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Evaluating the potential of rhizo-cyanobacteria as inoculants for rice and wheat

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Our investigation was aimed towards evaluation of a set of cyanobacteria isolated from the rhizosphere of diverse rice and wheat varieties as plant growth promoting agents and organic management of rice (*Oryza sativa* cv Pusa Basmati 1) and wheat (*Triticum aestivum* cv HD 2687) crop. Preliminary analyses of the extracellular filtrates of strains revealed the presence of IAA and hydrolytic enzymes. Pot culture experiments were undertaken using selected rhizo-cyanobacterial strains with rice and wheat crop, under glasshouse and controlled conditions of the National Phytotron Facility. Promising strains (C1, C6, C11, C20) were identified on the basis of enhanced soil microbiological activity (in terms of alkaline phosphatase activity and FDA hydrolysis), plant growth and yield parameters. Such strains possess important traits for effective establishment and are being evaluated for their promise at field level.

Key words: Cyanobacteria, Rice, Wheat, Rhizosphere, Microbial Activity, Plant Growth promotion

Introduction

Rice and wheat are grown in sequence in about 12 million hectares in South Asia to meet the growing demands of the human population globally. Extensive research has shown that microorganisms can play a significant role in improving the resource-use efficiency and improve complementarity of the agronomic practices for both these crops (Asghar *et al.,* 2002; Berg, 2009; Kloepper *et al.,* 1989). Soil–plant–microbe interactions are complex and there are many ways in which the outcome can influence the plant health and

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productivity. With the growing awareness of problems related to use of fertilizers/chemicals in agriculture, there has been a paradigm shift in agronomic practices, with increasing inclusion of organic practices. Cyanobacteria, more commonly known as blue green algae comprise an interesting group of phototrophic eubacteria whose diversity is unparalleled in the biological world, in terms of habitat, morphology or repertoire of metabolic activities. They proliferate in diverse types of ecosystems - ranging from the cold Tundra to the hot deserts, from surface waters of oceans to rhizosphere of plants and from barren lands/rock surfaces to polluted waters. Cyanobacterial inoculation has shown to enhance growth, root associated nitrogen fixation and yields of rice (Singh, 1961; Roger *et al.,* 1993; Mandal *et al.,* 1998).

Cyanobacteria represent a relatively untapped source of novel metabolites with a wide range of bioactivities including toxins (microcystins, nodularins, peptilides), antibiotics, protease inhibitors, immuno-modulators and antiviral agents (Namikoshi and Rinehart, 1996). However, their role in plant growth promotion, especially in relation to their functioning in the rhizosphere, has not been explored in depth. Most of the work on microbiology of rice/ wheat rhizosphere has been carried out on qualitative and quantitative analyses of populations of bacteria, fungi and actinomycetes and their activities, with very little emphasis on these ubiquitous prokaryotes (Arshad and Frankenberger, 1998). This has been mainly because cyanobacteria have been considered obligate photoautotrophs. However, few reports exist on epiphytic growth on phyllosphere/ rhizosphere and in aquatic / high humidity environments (Rippka, 1972; Freiberg, 1998; Prasanna *et al.,* 2009 a,b) and exhibit heterotrophic abilities. Most of the known cyanobacterial associations with plants are known to involve a mutual exchange of nutrients, especially related to the fixation of carbon or nitrogen (Rai and Bergman, 2002; Jaiswal *et al.,* 2008; Karthikeyan *et al.,* 2009).

Therefore, the rhizosphere represents a less explored frontier - in terms of cyanobacteria, especially in relation to their abundance, diversity and interactions with other components of the rhizosphere. This can have not only immense significance but also be critical for developing more efficient colonization of microbial inoculants and creation of new nitrogen fixing and plant growth promoting symbioses. By virtue of their abundance in diverse habitats, cyanobacteria are natural candidates for seedling inoculation, especially in reforestation and rehabilitation of diverse ecosystems. As rhizosphere cyanobacterial isolates can be better competitors, when deployed as inoculants in agriculture, due to their direct linkage with roots, their inclusion in biofertilizer/biocontrol consortia can improve the effectiveness of these inoculants. Our investigation was aimed at characterizing a set of

cyanobacterial isolates from the rhizosphere of rice and evaluating the influence of selected rhizo-cyanobacteria on soil microbiological and plant related parameters, in pot experiments with wheat and rice crop. This study can be useful as a prelude to their utilization in rice-wheat cropping systems for effective nutrient management and improved crop yields.

