *a* and chlorophyll *b* were statistically similar under treatments T3 and treatment to T1, whereas, total carotenoids were not influenced by different treatments under study. The influence of cyanobacterial inoculant could be attributed to the nitrogen fixing property with the potential of extracellular ammonia release and production of Indole Acetic Acid by the strain used. Further testing under outdoor conditions will help to assess its efficiency and exploitation in integrated nutrient management of rice crop.

Introduction

Rice is mainly grown under irrigated conditions where N-fertilizer efficiency is low due to large nitrogen losses from flooded soil (De Datta and Buresh, 1989). Hence, to cope with the increasing demand for food, increase in rice yield and production will be required without creating adverse impact of chemical fertilizers on environment. Therefore, to minimize environmental problem, we need to go for alternative strategy like use of microbial biofertilizers which play a role in plant productivity through biological nitrogen fixation with other plant growth promoting traits. Till date, only prokaryotic microorganisms *i.e.* symbiotic and free-living eubacteria, including cyanobacteria have been reported to possess the nitrogen fixation potential.

Cyanobacteria comprise a large group of structurally complex, photosynthetic gram negative prokaryotes which flourish in rice

field and play a major role in maintaining the fertility of its ecosystem. Cyanobacteria inhabiting soil improve its fertility through beneficial mechanisms like fixation of atmospheric nitrogen, inorganic phosphate solubilization by excretion of organic acids and extracellular phosphatases, production of phytohormones and siderophores (Bose and Nagpal, 1971; Rai and Sharma, 2006; Prasanna *et al.,* 2013). These, in turn, may influence growth and productivity of crops (Rodriguez *et al.,* 2006; Rastogi and Sinha, 2009). Further, exopolysaccharides (EPS) secretion by cyanobacteria helps in forming soil particle aggregate and humus formed after death and decay develops strong reducing condition in soil (Vaishampayan *et al.,* 2000) and both these conditions can also improve structure and fertility of soil (Abdel-Raouf *et al.,* 2012).

These contribute 20–30 kg fixed nitrogen per ha and can also add organic matter to the paddy fields (Issa *et al.,* 2014). Positive effects of cyanobacterial inoculation on shoot/root length, dry weight and yield of wheat crops has been reported (Spiller and Gunasekaran, 1990; Karthikeyan *et al.,* 2009). In view of this, present study was undertaken under controlled conditions of National Phytotron Facility of ICAR-IARI, New Delhi to understand the effect of potential cyanobacterial nitrogen fixer on plant growth, root architecture and photosynthetic pigments of basmati rice.

Materials and Methods

Co-cultivation approach was used to examine the effect of *Anabaena cylindrospermoides* on basmati rice (variety: PB-1121), under controlled conditions of temperature (30°C/25°C), light (14h) and dark cycles (10 h) with a relative humidity of 70% at National Phytotron Facility, ICAR-IARI, New Delhi for the complete experimental period.

Growth and maintenance of cyanobacterial strain

Cyanobacterial strain (*Anabaena cylindrospermoides*) was isolated from rhizospheric soil of basmati rice (variety: PB-1121) grown at Genetics Block field of ICAR-IARI was used as an inoculum for the study. The strain had an appreciable nitrogenase activity in terms of Acetylene reducing assay (3161.84 nmoleC_2H_4/mg chl/ h) with extracellular ammonia (22.80 µmole NH_4^+/ml) release potential and production of Indole acetic acid (25.04 µg/ml). The purity of cyanobacterial strain was checked regularly through microscopic observations. It was grown and maintained in nitrogen depleted BG-11 medium (Stanier *et al.,* 1971) with suitable pH (7.0-7.5) at 28±2°C under light: dark cycles of 16:8 h with light intensity of 50-55 µmole photons/ m^2/s. Growth of cyanobacterial strain was analyzed as chlorophyll content during exponential phase of growth (14^{th} day) (McKinney, 1941).

Raising of rice seedling and co-culturing experiment

The seeds of rice (variety: PB-1121) were surface sterilized with 70% alcohol for 30 seconds followed by dipping in mercuric chloride solution (0.1%) for 5min. Subsequently, the seeds were rinsed several times with sterilized distilled water and dipped overnight either in sterilized BG-11 (-N) medium for un-inoculated (control) treatment or in cyanobacterial suspension with chlorophyll concentration of 5.0 µg/ mL (inoculated treatment).Rice seedlings were raised under control as well treated conditions in plastic trays containing soil as growth medium under controlled conditions of National Phytotron Facility. One month old seedlings were used to undertake experiment adopting co-cultivation approach with rice seedlings and cyanobacterial suspension.

