



Evaluation and characterization of the plant growth promoting potentials of two heterocystous cyanobacteria for improving food grains growth

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ABSTRACT

Cyanobacteria are potential prokaryotes found in diverse environment of freshwater and marine water ecosystem. They are well known applicant practised in ancient agriculture technique for improving the soil fertility, water holding capacity and texture of the soil. The availability, low cost and fast growth has considered them as superior candidate for biotechnological applications. In the recent years, cyanobacteria are been used to produce plant growth promoting hormones or substances for facilitating effective plant growth. The present study is focussed on validating the plant growth promoting ability of two heterocystous cyanobacteria strains (*Anabaena variabilis*, *Nostoc calcicola*) in five crop plant. The cyanobacterial strains were characterized by biochemical and plant growth promoting parameters like Chlorophyll content and IAA production. As result of this study, in vitro experiments revealed 100% of seed germination by Cyanobacteria growth promoting substances (CGPS) of *Anabaena variabilis* in the grains of *Zea mays* and *Sorghum bicolor*, while 90% germination in *Nostoc calcicola*. Statistical analysis proved the growth root length, plant height, weight of fresh and dry root, number of leaves in CGPS treated and untreated plant in pot. Further the CGPS was characterized using thin-layer chromatography (TLC), FT-IR and HPLC analysis. The dominant auxins in the two isolates were IAA in the range of 0.0372–1.2327 $\mu\text{g g}^{-1}$ dry weight was detected. However, IAA production by *Anabaena variabilis* proved as potential candidate for promoting plant growth. In addition, *Anabaena variabilis* can also be utilized as a bio-fertilizer for the crops cultivated in normal fields.

1. Introduction

Global food crises have increased the production of food crop at a drastic rate to feed the fast growing population. Reducing the dependency of chemical fertilizers and pesticides for fast growth of agricultural crop is becoming a major criterion among researchers (Igiehon and Babalola, 2017). To solve this problem, there is an urgent need to explore microorganism as an alternative to chemical fertilizers. Certain microorganisms like bacteria, fungi, cyanobacteria are extremely useful in promoting plant growth via direct and indirect way. These microorganisms directly helps in nitrogen-fixation process, phosphate solubilization or modulating plant hormone levels and indirectly helps in minimizing the effects of phytopathogens on plant growth and development by acting as biocontrol agents (Glick, 2012; Shunmugam et al., 2015). Among them cyanobacteria is one of the suitable, eco-friendly, cheaply available and alternative source of natural fertilizer or

biofertilizers (Vaishampayan et al., 2001; Mishra and Pabbi, 2004). Cyanobacteria are known to produce extracellular compounds such as phytohormones (auxins, gibberellins and cytokines), polypeptides, vitamins and amino acids for growth and development of plants. So far cyanobacterial extracts are also known to have positive effect on food crops like wheat, maize, rice, tomato and cucumber (Hosain et al., 2006; Radha Prasanna, 2016; Priya et al., 2015; Bidyarani et al., 2016; Gayathri et al., 2017). Few studies have characterized the chemical constituent's influencing plant growth (Boopathi et al., 2013; Hashtroudi et al., 2013). Some cyanobacterial heterocyst form namely *Nostoc*, *Anabaena*, *Calothrix*, *Haplosiphon* enhanced soil microbial biomass, available nitrogen, soil microbiological parameter, seed germination and yield rate in rice and wheat (Obana et al., 2007; Prasanna et al., 2013; Hussain and Hasnain, 2011; Mazhar et al., 2013; Karthikeyan et al., 2007; Xu et al., 2013). In India, *Zea mays*, *Sorghum bicolor* and *Oryza sativa* are the most important food crops in terms of

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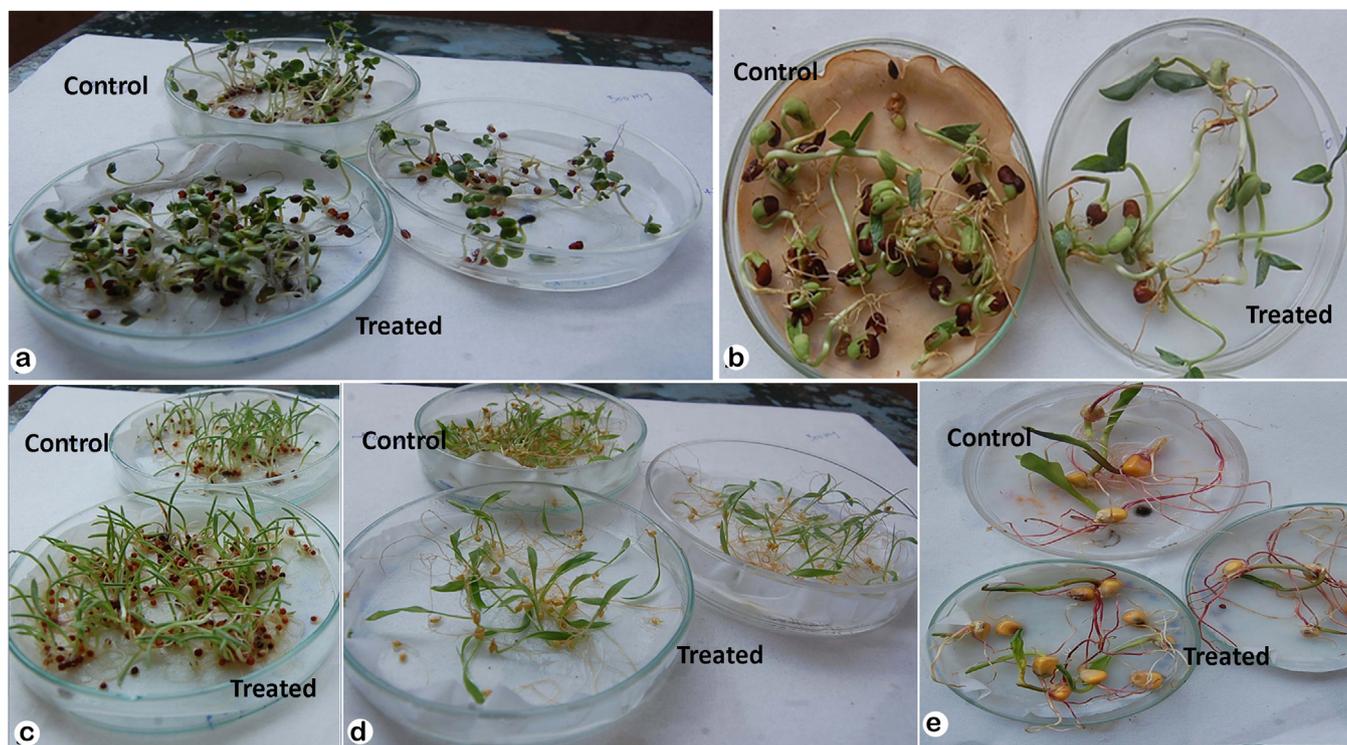


