VOLUME - 12, ISSUE - 07, JULY - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Agriculture

FIELD TRIAL OF CYANOBACTERIA BASED BIOFERTILIZER FOR CULTIVATION OF TRITICUM AESTIVUM PLANT

Deepak Kumar	Director, Bunshi Bioscience.
Rahul Anand	Research Associate, Bunshi Bioscience.
Akash Deka	Mewar University, Rajasthan
Kajal Gupta	Mewar University, Rajasthan

ABSTRACT The continuous growth of the global human population and the depletion of natural energy resources are posing a threat to the environment and the sustainable supply of food and energy. To address this issue, a solution known as "green technology" has been developed, focusing on the use of eco-friendly approaches. One of these approaches involves harnessing the power of cyanobacteria, which are ancient microorganisms that have existed on Earth for a long time. This approach was used to probe the diversity of phototrophic microorganisms in Wheat samples. The presence of nif genes allows cyanobacteria to fix nitrogen which plays a vital role in nitrogen cycle and biological processes such as plant growth and soil fertility. Wheat production test in current study evaluates the yield and quality of wheat crops. It shows factors such as improvement in growth, heat resistance and environmental adaptability, improvement in production; provide insights for optimizing cultivation practices and ensuring successful high yield. Identification of Cyanobacteria for their combination of nif and molecular identity is prior for production of bio fertilizers. Cyanobacteria can be cultivated on a large scale to produce biomass that has multiple uses, including biofertilizer, secondary metabolites, cosmetics, and medicines. In the field of agriculture, production of healthy crop cyanobacterial biofertilizer are being increasingly used in eco-friendly and sustainable practices. These biofertilizer are produced through mass cultivation of cyanobacteria and offer several benefits.

KEYWORDS : Cyanobacteria, biofertilizer, nitrogen fixation, wheat, agriculture, etc.

INTRODUCTION

Scientists have identified about 150 different kinds of cyanobacteria strains. These bacteria have been around for a very long time, even longer than 3.5 billion years. They are important because they have features that are similar to the oldest fossils ever found. Cyanobacteria are tiny organisms that were classified into different groups in 1985. These groups are called *Chroococcales, Nostocales, Oscillatoriales, and Stigonematales*. They belong to the phyla *Chroococcales, Gloeobacterales,* and *Pleurocapsales* (Patel *et al*., 2019). Cyanobacteria, a diverse group of photosynthetic bacteria, have the remarkable ability to convert atmospheric nitrogen into biologically useful forms through a process called nitrogen fixation. This process is facilitated by a specialized set of genes known as the *nif* genes, which play a pivotal role in nitrogen fixation.

The Nif Gene and Nitrogen Fixation:

The evolutionary patterns of nitrogen fixation ability and associated *nif* genes remain disputed, impacting our understanding of biogeochemical history. The nif gene cluster includes different genes that play roles in nitrogen fixation. These genes produce nitrogenase enzymes, which convert atmospheric nitrogen into ammonia through a series of reactions. The main components of the nitrogenase enzyme are the iron protein (nifH gene) and the molybdenum-iron protein (nifD and nifK genes). Other nif genes are also involved in controlling the nitrogenase enzymes' expression and activity (Kallas *et al*., 1985).

The expression of the nif genes is tightly regulated in cyanobacteria due to the energy-intensive nature of nitrogen fixation and the need for specific environmental conditions. Cyanobacteria have sophisticated mechanisms, including the NifA protein complex, which activates nif gene transcription when nitrogen is limited. Other regulatory proteins and environmental signals, like oxygen and fixed nitrogen levels, also play important roles in controlling nif gene expression (Mulligan *et al*., 1989)

Cyanobacteria's ability to fix nitrogen is ecologically important. Nitrogen is essential for life, but often limited in ecosystems. Cyanobacteria provide biologically available nitrogen to both aquatic and terrestrial ecosystems. This fuels the growth of other organisms, affecting productivity and biodiversity.