Materials and methods

Organisms and their characterization

A set of 20 cyanobacterial strains, isolated from the rhizosphere of different rice varieties, obtained from diverse agro ecologies of India were used for inoculation (Prasanna *et al.,* 2009 a,b). Homogenized suspensions of late log phase cultures were utilized for the biochemical and physiological analyses. The methodology as given by Herbert *et al.* (1977) was followed for the estimation of proteins, and the absorbance was read at 650 nm. The amount of proteins was calculated from the standard curve of known amounts of bovine serum albumin and expressed as µg of protein per ml of culture. The filtrate was checked for the production of IAA after four weeks of incubation, following the method of Gordon and Weber (1951).

Microscopic observations

Thin sections of roots (transverse and longitudinal) were prepared using microtome and fixed using standard procedures (Brandon *et al.,* 1964) and observed under light microscope to study the cyanobacterial association with the plant roots. Transmission electron microscopy of the root sections (was done using the FEI Philips 2680 model microscope at the Sophisticated Analytical Instrumentation Facility, All India Institute of Medical Sciences, New Delhi), after embedding in LR-White resin and fixation in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) at 4^0 C.

Experimental conditions for pot experiments

A pot culture experiment was designed to evaluate the effect of selected cyanobacterial strains on the yield of rice (*Oryza sativa* cv Pusa Basmati 1) and wheat (*Triticum aestivum* cv HD 2687) with varying doses of nitrogen in the glasshouse. Unsterile soil was used as medium for raising crop, which was taken from IARI fields. The physico-chemical properties of soil at the beginning of the experiment are given in Table 1. Plastic pots of 12" size were

employed in the study. The experiments was conducted with the treatments as given in Table 2, which included the use of individual strain, along with 1/3 N along with full dose of P and K fertilizers,. Control treatments involving 1/3 N, 2/3 N and full dose of N, and full dose of P and K fertilizers, were also included (F1, F2 and F3). The earthen pots were filled with 12 kg of soil per pot and irrigated regularly to maintain 60% water holding capacity (WHC) of soil. Recommended agronomic practices and NPK fertilizer doses were employed. Soil samples (0-20 cm depth) were collected at harvest stage. Further experiments were undertaken under controlled conditions of the National Phytotron Facility (using sterile potting mix - Vermiculite and sand in 3:1), IARI, New Delhi. The experimental facility provides suitable growing conditions for both the crops and recommended agronomic practices were followed. Each treatment was undertaken with three replications in both crops and experiments.

Table 1. Geographical details of experimental site and physicochemical properties of soil used in the pot culture experiment under glasshouse conditions

Altitude	228.16 m above mean sea level
Latitude	28° 4'N
Longitude	77° 12'E
pH	7.3
EC	0.64 dSm^{-1}
Organic carbon	0.43%
Available N	156 kg ha^{-1}
Available P	22.5 kg ha^{-1}
Soil classification	
Texture	Sandy clay loam
Order	Inceptisol
Family	Udic Ustocrept

Agronomic practices

Seedlings of rice variety *Pusa Basmati 1* (10 d old) were transplanted and irrigation was given after sowing (5 seedlings per pot). The recommended rate of fertilizers for rice crop - 120:80:60 NPK kg. ha^{-1} were applied one day before transplantation. 1/3 N, along with full dose of P and K was applied at the time of sowing. The split application of remaining portion of N, in the full dose and 2/3 N treatments was given at tillering stage.

The recommended rate of fertilizers for wheat crop is 120:80:60 NPK kg per hectare. Full dose of P and K were applied one day before sowing at the above-mentioned rate. 1/3 N was applied at the time of sowing. Five seeds of

wheat variety HD 2687 were sown per pot with equal spacing and irrigation was given after sowing. The split application of remaining portion of N was given at tillering stage, in the uninoculated treatments.