Fig. 1. Seeding growth images of control and experimental plants (a). *Paspalum scrobiculatum* (b). *Vigna unguiculata* (c). *Sorghum bicolor* (d). *Oryza sativa* (e). *Zea mays*.

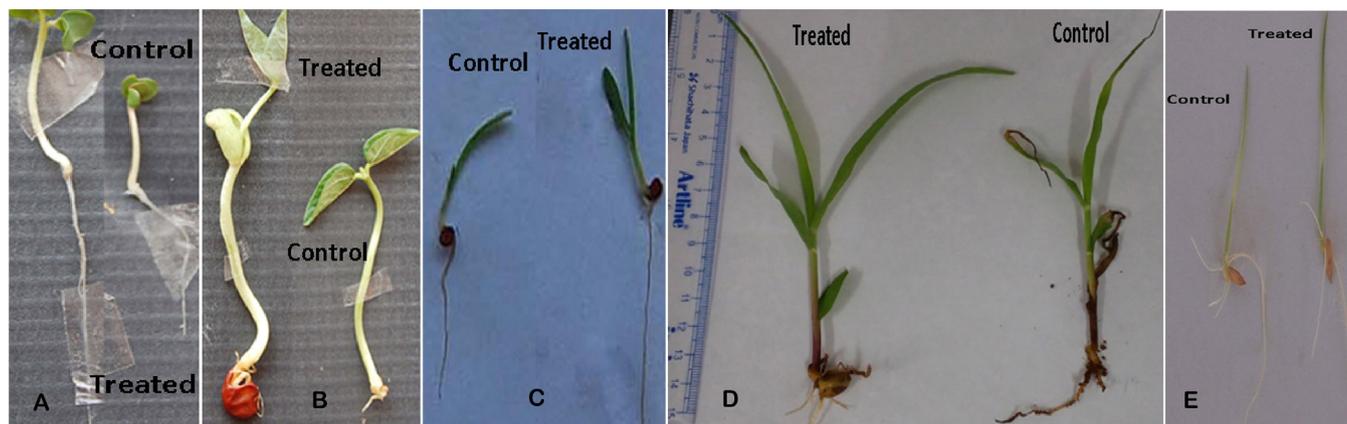


Fig. 2. Comparison of growth between cyanobacterial CGPS of the grain seeds with control and experimental plants (A). *Paspalum scrobiculatum* (B). *Vigna unguiculata* (C). *Sorghum bicolor* (D). *Zea mays* (E). *Oryza sativa*.

human consumption. However, one of the main problems for crop production in India is the intensive application of chemical fertilizers causing damage to the soil ecology and agricultural systems. Therefore, there is an alarming need of finding an eco-friendly and effective bio-fertilizers from cyanobacteria to promote the growth and yield of food crops. Considering the above fact the present study is focussed on utilizing two heterocyst cyanobacteria (*Anabaena variabilis* and *Nostoc calcicola*) to promote germination and growth of food crops like *Zea mays*, *Sorghum bicolor*, *Oryza sativa*, *Vigna unguiculata* and *Paspalum scrobiculatum* followed by characterization of these cyanobacterial isolates for growth parameters.

2. Materials and methodology

2.1. Culture growth and maintenance

The Cyanobacterial cultures *Anabaena variabilis* and *Nostoc calcicola* were obtained from the DBT sponsored National repository for Microalgae and Cyanobacteria – Freshwater (NRMCF), Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu. The cultures were cultivated and maintained in nitrogen free BG11 medium (Stanier et al., 1971; Rippka et al., 1979) under continuous fluorescent light at 3000 Lux and temperature $28 \pm 2^\circ\text{C}$ in baffled flasks. The morphological identification of the culture was done using keys of Desikachary (1959) and the culture tested for their different plant growth promoting activity

Table 1
Efficiency of CGPS in enhancing seed germination and crop plant growth.