Study was conducted in pots (dia-6") having well sieved IARI field soil (2 kg per pot) having specific physico-chemical properties (pH: 8.12, organic carbon: 0.47%, available nitrogen: 168 kg/ha, available P: 15 kg/ha and available K: 251 kg/ha). The pots were lined with polythene sheet before filling up with soil for the retention of water, maintenance of slight acidic pH and prevention of loss of nutrients from the hole at the bottom. Three rice seedlings were planted per pot and the pots were irrigated daily to maintain the water level. The weeding was also done regularly during experimental duration. Total treatments used in the study were as T1: 100% N, T2: 75% N, T3: 75% N + nitrogen fixer, T4: 50% N, T5: 50% N+ nitrogen fixer. Nitrogen was provided as urea and the doses were varied as per treatment on the basis of recommended dose of fertilizers (RDF) *i.e*. (120 kg N/ ha). Half of the nitrogen along with phosphorous (single super phosphate) and potassium (Muriate of Potash) in the ratio of 60 kgas P_2O_5/ha and 40 kg as K_2O/ha were provided as basal dose in soil. Cyanobacterial suspension (chlorophyll concentration: 5.0µg/mL) from exponential phase was added after five days of transplantation under the treatments T3 and T5 and the remaining half of nitrogen was given after 15 days of transplantation. Three replications were maintained for each treatment and the experiment was repeated thrice.

Plant biometric parameters

Plant samples were taken at 30 days after transplantation and total plants were uprooted from pots following complete precautions. Roots were washed thoroughly under running tap water. The morphological parameters (root and shoot height, number of leaves, root and shoot fresh weight) were recorded from each treatment. For dry weight measurement, plant samples were oven dried at 60^0C for 3-4 days till constant weight was achieved.

Root architecture through root scanner

Thoroughly washed roots were kept wet in water for the root scanner observations. Digital images of plant roots were acquired by root scanner (Model: Epson Perfection V 700 Photo Programme: Win-RHIZO Programme V. 2009 c 32-bit Software). Upon image processing, segmentation and analysis, various root parameters *viz*., total length (cm), total surface area (cm^2), average diameter (mm) and total root volume (cm^3) were measured.

Estimation of plant photosynthetic pigments (chlorophyll and carotenoids)

Modified hot extraction protocol involving Dimethyl Sulphoxide (DMSO) was used for the estimation of chlorophyll and carotenoids in the fresh leaves from different treatments (Jeffrey and Humphrey 1975). Spectrophotometric absorbance was recorded at 663, 645 and 480 nm and the pigments were expressed as mg/g fresh weight. Chlorophyll *a*, chlorophyll *b* and total chlorophyll were estimated using the formula by Arnon (1949) while carotenoids were determined following the formula by Lichtenthaler and Welburn (1983). The formulae used for the calculation of pigments are given as under.

Chlorophyll *a* = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times V/W \times 1000$

Chlorophyll *b* = $(22.9 \times A_{645} - 4.68 \times A_{663}) \times V/W \times 1000$

Total chlorophyll = $(20.2 \times A_{645} + 8.02 \times A_{663}) \times V/W \times 1000$

Total carotenoids = $(A_{480} + (0.114 \times A_{663}) - (0.638\text{-}A645)) \times V/W \times 1000$

Where, A is the absorbance at different

wavelengths, W = Sample weight in g and V= Volume of solvent (mL).

Statistical analysis

The data was analyzed using MS-Excel for measuring mean, standard deviation and OPSTAT online software was used for calculating critical difference and standard errors. All the values were mean of three replications.

Results and Discussion

Plant biometric parameters

Plant growth parameters were assessed for root and shoot length, root and shoot fresh and dry weight and number of leaves per plant. The maximum total plant length was exhibited in treatment T3 (85.03 cm) followed by treatment T1 (84.59 cm) with the lowest recorded in treatment T4 (66.37 cm). The total plant length in treatments T2 (76.15 cm) and T5 (76.96 cm) were almost *at par* (Fig. 1). The root length per plant was highest under the treatment T3 followed by treatments T1, T5 and T2 with lowest root length under the treatment T4. This data clearly showed the positive influence of cyanobacterial strain on total as well as root length as the treatment T3 was better over treatment T1. Shoot length per plant was maximum under treatment T1 followed by treatments T3, T2 with the lowest under treatment T4 (Table 1).