Suspensions of log phase (12-14 d) cultures of selected cyanobacterial strains (prepared by high speed centrifugation at 8000 rev min^{-1} for 10 min, followed by washing and dissolution of the pellet using sterile water) and added at the rate of 5 µg chlorophyll g^{-1} per pot. Seedlings from nursery were soaked in culture suspensions for 2 h and then transplanted in pots . The pots were irrigated regularly to maintain 60% WHC with water, as per the crop and soil moisture status.

Plant, soil and microbiological parameters

Samples were collected from 0-20 cm depth of soil, in triplicates from each pot, for assessing the microbiological parameters. A minimum of three plants were taken for analyzing the plant related parameters. The biomass and 1000 grain weight (given as g per pot in Tables and Figures) were recorded at the time of harvest. Alkaline phosphatase activity was assayed in soil suspended in modified universal buffer (pH 11), along with substrate p- nitro phenyl phosphate (Tabatbai and Bremner, 1969). The absorbance was measured at 440 nm and the enzymatic activity was expressed as µg p-nitro phenol released g^{-1} soil h^{-1}.

FDA (Fluorescein diacetate) hydrolysis assay was carried out by measuring the absorbance of the supernatant at 490 nm using Fluorescein standard (Adam and Duncan, 2001). The values were represented as µg Fluorescein released $g^{-1}h^{-1}$. The activity of hydrolytic enzymes were measured as given earlier (Prasanna *et al*., 2008).

Statistical analyses

The triplicate sets of data for the various parameters evaluated were subjected to ANOVA (Analysis of Variance) in accordance with the experimental design (Completely Randomized Design) using MSTAT-C statistical package to quantify and evaluate the source of variation, and CD (Critical Differences) values were calculated at P level of 0.05%. SD (Standard deviation) values are depicted in the graphs as bars.

Results and discussion

The intensive agricultural practices prevalent in the modern times are heavily dependent on the application of chemical inputs; including fertilizers,

which affect soil health and fatigue in the long run. During the last few decades, a significant increase in growth and yield of agronomically important cereal crops and improved soil fertility, in response to inoculation with PGPR has been reported (Asghar *et al.,* 2002; Morrissey *et al.,* 2004; Richardson *et al.,* 2009). Rice (*Oryza sativa* L.) is one of the most prominent food crops globally, and represents the staple diet for almost half of the human population of the world. It is estimated that there will be about 8 million people by the year 2020 of which 760 million tons of rice. Wheat is the staple food for 35% of the world's population. It provides more calories and proteins in the diet than any other crop. Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality (Kennedy, 2004; Khalid *et al.,* 2004).

Cyanobacteria represent an ancient diverse group of photosynthetic prokaryotes, which form an integral component of waterlogged rice fields, and supply around 86% of the global requirement of rice (Venkataraman, 1972). The cyanobacterial strains , evaluated in this study, belong to the germplasm of axenized unicyanobacterial isolates which had been previously identified using the taxonomic keys of Desikachary (1959) after careful observations related to the growth pattern on agar/ in liquid media and microscopic observations on color, shape and size of cells (Prasanna *et al.,* 2009). Preliminary observations revealed the dominance of the genera-*Anabaena* and *Nostoc* among the isolates. Such observations have also been recorded earlier, however, mainly with respect to the surface layers of soil/soil-water interface (Nayak *et al.,* 2001, 2004). The competitive nature of the genera-*Nostoc* and *Anabaena* is well documented (Nayak and Prasanna, 2007; Prasanna and Nayak, 2007) and is intimately related to the diversity in nutritional modes, ranging from phototrophy to heterotrophy (Vasudevan *et al.,* 2007), adaptation to different habitats and ability to exist as free-living or in associations with diverse members of the plant kingdom (Rai and Bergman, 2002; Nilsson *et al.,* 2005).