Plant parameters		<i>Anabaena variabilis</i> CGPS Concentration			<i>Nostoc calcicola</i> CGPS Concentration		
<i>Paspalum scrobiculatum</i>	Control	100 mg/ml	200 mg/ml	500 mg/ml	100 mg/ml	200 mg/ml	500 mg/ml
Germination (%)	50	90	55	90	70	50	25
Fresh weight (g)	0.02 ± 0.0	0.06 ± 0.05	0.00 ± 0.00	0.19 ± 0.12	0.01 ± 0.00	0.03 ± 0.00	0.53 ± 0.27
Dry weight (g)	0.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.02 ± 0.11
Root length (cm)	3.3 ± 0.63	6.16 ± 0.82	8.33 ± 1.01	5.26 ± 0.29	2.63 ± 0.08	5.80 ± 0.20	4.56 ± 0.75
Shoot length (cm)	2.8 ± 0.73	3.56 ± 0.92	4.93 ± 0.13	3.83 ± 0.40	0.83 ± 0.17	1.50 ± 0.075	2.70 ± 0.76
No of leaves (Nos)	2.0 ± 0.0.0	2.33 ± 0.33	3.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.0.0	2.00 ± 0.0	2.00 ± 0.00
<i>Vigna unguiculata</i>	Control	100 mg/ml	200 mg/ml	500 mg/ml	100 mg/ml	200 mg/ml	500 mg/ml
Germination (%)	50	90	45	100	5	30	60
Fresh weight (g)	0.15 ± 0.01	0.25 ± 0.03	0.277 ± 0.03	1.10 ± 0.49	0.60 ± 0.04	0.27 ± 0.02	0.61 ± 0.20
Dry weight (g)	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.155 ± 0.08	0.41 ± 0.23
Root length (cm)	4.26 ± 0.78	7.83 ± 0.202	7.63 ± 0.078	5.86 ± 0.33	6.76 ± 0.060	8.50 ± 0.75	5.20 ± 0.57
Shoot length (cm)	4.83 ± 0.88	5.93 ± 0.29	5.26 ± 0.31	3.63 ± 0.80	3.93 ± 0.63	4.26 ± 0.52	3.43 ± 0.49
No of leaves (Nos)	2.00 ± 0.00	2.33 ± 0.033	2.66 ± 0.33	2.00 ± 0.00	2.00 ± 0.00	2.33 ± 0.33	2.00 ± 0.00
<i>Sorghum bicolor</i>	Control	100 mg/ml	200 mg/ml	500 mg/ml	100 mg/ml	200 mg/ml	500 mg/ml
Germination (%)	45	5	100	100	45	35	90
Fresh weight (g)	0.01 ± 0.00	0.16 ± 0.019	0.04 ± 0.00	0.04 ± 0.02	0.48 ± 0.012	0.42 ± 0.11	0.19 ± 0.07
Dry weight (g)	0.00 ± 0.00.0	0.14 ± 0.0085	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.27	0.17 ± 0.24	0.00 ± 0.00
Root length (cm)	2.60 ± 0.49	4.53 ± 0.60	5.70 ± 0.49	5.00 ± 1.16	6.43 ± 0.46	4.83 ± 0.57	3.86 ± 0.12
Shoot length (cm)	1.00 ± 0.28	0.86 ± 0.578	3.66 ± 0.16	2.73 ± 0.788	1.76 ± 1.36	1.36 ± 0.072	2.80 ± 0.52
No of leaves (Nos)	2.00 ± 0.0.00	2.66.66	2.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	1.66 ± 0.033	1.66 ± 0.33
<i>Oryza sativa</i>	Control	100 mg/ml	200 mg/ml	500 mg/ml	100 mg/ml	200 mg/ml	500 mg/ml
Germination (%)	50	5	35	85	25	50	50
Fresh weight (g)	0.15 ± 0.01	0.25 ± 0.00	0.27 ± 0.00	0.10 ± 0.028	0.72 ± 0.00	0.27 ± 0.00	0.61 ± 0.20
Dry weight (g)	0.03 ± 0.01	0.03 ± 0.03	0.03 ± 0.03	0.05 ± 0.49	0.60 ± 0.04	0.04 ± 0.02	0.27 ± 0.20
Root length (cm)	3.00 ± 0.0.28	4.90 ± 0.66	4.73 ± 0.54	3.70 ± 0.28	6.43 ± 0.61	8.20 ± 0.073	6.20 ± 0.50
Shoot length (cm)	2.00 ± 0.28	2.83 ± 0.33	2.40 ± 0.20	2.16 ± 0.24	2.60 ± 0.34	4.56 ± 0.10	3.60 ± 0.26
No of leaves (Nos)	2.00 ± 0.00	2.66 ± 0.33	2.66 ± 0.033	2.33 ± 0.033	3.00 ± 0.00	3.0 ± 0.00	2.00 ± 0.00
<i>Zea mays</i>	Control	100 mg/ml	200 mg/ml	500 mg/ml	100 mg/ml	200 mg/ml	500 mg/ml
Germination (%)	25	70	100	35	25	10	10
Fresh weight (g)	0.34 ± 0.14	0.76 ± 0.10	0.90 ± 0.19	1.13 ± 0.08	0.60 ± 0.04	0.90 ± 0.11	0.76 ± 0.47
Dry weight (g)	0.10 ± 0.05	0.05 ± 0.03	0.06 ± 0.003	0.01 ± 0.00	0.04 ± 0.02	0.41 ± 0.23	0.031 ± 0.01
Root length (cm)	0.70 ± 0.15	1.76 ± 0.88	4.86 ± 1.84	3.16 ± 0.41	8.20 ± 0.17	10.26 ± 0.95	7.66 ± 1.03
Shoot length (cm)	0.60 ± 0.10	1.50 ± 0.57	2.26 ± 0.84	3.70 ± 0.10	2.75 ± 0.075	5.50 ± 0.43	3.96 ± 0.93
No of leaves (Nos)	1.33 ± 0.33	1.66 ± 0.66	2.66 ± 0.033	3.00 ± 0.00	2.00 ± 0.00	2.66 ± 0.033	2.33 ± 0.33