Total fresh weight per plant was also highest in treatment T3 (3.95g) followed by T1 (3.74g) and lowest in T4 (2.34g) (Fig. 1). Root fresh weight was highest in treatment T3 which was *at par* with treatment T1 followed by treatments T5, T2 and treatment T4 recorded lowest root fresh weight. Similar trend was observed for shoot fresh weight as well, wherein maximum was observed in treatment T3 followed by treatments T1, T5 with minimum in treatment T4. The reduced fresh weight per plant under T4 could be due to lesser application of chemical nitrogenous fertilizer as compared to other treatments (Table 1).

Total dry weight was recorded to be highest in treatment T3 (0.39 g/plant) followed by treatment T5 (0.33g/plant), and the total dry weight under the treatment T2 was *at par* with treatment T1 and it was lowest in treatment T4 (0.20g/plant) (Fig. 1). Root and shoot dry weight and number of leaves per plant remained more or less similar under different treatments at 30^{th} day of observations (Table 1). The results were consistent with the reports of Saadatnia and Riahi, (2009) who reported the significant effect of cyanobacteria on rice plants in pot experiment over control for various parameters *viz*., faster germination, increase of 53% in plant height, 66% in roots length, 80% in root fresh weight, 150% in root dry weight,58% in leaf and stem fresh weight, 125% in leaf and stem dry weight. They also reported an increase of 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. Prasanna *et al.,* (2009) also observed the positive influence of cyanobacterial strains isolated from the rhizosphere of diverse rice and wheat varieties on enhancing soil microbial biomass carbon, available nitrogen, and related soil microbiological parameters as well as increased grain yields and grain weight of rice crop. The treatments in which *Calothrix ghosei* (K1), *Hapalosiphon intricatus* (K2) and *Nostoc* sp. (K3) were applied along with 1/3 N + P + K gave statistically equivalent results as compared to application of full dose of chemical fertilizers in terms of grain yield in wheat crop (Karthikeyan *et al.,* 2007).

Table.1 Comparative plant length (cm / plant), plant weight (fresh and dry weight; g/plant) and number of leaves per plant in basmati rice (variety: PB-1121) at 30 days after transplantation (DAT) under selected treatments

Treatments	Root length	Shoot length	Root fresh weight	Root dry weight	Shoot fresh weight	Shoot dry weight	Number of leaves
T1	15.88±1.832	68.70±3.564	1.05±0.153	0.086±0.017	2.69±0.230	0.22±0.037	6.55±0.508
T2	12.04±0.940	64.11±3.006	0.71±0.020	0.065±0.003	1.90±0.046	0.24±0.142	5.53±0.503
T3	17.07±1.008	67.96±2.625	1.07±0.155	0.094±0.030	2.88±0.755	0.31±0.200	6.92±1.508
T4	10.66±1.527	55.70±3.719	0.51±0.044	0.078±0.008	1.83±0.115	0.13±0.022	5.79±0.262
T5	13.41±0.523	63.55±3.151	0.80±0.095	0.082±0.002	2.30±0.400	0.25±0.102	4.96±0.503
SE (±)	0.724	1.869	0.063	0.010	0.231	0.070	0.455
CD (0.05p)	2.312	5.966	0.200	NS	0.737	NS	NS

* All treatments include Recommended Dose of Fertilizers (RDF) as P and K while N of RDF is varied as T1= 100% N; T2=75% N; T3= 75% N + N_2 fixer; T4= 50% N; T5=50% N + N_2 fixer;
N_2 fixer: *Anabaena cylindrospermoides*. Each value is a mean of three replications

Table.2 Comparative total length (cm), surface area (cm^2), average diameter (mm), volume (cm^3) of roots per plant in basmati rice (variety: PB-1121) at 30 days after transplantation (DAT) under selected treatments

Treatments	Total length	Total surface area	Average diameter	Root volume
T1	182.70±6.854	8.50±0.500	1.09±0.269	2.82±0.331
T2	152.11±7.670	6.80±0.283	0.77±0.049	1.47±0.081
T3	177.51±4.169	8.30±0.317	0.95±0.028	2.76±0.108
T4	101.89±3.213	5.63±0.293	0.64±0.020	0.88±0.055
T5	117.41±4.856	6.23±0.110	0.88±0.073	1.26±0.074
SE (±)	3.236	0.188	0.074	0.096
CD (0.05p)	10.330	0.600	0.236	0.305