Environmental Implications:

The nif gene cluster in cyanobacteria and its regulation have broader implications for environmental management. Harmful algal blooms caused by excessive nitrogen and phosphorus inputs are a concern in freshwater bodies and coastal areas. Manipulating the nif genes in cyanobacteria could help control or reduce these blooms by limiting nitrogen availability (Rubio *et al*., 2008)

Cyanobacteria use as a biofertilizer:

The world's population is growing rapidly and is expected to reach 9.7 billion people in the next 30 years. India will contribute significantly to this growth. With more people, there is a greater demand for safe and healthy food. To meet this demand, the World Health Organization aims to increase global food production by 50% by 2029. The "Green Revolution" practices help achieve this by improving agricultural productivity and reducing the negative effects of chemical-based fertilizers on human health and the environment. Green technology, using cyanobacteria, is being used to create an eco-friendly environment, improving crop productivity and soil fertility. As the population increases, populations need more safe and healthy food. The Green Revolution practices and green technology help increase food production while protecting our health and the environment. Cyanobacteria help clean up pollution and support healthy soil for growing food.

Cyanobacteria can be used to explore the potentiality of biofertilizers. These biofertilizers are accessible and affordable, and can address nitrogen deficiency in plants, improve soil aeration, increase water-holding capacity, and provide vitamin B12. Effective nitrogen-fixing cyanobacteria for biofertilizers include *Nostoc linkia, Anabaena variabilis, Aulosira fertilisima, Calothrix sp., Tolypothrix sp.,* and *Scytonema sp.*, which can be found in rice-growing areas (Tsygankov, 2007).

Anabaena and *Nostoc* are cyanobacteria that live on soil and rocks, fixing about 20-25 kilograms of atmospheric nitrogen per hectare. *Anabaena* can fix even more nitrogen, up to 60 kilograms per hectare per growing season, while also enriching the soil with organic matter. Cyanobacteria, such as Azolla-Anabaena, form beneficial associations for nitrogen fixation and nutrient enrichment in rice paddy fields. These compounds promote abundant spore production and have been successfully used as biofertilizers in various crops (Rai *et al*., 2019).

16S rRNA to amplify cyanobacteria genes

Studying cyanobacteria in their natural environment alongside isolated strains is important for understanding them better. However, there are challenges in doing so. Inadequate culture conditions can change Cyanobacteria's physical characteristics, some organisms cannot be grown in the lab, and misidentification can occur in culture collections. This makes it difficult to apply taxonomic classifications based on cultures to field populations. The 16S rRNA has conserved regions for function and hyper variable regions that act as genetic fingerprints to differentiate between species and infer evolutionary relationships. The current most promising approach for classifying cyanobacteria is analyzing the sequence of genes that encode 16S rRNA (Nubel U *et al.,* 1997; Steindler *et al*., 2005).

Wheat production in India:

Wheat is an important crop that is grown during the winter in north-western India. It is usually grown after rice and cotton, forming cropping systems like rice-wheat and cotton-wheat. It contributes to about one-fifth of the daily calorie and protein needs of humans. The crop yield has not been increasing significantly over the years, and there has been a decline in the water table underground, which means less water is available for crops. The soil quality has also been degrading due to a decrease in organic matter in the soil. Additionally, this cropping system has been causing environmental pollution. To ensure that we can feed the growing population, which is expected to reach about 1.35 billion by 2025, India needs to increase its crop production by about 25%. It is important to address these sustainability issues in the rice-wheat cropping system to overcome the challenges and ensure a secure food supply for the future (Singh P *et al*., 2019; Joshi *et al*., 2007).

MATERIAL AND METHODS:

Biofertilizer TEST:

Cyanobacterial culture provided from Aakriti Biotechnology, Ranchi, Jharkhand, India, was maintained and monitored for growth. Poly house 4ft of height and 16^2ft with plastic covering was prepared for the biofertilizer test. Soil collected from fertile land, sterilized using an autoclave. Biofertilizer was prepared with Cyanobacteria culture with two and half months. Wheat Seeds germination was carried out after sterilizing the seeds using cleaning with soap solution followed with fungicide. The biofertilizer was cleaned to eliminate salts further diluted with 5L of water and spread on the test plot soil surface uniformly. Growth parameters were monitored for plant height, shoot length, root length, hair formation, and overall health. Proper conditions were maintained in the poly house for seed germination, including moisture, temperature, light, and air circulation. Monitoring and adjustment of environmental conditions were done throughout the process.