Table 2
The biochemical range of crop plants.

S. No	Crop plant	Chlorophyll a (mg /g)	Chlorophyll b (mg /g)	Total Chlorophyll	Carbohydrate (mg /g)
1	<i>Paspalum scrobiculatum</i>	1.44	1.95	2.28	86.33
2	<i>Vigna unguiculata</i>	2.06	0.86	3.32	10.23
3	<i>Sorghum bicolor</i>	2.21	0.61	3.59	10.33
4	<i>Oryza sativa</i>	2.39	0.00	3.70	11.76
5	<i>Zea mays</i>	11.51	12.13	23.37	38.33

2.2. Preparation of Cyanobacteria growth promoting substances

The cyanobacteria were harvested after 15 days of cultivation. Fresh biomass of cyanobacteria (100 mg, 200 mg and 500 mg) was taken and homogenised with 100 ml of distilled water in a sterile mortar and pestle. The suspension was centrifuged and the cell free supernatant was collected. The Cyanobacteria growth promoting substances (CGPS) from the harvested biomass was extracted in different concentration (0.1%, 0.2% and 0.5%).

2.3. Seed germination study

The seed germination studies were performed among five types of grains (*Zea mays*, *Sorghum bicolor*, *Oryza sativa*, *Vigna unguiculata* and *Paspalum scrobiculatum*) collected from Crop processing Institute, Kolli Hills, Namakkal District, Tamil Nadu, India. The seeds were surface sterilized in 70% ethanol for 1 min followed by 0.1% HgCl₂ for 4 min then further washing them four times with sterile distilled water. After disinfection, germination of grain seeds was carried out separately by spreading 10 seeds on filter papers placed in glass petri-dishes containing 10 ml of CGPS (0.1%, 0.2% and 0.5%). Petri dishes containing

seeds with 10 ml of distilled water served as a control. Germination of seeds incubated in 16 h dark cycle for 8–10 days. The morphometric parameters of seeds such as germination percentage, shoot length, root length fresh weight, dry weight and number of leaves were measured.

2.4. Extraction, purification and detection of IAA

The 100 mg lyophilized cells of cyanobacteria were dissolved in 100 ml of water and (Intracellular culture filtrates) were centrifuged at 10000 rpm for 10 min. Initial pH of the culture filtrate was adjusted to 11 by 7 M HCl or 7 M NaOH and it was hydrolysed at 70 °C for 1 h. Again pH of the culture filtrate was adjusted to 2.5 by 7 M HCl or 7 M NaOH and it was extracted 3 times with 15 ml of ethyl acetate (Karadeniz 2006).

2.5. Thin-layer chromatography

The CGPS was fractionated on TLC. Commercially available silica gel-G coated plate was used for the experiment (1 × 10 cm at 0.5 mm thickness). Around 10 µl of the extract and standard IAA (10 µg/ml) were loaded on the TLC plate. Isopropanol/ammonia/ethyl acetate

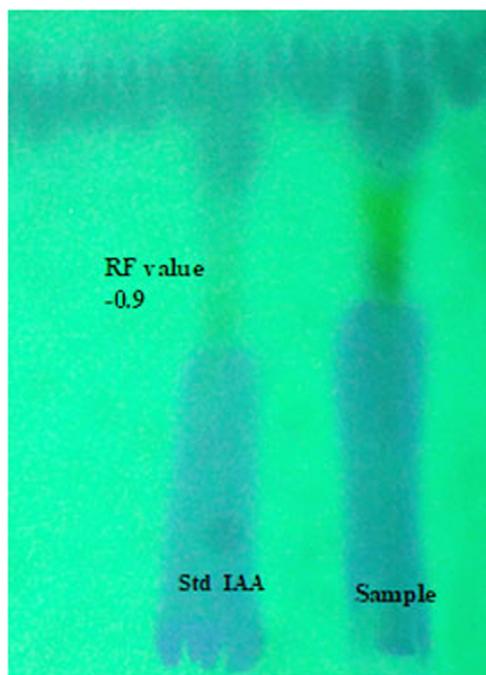


Fig. 3. Thin layer Chromatography UV visible spot of Cyanobacterial extracted IAA.

(10:1:1) was used for solvent system. After separation, the plates were sprayed with Salkowski reagent (Sridevi and Mallaiah, 2007) and dried in oven 100 °C for about 10–15 min to detect the presence of IAA. Hence, a red spot appeared only in this exposed portion. The corresponding part of the silica layer (adjacent to the reagent-exposed IAA spot) was collected in methanol (HPLC grade, Fisher Scientific, India).