The axenized isolates were characterized for their growth in terms of protein content, hydrolytic enzymes and production of IAA. Protein content ranged between 60 - 196 ug/ml while IAA production was observed in the range of 0.02 – 0.34 ug/ml (Table 2). Benefits of algal inoculation have often been interpreted as an action of biologically potent substances, such as IAA produced by these organisms (Misra and Kaushik, 1989; Sergeeva *et al.,* 2002). In terms of hydrolytic enzymes, strains C2 and C3 recorded highest values for FPase (Filter paperase activity) and amylase activity. C1 recorded highest values for xylanase activity while chitosanase activity was highest in strains C8 and C9 (Table 3). Cyanobacteria are generally recognized as phototrophs; hence, activity related to hydrolytic enzymes has been less documented

(Prasanna *et al.,* 2008). In our study, as these cyanobacterial strains are isolates from the rhizosphere, the activity related to hydrolytic enzymes assumes significance for their multifaceted role in that niche. Such enzymes (chitinases, cellulases) may help to compete against phytopathogenic microflora, besides aiding in the entry of cells into the host tissues for forming associative/symbiotic relationships with plants. Plant growth promoting rhizobacteria (PGPR) are generally screened on the basis of such biochemical traits-IAA production, production of hydrolytic enzymes etc.; therefore these cyanobacterial strains hold promise as PGPRs.

Table 2. Total soluble proteins and IAA production in cyanobacterial strains

Treatments	Genus	Proteins (µg/ml)	IAA (µg/ml)
C1	*Anabaena* sp.	155.30^{BC}	0.08^{BCDEF}
C2	*Nostoc* sp.	168.00^{B}	0.29^{A}
C3	*Nostoc* sp	132.00^{CDE}	0.05^{CDEF}
C4	*Nostoc* sp	$76\ .00^{HIJ}$	0.13^{BC}
C5	*Nostoc* sp.	108.00^{EFG}	0.07^{CDEF}
C6	*Calothrix sp*.	121.30^{DEF}	0.04^{EF}
C7	*Nostoc* sp.	138.70^{CD}	0.12^{BCDE}
C8	*Hapalosiphon* sp.	92.67^{GHI}	0.05^{CDEF}
C9	*Anabaena* sp.	57.33^{J}	0.04^{EF}
C10	*Nostoc* sp.	99.33^{FGHI}	0.06^{CDEF}
C11	*Anabaena* sp.	158.00^{BC}	0.05^{CDEF}
C12	*Anabaena* sp.	122.70^{DEF}	0.05^{CDEF}
C13	*Nostoc* sp.	72.67^{IJ}	0.34^{A}
C14	*Nostoc* sp	60.00^{J}	0.08^{F}
C15	*Anabaena* sp.	196.00^{A}	0.03^{EF}
C16	Cyanobiont from *Azolla* sp.	76.67^{HIJ}	0.02^{F}
C17	Cyanobiont from *Azolla* sp	100.70^{FGH}	0.08^{BCDEF}
C18	*Calothrix* sp.	109.30^{EFG}	0.15^{B}
C19	*Hapalosiphon* sp.	$74\ .00^{HIJ}$	0.04^{BCD}
C20	*Nostoc* sp.	105.30^{EFG}	$0.13^{\ BC}$
SEM		8.43	0.03

Table 3. Activity of selected hydrolytic enzymes (IU/ ml) in culture filtrates of cyanobacterial strains