DNA Isolation and PCR Sequencing:

Cyanobacteria strain was collected from local pond, purified and maintained in BG-11 media at Aakriti Biotechnology lab, Ranchi, India. DNA isolation performed using DNA isolation Kit (Bunshi Bioscience Pvt Ltd). DNA was obtained and observed on 0.8% agarose gel under Gel imagining system (BioEra). 40ng of DNA was amplified by Thermalcycler (BioEra Thermalcycler neo). Overall 35 cycles of reaction was performed involved denaturation at 94 ^{0}C for 40 sec, followed by annealing at 50 ^{0}C for 30 seconds, with extension at 72 ^{0}C for 30 seconds. Program was finally extended at 72 ^{0}C for 10 min. Amplified PCR product were observed using 1% Agarose gel. The PCR amplicon was extracted from gel and sequenced using ABI Prism Platform (Applied Biosystems, USA).

RESULTS:

Pure culture of Cyanobacteria, grown at optimum at 1.5 OD at 680nm was taken for study. 50μg of genomic DNA was obtained and diluted to 100ng per μl and used for PCR. The 16S rRNA gene gave 1.5kb amplification; *Nif*H gene gave 200bp amplification along with positive control. The cyanobacteria strain taken for biofertilizer testing was found to be close to *Synechococcus sp*. (Figure – 4).

The cyanobacteria biofertilizer had highly positive impact on the growth of wheat plant and overall health of the plant. The wheat plantlets grown on soil supplemented with cyanobacteria biofertilizer had shoot length almost 45.55% more than the control plants, after 30 days of growth, root length 14.54% more than the control plant. The hairy roots projections in the test plantlets were more than the control plantlets. The overall plant height after 30 days of growth was 28.79% more than the control plant.

Table – 1: Growth parameters of the wheat.

Growth Parameter	Date	Normal Avg.	Tested Avg.
Height	10 days	14cm	17cm
	15 days	17.3cm	25cm
	30 days	23cm	32.3cm
Length of Shoot	10 days	9.5cm	11cm
	15 days	11.5cm	13.3cm
	30 days	12cm	22cm
Length of Root	10 days	4.6cm	5.4cm
	15 days	5.5cm	11.5cm
	30 days	9.4cm	11cm
Hair formation in Root	10 days	good	good
	15 days	good	Multiple
	30 days	less	Multiple
Overall health	10 days	normal	normal
	15 days	normal	strong
	30 days	normal	strong

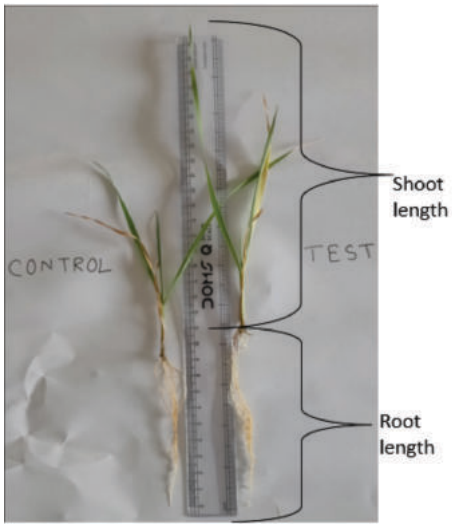

Figure 1: Difference in growth of Wheat after 30 Days and hairy root development comparatively.

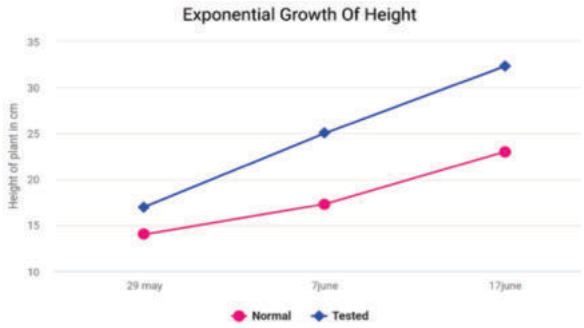

Figure – 2: Growth of wheat on cyanobacteria biofertilizer and control.