2.6. Fourier transform infrared analysis

The partially purified fractions (*A. variabilis* and *N. calcicola*) were scrapped from the TLC plate and analyzed using a Fourier transform infrared (FTIR) spectrophotometer (Perkin Elmer) (Kamnev et al., 2000). The result of TLC-purified IAA was compared with the IR pattern of standard IAA. The set scanning range was 400–4000 cm^{-1} in transmission mode.

2.7. Identification of IAA by HPLC

Chromatographic separation was performed on an Agilent 1200 series high-performance liquid chromatography (HPLC) system including a quaternary pump and a degasser equipped with a G1315D diode array detector and a G1321A fluorescence detector. HPLC was performed for the presence of IAA from crude extract CGPS against the standard IAA. Hormones were quantified by reference to the peak area obtained for the IAA (Sigma, I-2886).

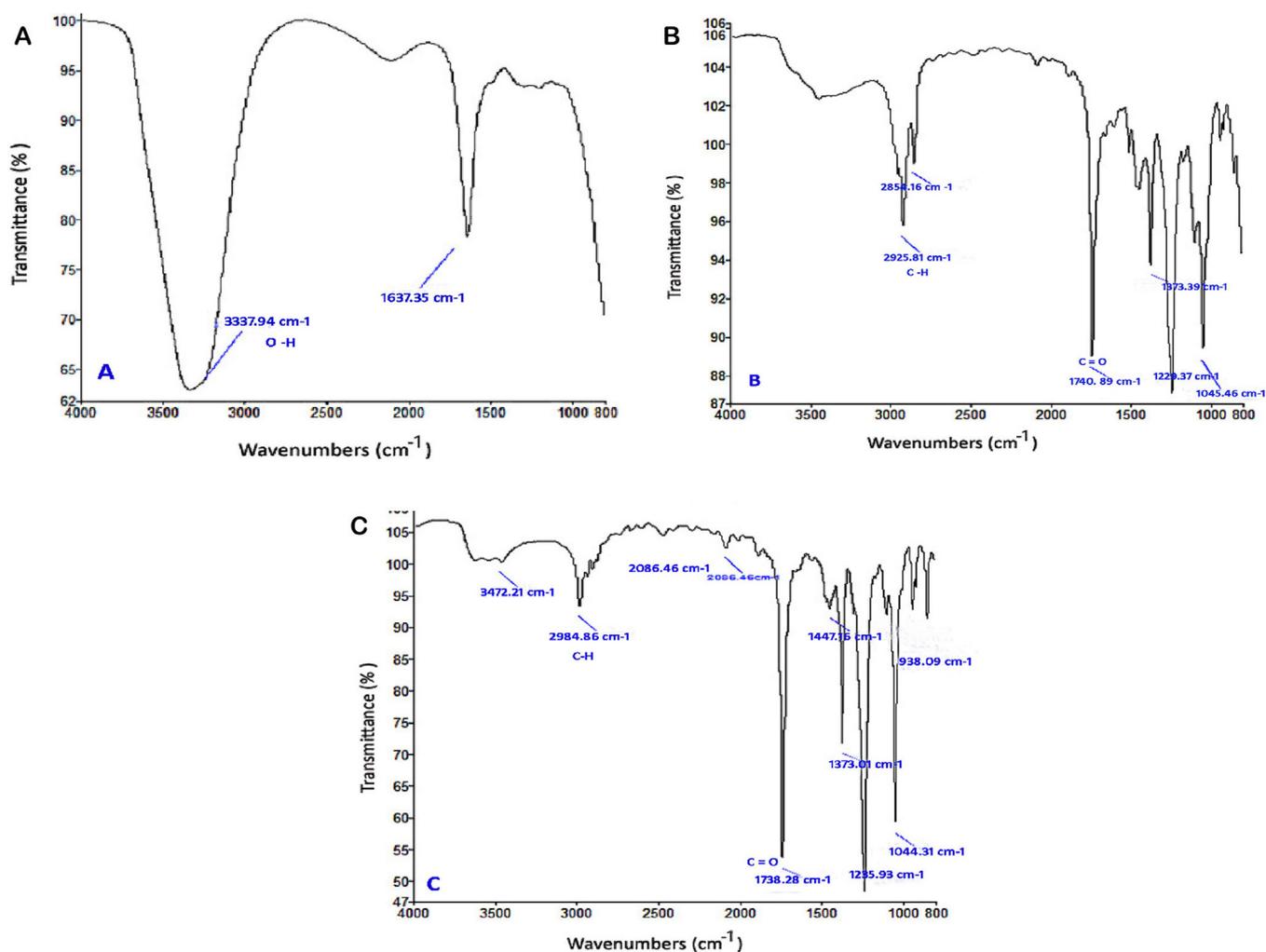


Fig. 4. Infra Red Spectra of Standard IAA and cyanobacterial extracted IAA (A) Standard IAA (B). *Anabaena variabilis* (C). *Nostoc calcicola*.