* All treatments include Recommended Dose of Fertilizers (RDF) as P and K while N of RDF is varied as T1= 100% N; T2=75% N; T3= 75% N + N_2 fixer; T4= 50% N; T5=50% N + N_2 fixer;
N_2 fixer: *Anabaena cylindrospermoides*. Each value is a mean of three replications

Table.3 Comparative chlorophyll (*a*, *b*, total: mg/g fresh weight) and carotenoids (mg/g fresh weight) in basmati rice (variety: PB-1121) at 30 days after transplantation (DAT) under selected treatments

Treatments	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Total carotenoids
T1	3.39±0.201	0.90±0.066	4.46±0.543	0.29±0.055
T2	2.84±0.231	0.64±0.051	4.05±0.263	0.24±0.028
T3	3.28±0.041	0.95±0.046	4.19±0.046	0.28±0.020
T4	2.96±0.093	0.77±0.108	3.80±0.140	0.22±0.019
T5	2.79±0.246	0.61±0.173	3.42±0.420	0.23±0.010
SE (±)	0.105	0.058	0.194	0.018
CD (0.05p)	0.335	0.186	0.619	NS

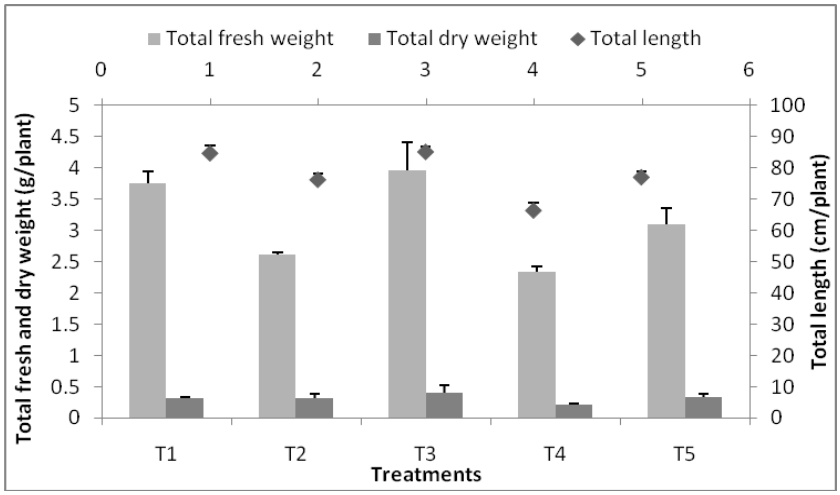
* All treatments include Recommended Dose of Fertilizers (RDF) as P and K while N of RDF is varied as T1= 100% N; T2=75% N; T3= 75% N + N_2 fixer; T4= 50% N; T5=50% N + N_2 fixer;
N_2 fixer: *Anabaena cylindrospermoides*. Each value is a mean of three replications

Table.4 Comparative total pigments (mg/g fresh weight) and their ratio in basmati rice (variety: PB-1121) at 30 days after transplantation (DAT) under selected treatments

Treatments	Total pigments	Chl/pigments	Carot/pigments	Chl/ carot
T1	4.750381	0.939792	0.060208	15.609
T2	4.304147	0.94312	0.05688	16.58077
T3	4.476187	0.938222	0.061778	15.18695
T4	4.030002	0.944994	0.055006	17.1797
T5	3.649319	0.937416	0.062584	14.97853

* All treatments include Recommended Dose of Fertilizers (RDF) as P and K while N of RDF is varied as T1= 100% N; T2=75% N; T3= 75% N + N_2 fixer; T4= 50% N; T5=50% N + N_2 fixer;
N_2 fixer: *Anabaena cylindrospermoides*. Each value is a mean of three replications

Fig.1 Comparative total plant length, fresh weight and dry weight in basmati rice (variety: PB-1121) at 30 days after transplantation (DAT) under selected treatments



* All treatments include Recommended Dose of Fertilizers (RDF) as P and K while N of RDF is varied as T1= 100% N; T2=75% N; T3= 75% N + N_2 fixer; T4= 50% N; T5=50% N + N_2 fixer;
N_2 fixer: *Anabaena cylindrospermoides.* Each value is a mean of three replications

Significant differences were recorded in pot culture study involving application of algal extract of *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89, on vegetable crops for plant height, root length, dry and fresh weight of plant as well as leaf number after 40 days from planting (Shariatmadari *et al.*, 2013).