Treatments	Genus	Filter paperase	Xylanase	Amylase	Chitosanase
C1	*Anabaena* sp.	0.14^{BCD}	0.09^{A}	29.20^{B}	10.20^{A}
C2	*Nostoc* sp.	0.22^{A}	0.06^{A}	58.12^{A}	5.57^{BCD}
C3	*Nostoc* sp	$0.O4^{E}$	0.07^{B}	23.25^{D}	0.87^{GH}
C4	*Nostoc* sp	0.18^{ABC}	0.03^{C}	00.93^{O}	6.31^{B}
C5	*Nostoc* sp.	0.20^{AB}	0.06^{C}	7.90^{I}	2.92^{EFG}
C6	*Calothrix* sp.	0.04^{E}	0.04^{D}	15.81^{F}	6.94^{B}
C7	*Nostoc* sp.	0.14^{BCD}	0.02^{D}	27.43^{C}	3.96^{CDE}
C8	*Hapalosiphon* sp.	0.14^{CDE}	0.01^{D}	8.83^{H}	5.80^{BC}
C9	*Anabaena* sp.	0.10^{DE}	0.07^{D}	20.46^{E}	11.03^{A}
C10	*Nostoc* sp.	0.08^{DEF}	0.01^{D}	6.97^{J}	0.75^{H}
C11	*Anabaena* sp.	0.11^{DE}	0.05^{D}	7.90^{I}	7.03^{B}
C12	*Anabaena* sp.	0.10^{DE}	0.01^{E}	6.50^{K}	5.54^{BCD}
C13	*Nostoc* sp.	011^{DE}	0.04^{F}	15.81^{F}	1.09^{GH}
C14	*Nostoc* sp	0.14^{CDE}	0.06^{F}	13.48^{G}	3.23^{EF}
C15	*Anabaena* sp.	0.10^{DE}	0.07^{F}	1.86^{N}	3.66^{DE}
C16	Cyanobiont from *Azolla* sp.	0.12^{DE}	0.07^{F}	8.84^{H}	1.41^{FGH}
C17	Cyanobiont from *Azolla* sp	0.10^{DE}	0.04^{F}	0.92^{O}	1.28^{EFGH}
C18	*Calothrix* sp.	0.11^{DE}	0.09^{G}	0.46^{O}	2.44^{EFGH}
C19	*Hapalosiphon* sp.	0.11^{DE}	0.01^{G}	3.72^{L}	2.67^{EFGH}
C20	*Nostoc* sp.	0.08^{DE}	0.01^{G}	2.78^{M}	1.47^{FGH}
SEM		0.02	0.02	0.02	0.63

In order to understand their agronomic utility as inoculants, pot culture experiments were then undertaken using the set of twenty isolates as inoculants in rice and wheat crop under glasshouse conditions. A significant enhancement in FDA hydrolysis and alkaline phosphatase activity in inoculated pots especially in treatments involving strains C1, C6 and C20 for rice. In wheat crop, strains C6, C11 and C20 performed best and recorded significantly higher values compared to 1/3N+PK treatment. This reflected the role of cyanobacteria in promoting the growth of other microflora and intensification of microbial activity in the rhizosphere. Such phenomena are known to be influenced by the size of microbial populations, which, in turn can compete with the plant for nutrients, or cause disease or stimulate plant growth or suppress pathogens; such interactions alter the biological and chemical composition of the rhizosphere and thereby influence plant health. In our study, microbial activity, in terms of FDA hydrolysis showed a 40-80% enhancement in wheat over rice crop; however, alkaline phosphatase activity was almost 3-folds lower in wheat crop (Table 4).

Table 4. Microbial Activity of soil as influenced by inoculation of cyanobacterial strains in pot culture experiments under glasshouse conditions in rice and wheat crop