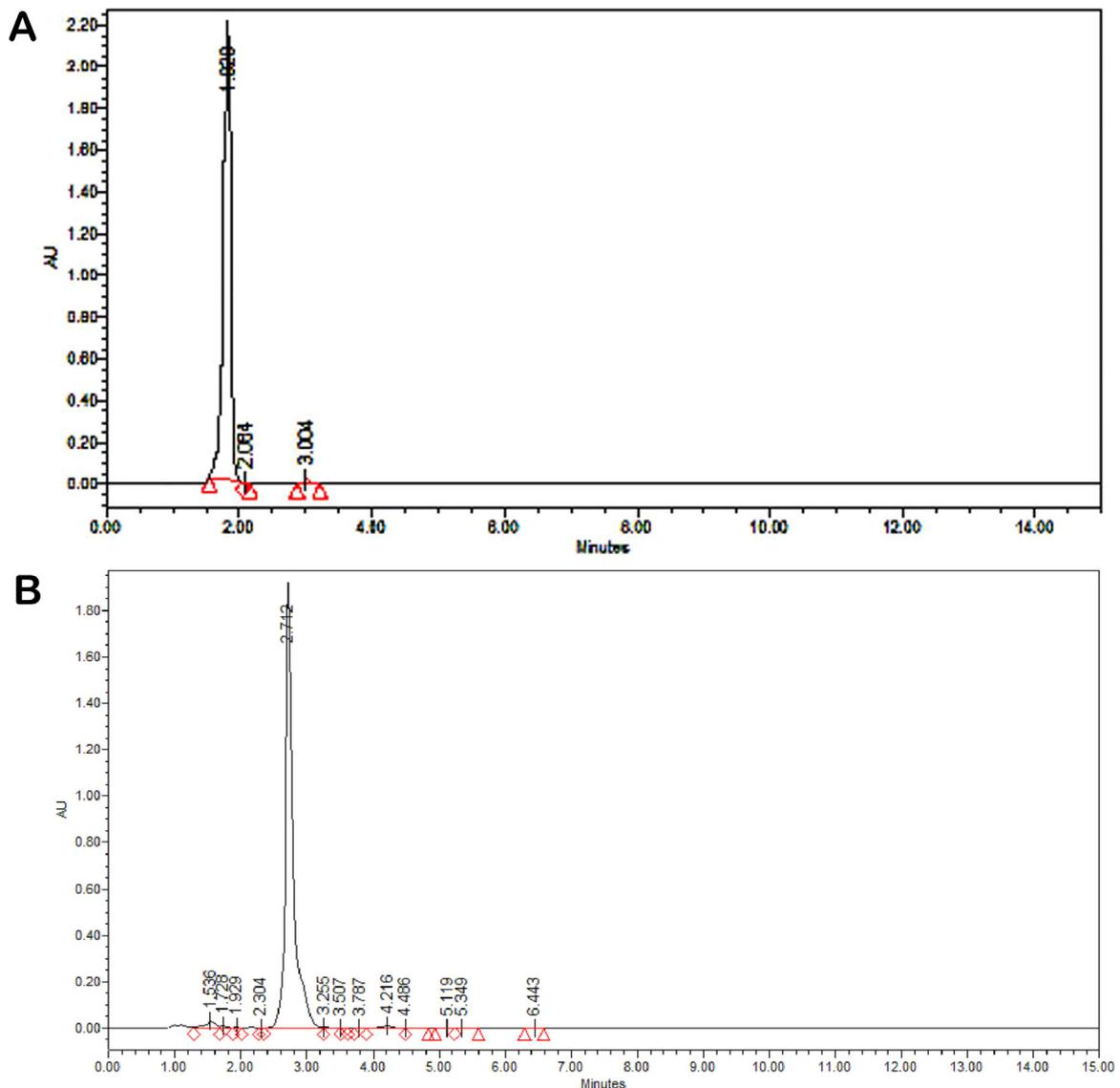


Fig. 5. High performance liquid chromatography (HPLC) chromatograms of the (A). Standard IAA (B). *Anabaena variabilis*.

2.8. Biochemical analysis of treated plants

The chlorophyll content of the cyanobacterial wet biomass was estimated using the method of Arnon (1949).

2.9. Statistical analysis

All the experiments were done in triplicate, unless specified otherwise. Appropriate statistical methods were used to draw valid conclusions from the data using SPSS software. All data are given as mean \pm standard error (SE) at a significance of $p \leq 0.005$ and the error bar in the graphs represents with 5% error percentage

3. Results and discussion

In the present study, two heterocyst Cyanobacterial strains were selected and their CGPS were tested for their plant growth promoting ability in four grain plants with three concentrations (0.1%, 0.2%, 0.5%). Morphometric parameters such as shoot length, root length, fresh and dry weight and total seedlings were measured after seed germination and compared with the control (Figs. 1 and 2). The 100% germination percentage was observed at 0.2%, 0.5% concentration of

A. variabilis CGPS in the seeds of *Zea mays* and *Sorghum bicolor* respectively while the lowest germination of 10% was recorded from 0.1%, 0.2% concentration of *A. variabilis* CGPS in grains of *Vigna unguiculata* when compared to the control (Table 1). The *A. variabilis* CGPS may not be a universal plant growth promoter as show different germination rate between all the three seeds

In pot culture experiment, the highest shoot length (5.93 cm/seedings) in *Vigna unguiculata*, root length (7.83 cm/seedings) in *Vigna unguiculata*, fresh weight (0.90 g/seedings) in *Anabaena variabilis*, dry weight (0.14 g/g^{-1}) in *Sorghum bicolor* was observed after treating them with different concentrations of *A. variabilis* CGPS. While the highest root length (10.26 cm/seedling) in *Zea mays*, shoot length (5.50 cm/seedings) in *Zea mays*, fresh weight (0.9 g/seedings) in *Zea mays* and dry weight (0.6 g/seedling) in *Oryza sativa* was observed in seeding treated with different concentration of *N. calcicola* CGPS. Our data revealed that 0.2% and 0.5% concentration clearly induced the germination percentage as well as the root, shoot and total seedling lengths when compared to the control (Table 1).