Root architecture

Root scanning results revealed the maximum total length of roots under treatment T1 (182.70 cm), followed by treatments T3 (177.51 cm), T2 (152.11 cm), T5 (117.41 cm) with the lowest under treatment T4 (101.89 cm). Total surface area also exhibited the similar pattern wherein, treatment T1 showed highest value followed by treatments T3, T2, T5 and T4. Average diameter was seen to be maximum under treatment T1 followed by treatments T3, T5 with lowest observed under treatment T4. Root volume recorded was highest under treatment T1 (2.82 cm^3) followed by treatments T3 (2.76 cm^3), T2 (1.47 cm^3) and lowest in treatment T4 (0.88 cm^3), and the value under treatment T3 was statistically *at par* with treatment T1 (Table

2). The root system architecture can be regulated by the availability and composition of various N forms present in soil which exposes roots to local N signals in combination with systemic signals reflecting the N nutritional status of the shoot (Jia and Wirén, 2020). Singh *et al.,* (2020) studied the effect of two siderophore-producing endophytes (*Arthrobacter sulfonivorans* DS-68 and *Enterococcus hirae* DS-163) in four genotypes of wheat (*Triticum aestivum* L.) for biofortifcation of grains with Fe and enhance yield. They reported that endophytic inoculation led to increase in surface area, volume, length of roots and number of root tips by 78.27%, 75%, 71% and 44%, relative to the uninoculated control (recommended dose of fertilizers), across genotypes and soil types. Irizarry and White, (2017) also reported the positive effects of *Bacillus amyloliquefaciens* inoculation on greater seedlings percentage with expanded cotyledons after eight days with enhanced primary and lateral root growth and altered root architecture.

Photosynthetic pigments

Total chlorophyll was highest under treatment T1 (4.46 mg/g fresh weight), followed by treatments T3 (4.19 mg/g fresh weight), T2 (4.05 mg/g fresh weight), T4 (3.80 mg/g fresh weight) and lowest in treatment T5 (3.42 mg/g fresh weight). Chlorophyll *a* was maximum under treatment T1 followed by treatments T3, T4, T2 and lowest in treatment T5 and the chlorophyll content under treatment T3 was statistically *at par* with treatment T1. On the other hand, chlorophyll *b* was recorded to be highest in treatment T3 followed by treatments T1, T4,T2 and lowest in treatment T5.Carotenoids observed were more or less similar in different treatments, indicating no effect of treatments on this parameter (Table 3). Total pigments were maximum in treatment T1 (4.75 mg/g fresh weight) and minimum in treatment T5 (3.64 mg/g fresh weight). Chlorophyll to pigment ratio was lowest in treatment T5 (0.937) and highest in treatment T4 (0.944), whereas carotenoids to pigment ratio varied from lowest in treatment T4 (0.055) to the highest in treatment T5 (0.062). On the other hand, chlorophyll to carotenoid ratio was highest under treatment T4 (17.17) and lowest under T5 (14.97) treatment (Table 4). Grzesik *et al.,* (2017) studied the growth and physiological response of willow (*Salix viminalis* L.) plants to triple foliar biofertilization with *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120, and *Chlorella* sp. under the conditions of limited use of chemical fertilizers and reported the increased stability of cytomembranes, chlorophyll content, intensity of net photosynthesis, transpiration, stomatal conductance, and decreased intercellular CO_2 concentration. The soil amended with varied level of fly ash and supplementation with N_2-fixing cyanobacteria masses as biofertilizer resulted in increased pigments and enzyme activities along with other parameters in rice crop (Padhy *et al.,* 2016). Inoculation of wheat plants with cyanobacteria plus K, P and S significantly increased dry weight, total nitrogen, and pigments over control and other treatments (Abd-Alla *et al.,* 1994).

In conclusions the inoculation of basmati rice with exponentially growing suspension of cyanobacterium in combination with different levels of nitrogen resulted in positive influence on growth parameters and photosynthetic pigments of crop. Observations on short term experiment under controlled environmental showed that nitrogen fixing cyanobacterium can save about 20% to 25% of nitrogenous fertilizers, hence, can be utilized as an important component in integrated nutrient management of rice crop.

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