Treatments	Inoculated Strains	Rice		Wheat	
		FDA (µg fluorescein /g/h)	Alkaline Phosphatase (µg PNP /g/h)	FDA (µg flourescein /g/h)	Alkaline Phosphatase (µg PNP /g/h)
C1	*Anabaena* sp.	0.93^{A}	49.82^{EFGHI}	2.62^{BCD}	2.21^{F}
C2	*Nostoc* sp.	0.37^{CDE}	10.1^{CDE}	1.97^{CDE}	11.97^{DEF}
C3	*Nostoc* sp	0.22^{EF}	200.9^{A}	2.11^{BCDE}	17.32^{BCDE}
C4	*Nostoc* sp	0.23^{DE}	11.78^{I}	2.00^{CDE}	9.96^{EF}
C5	*Nostoc* sp.	0.34^{DE}	122.8^{BCD}	1.74^{DEF}	11.17^{DEF}
C6	*Calothrix* sp.	0.83^{A}	46.05^{FGHI}	2.74^{ABC}	35.69^{A}
C7	*Nostoc* sp.	0.42^{ABC}	68.38^{DEFGH}	2.13^{BCDE}	14.92^{BCDEF}
C8	*Hapalosiphon* sp.	0.34^{DE}	96.48^{DEF}	2.02^{BCDE}	16.47^{BCDEF}
C9	*Anabaena* sp.	0.32^{DE}	46.31^{FGHI}	1.71^{DEF}	16.32^{BCDEF}
C10	*Nostoc* sp.	0.39^{BCD}	10.7^{CDE}	2.44^{BCDE}	13.66^{BCDEF}
C11	*Anabaena* sp.	0.59^{AB}	172.4^{AB}	2.95^{AB}	27.81^{AB}
C12	*Anabaena* sp.	0.20^{FGH}	21.83^{HI}	2.06^{BCDE}	22.77^{ABCDE}
C13	*Nostoc* sp.	0.20^{FGH}	16.14^{HI}	1.94^{CDE}	9.64^{EF}
C14	*Nostoc* sp	0.17^{K}	153.4^{ABC}	1.79^{DE}	25.45^{ABCD}
C15	*Anabaena* sp.	0.10^{H}	81.90^{DEFG}	2.04^{BCDE}	21.75^{BCDE}
C16	Cyanobiont from *Azolla* sp.	0.20^{FGH}	123.6^{BCD}	2.75^{ABC}	19.29^{BCDE}
C17	Cyanobiont from *Azolla* sp	0.26^{DEF}	94.72^{DEF}	2.33^{BCDE}	8.53^{EF}
C18	*Calothrix* sp.	0.22^{EF}	10.84^{I}	1.61^{EF}	9.17^{EF}
C19	*Hapalosiphon* sp.	0.31^{DE}	83.47^{DEFG}	2.31^{BCDE}	16.53^{BCDEF}
C20	*Nostoc* sp.	0.29^{DE}	117.6^{CD}	3.57^{A}	27.11^{ABC}
F1	Full Dose NPK	0.28^{DE}	37.51^{GHI}	1.99^{CDE}	18.95^{BCDE}
F2	2/3 NPK	0.25^{DEF}	103.2^{CDE}	0.89^{F}	12.58^{CDEF}
F3	1/3NPK	0.42^{ABC}	49.82^{EFGHI}	2.20^{BCDE}	12.54^{CDEF}
SEM		0.05	818.0	0.22	54.86

Alkaline phosphatases are of importance in soil organic P mineralization and plant nutrition (Klose and Tabatbai, 2002). Plant parameters such as biomass and grain yields (measured as 1000 grain weight) also recorded significant increase in pots inoculated with cyanobacterial trains in rice and wheat crop (Fig. 1). Most researchers are of the opinion that the growth promoting substances are produced by cyanobacteria may be hormones *i.e.* auxins (Aiyer *et al.,* 1971; Venkataraman and Neelakantan, 1967; Misra and Kaushik, 1989; Sergeeva *et al.*, 2002), gibberellin like, cytokinin like or abscissic acid, vitamins or amino acids.

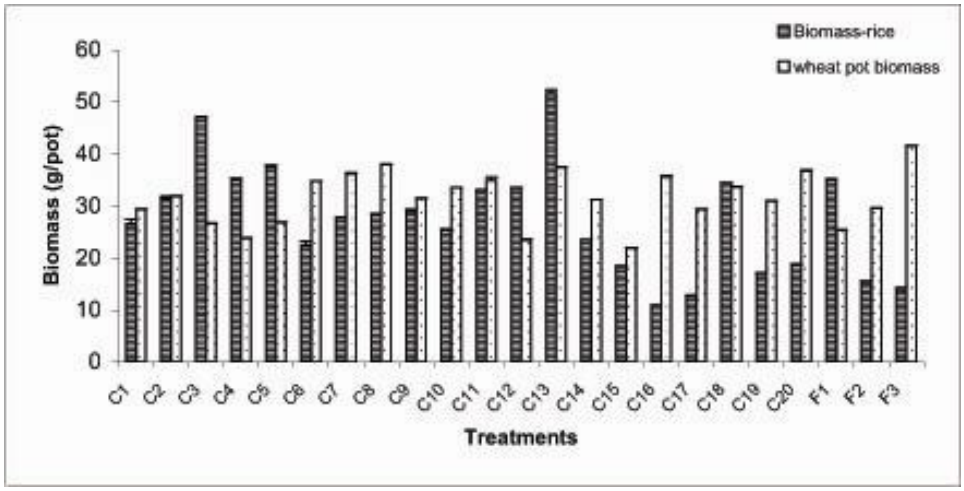

Fig. 1. Plant biomass (dry weight) of rice and wheat plants grown under glasshouse conditions (in unsterile soil) as influenced by inoculation of rhizo-cyanobacterial strains. Vertical bars on columns represent SD (n=3).