Further the biochemical quantification of the leaves was performed. The results revealed that the total chlorophyll content was high (23.37 mg/g) in *Zea mays* and carbohydrate value was high in (86.33 mg/g) *Paspalum scrobiculatum* in comparison with other grain

plants Table 2. The increased chlorophyll content in the leaves is due to the uptake of more nutrients from the soil along with the synergistic effect of cyanobacterial CGPS on the plant (Varalakshmi and Malliga, 2012). Additionally, Elanwar et al. (2010), stated on their studies on pea plant that increase in carbohydrate contents in plant may be attributed due to the activity of the growth promoters from cyanobacteria which could be the reason behind activating photosynthesis in plants. The wide range of plant growth promoting substances (PGPS) like auxins, cytokinins, gibberellins, amino acids, sugars, vitamins and free volatile fatty acids present in the cyanobacterial filtrate (CF) may amplify germination, root and shoot lengthening in germinating plants (Misra and Kaushik, 1989a, 1989b; Priya et al., 2015; Wuang et al., 2016; Gayathri et al., 2017). These substances of cyanobacteria have important role several metabolism of plants like growth regulation, metabolism and plant development (Hashtroudi et al., 2013).

In correlation with previous reports, the present study clearly shows increase that CGPS of *A. variabilis* (0.2% and 0.5% concentration) helps in seed germination, root and shoot lengthening of gram seedlings. This could be also due to the effect of cyanobacterial exudates on the growth promoting substances especially auxins, cytokinins (Stirk et al., 1999), and gibberellins (Serdyuk et al., 1992). Among them IAA is the most important auxins in plants which promote rooting and regulates essential physiological functions like cell elongation, division, tissue differentiation, responses to light and gravity and root colonization (Boopathi et al., 2013; Hussain et al., 2015). In our study the concentration of IAA was ranging from 0.0372 to 1.2327 $\mu\text{g g}^{-1}$ dry cell weight in both the strains of cyanobacteria. Therefore the present result clearly predicted that lower concentration of IAA in the studied cyanobacteria has promoted the root enhancement in the plants used for the study. In similarity with the present study, Minakshi, Lingakumar (2011) has also reported that low concentrations of IAA can increase some growth parameters such as root length and leaf area.

Further, IAA in the CGPS was separated by TLC and identified as a red spot by spraying with Salkowski reagent (Fig. 3). The RF value was determined as 0.45 cm for IAA. The isolated IAA from the extract was further taken for FT-IR and HPLC analysis. The FTIR results clearly showed the similarity in the functional group of the standard and the sample. The presence of stretching at 2984.94 cm^{-1} indicated NH group, 1740.89 cm^{-1} indicated the C=O stretch, 1045.46 and 1255.93 indicated the presence of C-C aromatic ring (Fig. 4). Therefore comparing the standard IAA and CGPS clearly indicated the presence of IAA in the CGPS of both the cyanobacteria.

Finally, HPLC analysis was performed for analysing the presence and purity of IAA in the CGPS. The HPLC chromatograms of the standard and samples are shown in Fig. 5A. The peaks were identified by comparing the retention times of authentic standards, the UV spectra, and also spiking the individual standards to the cyanobacteria extracts. The observed peaks at 9.130, 9.147, and 9.288 min were related to IAA, when compared to the peak of IAA in the chromatogram of standard mixture at 9.127 min (Fig. 5B). The results of our study showed that improvement of rooting in the studied plants may be promoted by phytohormones in IAA. Apart from these factors, the CGPS must also contain other growth promoting macro and micromolecules like vitamins, nicotinic acid, folic acid, pantothenic acid etc, which could have also supported the efficient growth and yield of the plant (Venkataraman and Neelakantan, 1967).

4. Conclusion

In conclusion, the application of *Anabaena variabilis* in 0.2%, 0.5% concentration showed better result in terms of morphological, biochemical and yield parameters when compared with other concentrations and control. Finally our results showed that the *Anabaena variabilis*, have the ability to promote grain seeding growth with two crop

plant *Zea mays*, *Sorghum bicolor* and they are appropriate candidate for the formulation of a biofertilizer. Further detailed survey on *Anabaena variabilis* will develop a potential biofertilizer for the growth of *Zea mays* and *Sorghum bicolor* in field study.

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References

- Bidyarani, N., Prasanna, R., Babua, S., Hossainb, F., Saxena, A.K., 2016. Enhancement of plant growth and yields in chickpea *Cicer arietinum* L through novel cyanobacterial and biofilmed inoculants. *Microbiol. Res.* 188, 97–105.
- Boopathi, T., Balamurugan, V., Gopinath, S., Sundararaman, M., 2013. Characterization of IAA production by the mangrove cyanobacterium *Phormidium* sp. MI405019 and its influence on tobacco seed germination and organogenesis. *J. Plant Growth Regul.* 32, 758–766.
- Desikachary, T.V., 1959. Cyanophyta. Indian Council of Agriculture Research, Bombay, India.
- Gayathri, M., Shunmugam, S., Thajuddin, N., Muralitharan, G., 2017. Phytohormones and free volatile fatty acids from cyanobacterial biomass wet extract (BWE) elicit plant growth promotion. *Algal Res.* 26, 56–64.
- Glick, B.R., 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 1–15.
- Hashtroudi, M.S., Ghassempour, A., Riahi, H., Shariatmadari, Z., Khanjir, M., 2013. Endogenous auxin in plant growth-promoting Cyanobacteria-*Anabaena vaginicola* and *Nostoc calcicola*. *J. Appl. Phycol.* 25, 379–386.
- Hussain, A., Hasnain, S., 2011. Phytostimulation and biofertilization in wheat by cyanobacteria. *J. Ind. Microbiol. Biotechnol.* 38, 85–92.
- Igiehon, N.O., Babalola, O.O., 2017. Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. *Appl. Microbiol. Biotechnol.* 101, 4871–4881.
- Kamnev, A., Kuzmann, E., Perfiliev, Y., Vertes, A., Ristic, M., Popovic, S., 2000. Composite ferric oxyhydroxide-containing phases formed in neutral aqueous solutions of tryptophan and indole-3-acetic acid. *J. Radioanal. Nucl. Chem.* 246, 123–129.
- Karthikeyan, N., Prasanna, R., Lata, N., Kaushik, B.D., 2007. Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *Eur. J. Soil Biol.* 43, 23–30.
- Mazhar, S., Cohen, J.D., Hasnain, S., 2013. Auxin producing non-heterocystous cyanobacteria and their impact on the growth and endogenous auxin homeostasis of wheat. *J. Basic Microbiol.* 9999, 1–8.
- Minakshi, V., Lingakumar, K., 2011. The role of IAA and 2, 4-D on growth and biochemical constituents in vegetatively propagated *Mentha arvensis* L. *J. Bio Sci.* 2, 10–15.
- Misra, S., Kaushik, B.D., 1989a. Growth promoting substances of cyanobacteria I. Vitamins and their influence on rice plant. The Proceedings of the National Academy of Sciences, India, Section B: *Biol. Sci.* B55, 295–300.
- Misra, S., Kaushik, B.D., 1989b. Growth promoting substances of cyanobacteria II. Detection of amino acids, sugars and auxins. The Proceedings of the National Academy of Sciences, India, Section B: *Biol. Sci.* B55, 499–504.
- Obana, S., Miyamoto, K., Morita, S., Ohmori, M., Inubushi, K., 2007. Effect of *Nostoc* sp. on soil characteristics, plant growth and nutrient uptake. *J. Appl. Phycol.* 19, 641–646.
- Prasanna, R., Chaudhary, V., Gupta, V., Babu, S., Kumar, A., Singh, R., Shivay, Y.S., Nain, L., 2013. Cyanobacteria mediated plant growth promotion and bioprotection against *Fusarium* wilt in tomato. *Eur. J. Plant Pathol.* 136, 337–353.
- Priya, H., Prasanna, R., Ramakrishnan, B., Bidyarani, N., Babu, S., Thapa, S., Renuka, N., N., 2015. Influence of cyanobacterial inoculation on the culturable microbiome and growth of rice. *Microbiol. Res.* 171, 78–89.
- Rippka, R., Deruells, J., Waterbury, J.B., Herdman, M., Stanier, R.Y., 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* 111, 1–61.
- Serdyuk, O.P., Smolygina, L.P., Kobzar, E.V., Gogotov, I.N., 1992. Phytohormones formed by the nitrogen fixing association of *Anabaena-Azollae*. *Dokl. Biochem.* 325, 149–151.
- Shunmugam, S., Muralitharan, G., Thajuddin, N., 2015. Cyanobacteria and Algae Potential Sources of Biological Control Agents Used Against Phytopathogenic Bacteria. CRC press, pp. 241–254.
- Sridevi, M., Mallaiiah, K.V., 2007. Production of indole-3-acetic acid by *Rhizobium* isolates from *Sesbania* species. *Afr. J. Microbiol.* 1, 125–128.
- Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue green algae (order: Chroococcales). *Bacteriol. Rev.* 35, 171–205.
- Stirk, W.A., Ordog, V., Van staden, J., 1999. Identification of the cytokinin isopentenyladenine in a strain of *Arthonema africanum* (Cyanobacteria). *J. Phycol.* 35, 89–92.
- Varalakshmi, P., Malliga, P., 2012. Evidence of production of indole-3-acetic acid from a fresh water cyanobacteria (*oscillator annae*) on the growth of *H. annuus*. *Int. J. Sci. Res. Publ.* 2, 1–15.
- Wuang, S.C., Khin, M.C., Chua, P.Q.D., Luo, Y.D., 2016. Use of *Spirulina* biomass produced from treatment of aquaculture wastewater as agricultural fertilizers. *Algal Res.* 15, 59–64.
- Xu, Y., Rossi, F., Colica, G., Deng, S., De Philippis, R., Chen, L., 2013. Use of cyanobacterial polysaccharides to promote shrub performances in desert soils: a potential approach for the restoration of desertified areas. *Biol. Fertil. Soils* 49, 143–152.