A set of ten promising strains were further evaluated under controlled conditions of the National Phytotron Facility, based on their influence on microbial activity and degree of association with roots. Most of the strains (especially C1, C6, C11, C15, and C20) recorded significantly higher or statistically at par values as compared to fertilizer controls (Table 5; Fig. 2). Electron microscopic observations of root sections of wheat plants revealed the intracellular presence of short filaments of *Calothrix* sp. and single cells of *Nostoc* sp. (Fig. 3). Earlier studies from our laboratory had shown the presence of cells/ short filaments of cyanobacteria inside the roots (Karthikeyan *et al.,* 2009; Jaiswal *et al.,* 2008). Such strains can be promising candidates for developing plant growth promoting associations for wheat crop, besides serving as model systems for understanding the metabolic interactions of cyanobacteria with host plant, such as wheat.

Table 5. Microbial Activity of soil and plant biomass as influenced by inoculation of cyanobacterial strains in pot culture experiment under controlled conditions of National Phytotron Facility in rice and wheat crop after four weeks of growth

Treatments	Inoculated Strains	Rice			Wheat		
		Alkaline Phosphatase (µg PNP /g/h)	FDA (µg fluorescein /g/hr)	Biomass (g/pot)	Alkaline Phosphatase (µg PNP/g/h)	FDA (µg flourescein /g/h)	Biomass (g/pot)
C1	*Anabaena* sp.	65.62^{AB}	3.35^{A}	0.12^{A}	45.40^{ABC}	2.01^{A}	3.90^{ABC}
C2	*Nostoc* sp.	38.72^{AB}	1.058^{D}	0.035^{BC}	52.75^{ABC}	0.52^{C}	3.81^{ABCD}
C5	*Nostoc* sp.	24.82^{B}	1.79^{C}	0.032^{BC}	51.88^{ABC}	0.76^{BC}	2.36^{F}
C6	*Calothrix* sp.	88.32^{A}	1.74^{C}	0.047^{AB}	58.09^{AB}	1.97^{A}	4.51^{A}
C11	*Anabaena* sp.	58.72^{AB}	1.96^{BC}	0.087^{AB}	59.39^{A}	1.59^{AB}	3.75^{ABCD}
C12	*Anabaena* sp.	29.12^{AB}	0.159^{F}	0.021^{DE}	53.31^{ABC}	0.77^{BC}	3.18^{CDEF}
C14	*Nostoc* sp.	36.67^{AB}	0.59^{E}	0.034^{BC}	40.78^{CD}	0.68^{C}	4.46^{A}
C15	*Anabaena* sp.	34.25^{AB}	0.44^{EF}	0.06^{BC}	50.28^{ABC}	1.09^{BC}	3.82^{ABCD}
C16	Cyanobiont from *Azolla* sp.	33.43^{AB}	0.76^{DE}	0.09^{BC}	55.46^{AB}	0.75^{BC}	2.64^{EF}
C20	*Nostoc* sp.	27.34^{AB}	1.96^{BC}	0.04^{BC}	55.89^{AB}	1.03^{BC}	2.89^{DEF}
Control 1	PGPR strains	30.74^{AB}	0.49^{EF}	0.07^{BC}	29.13^{DE}	0.86^{BC}	3.49^{BCDE}
Control 2	TY media	56.15^{AB}	0.74^{DE}	0.03^{BC}	46.51^{ABC}	1.22^{ABC}	2.63^{EF}
Control 3	BG11	53.58^{AB}	2.18^{B}	0.01^{BC}	56.74^{AB}	0.65^{C}	4.41^{AB}
Control 4	FD NPK	27.32^{AB}	0.58^{E}	0.02^{BC}	44.12^{BC}	1.01^{BC}	3.26^{CDEF}
Control 5	1/3 NPK	35.46^{AB}	0.68^{DE}	0.05^{BC}	16.95^{E}	0.33^{C}	3.83^{ABCD}
SEM		53.85	0.04	0.01	973.7	0.21	1.81

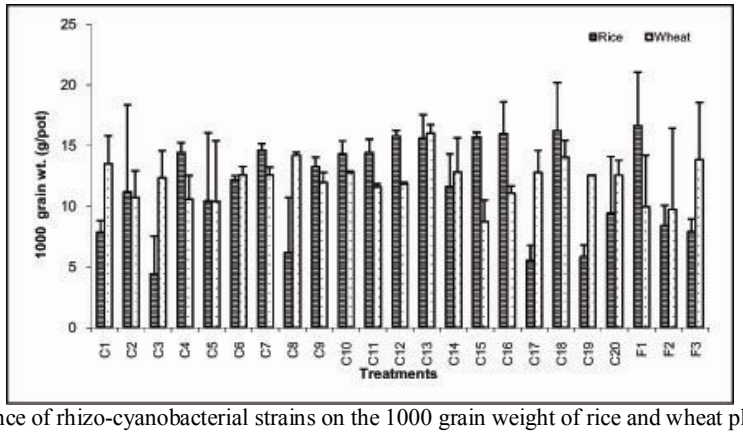

Fig. 2. Influence of rhizo-cyanobacterial strains on the 1000 grain weight of rice and wheat plants, grown under glasshouse conditions (in unsterile soil) Vertical bars on columns represent SD (n=3).

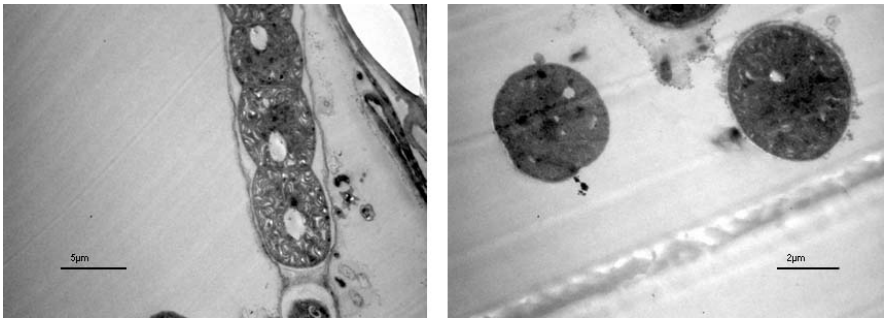

Fig. 3. Transmission Electron micrographs of wheat root sections, showing the intracellular location of cyanobacterial cells and filaments.

Co-cultivation of plant seedlings (wheat, maize, sugarbeet) and establishment of cells, tissues, plant regenerates, calli with introduced cyanobacteria have also been successful (Gusev *et al.,* 1986; Rai *et al.,* 2002). Colonization of rice and wheat roots by *Nostoc* sp. is also reported (Gantar *et al.,* 1991; Rai *et al.,* 2002; Karthikeyan *et al.,* 2009), but the main emphasis has been on nitrogen fixation. In our study, electron microcopy clearly revealed the entry of cyanobacterial cells/short filaments into the cells of wheat roots, *vis a vis* lack of any such cells in control treatments, in which no inoculation was done. Hence, their potential for developing associations which can promote growth of surrounding microflora in the rhizosphere and enhance plant growth and yields is evident, especially strains - C1, C6, C11 and C20 which ranked among the top ranks for most of the attributes evaluated.

Conclusions

Our investigations clearly illustrate and emphasize the significance of these rhizosphere isolates, which exhibit multiple useful traits as inoculants, including entry into roots and ability to form tight associations. This study also illustrates their diverse functions in this ecological niche – including conservation of supply of nutrients, providing a stable community that can minimize invasion by antagonists/stress factors and associate mutually with plant roots for improved plant growth and soil productivity. Such strains can prove to be “green options” and serve as effective supplements for inclusion under organic agriculture practices.

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