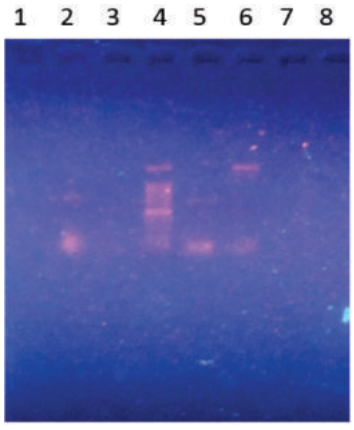

Figure 3: PCR Amplification of 16S rRNA gene. Lane 2: +ve control, Lane 4: Ladder, Lane 5: NifH gene, Lane 6: 16S rRNA gene.

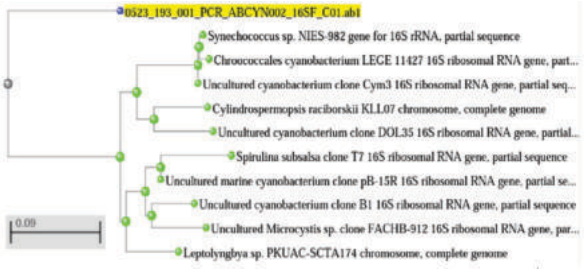

Figure 4: Phylogenetic Tree after sequence Cyanobacteria DNA

DISCUSSION:

Cyanobacteria were cultured in BG11 media and used as a biofertilizer for wheat plants. After 30 days of treatment, noticeable improvements were observed in the treated wheat compared to the control group. The treated plants showed increased height, longer root length, Development of hairy formation and enhanced overall health. These results indicate that cyanobacteria positively influenced wheat growth and development, suggesting their potential as a sustainable method to enhance wheat production. Further research is needed to understand the mechanisms and optimize application techniques for cyanobacterial treatments in wheat cultivation. Isolating, amplifying (using PCR) and sequencing the 16S rRNA gene can be considered a reliable and established method for characterizing the morphology of bacteria.

CONCLUSION:

The application of cyanobacteria as a biofertilizer for wheat plants resulted in significant improvements in growth and development. The treated wheat showed increased height, longer roots length, development of hairy formations, and enhanced overall health. These findings highlight the positive impact of cyanobacteria on wheat production and suggest their potential as a sustainable approach. Further research is needed to explore the underlying mechanisms and optimize the application techniques for cyanobacterial treatments in wheat cultivation. Additionally, isolating, amplifying (using PCR), and sequencing the 16S rRNA gene is a reliable method for characterizing the morphology of bacteria.

REFERENCES:

1. Patel, H. M., Rastogi, R. P., Trivedi, U., & Madamwar, D. (2019). Cyanobacterial diversity in mat sample obtained from hypersaline desert, Rann of Kachchh. *3 Biotech*, 9(8), 304. https://doi.org/10.1007/s13205-019-1837-y
2. Rubio, L. M., & Ludden, P. W. (2008). Biosynthesis of the iron-molybdenum cofactor of nitrogenase. *Annual review of microbiology*, *62*.
3. Rai, A. N., Singh, A. K., & Syiem, M. B. (2019). Plant growth-promoting abilities in cyanobacteria. In *Cyanobacteria* (pp. 459-476). Academic Press.
4. Nübel, U., Garcia-Pichel, F., & Muyzer, G. (1997). PCR primers to amplify 16S rRNA genes from cyanobacteria. *Applied and environmental microbiology*, *63*(8), 3327-3332.
5. Singh, P., Singh, G., & Sodhi, G. P. S. (2019). Applying DEA optimization approach for energy auditing in wheat cultivation under rice-wheat and cotton-wheat cropping systems in north-western India. *Energy*, *181*, 18-28.
6. Kallas, T., Coursin, T., & Rippka, R. (1985). Different organization of nif genes in nonheterocystous and heterocystous cyanobacteria. *Plant molecular biology*, *5*, 321-329.
7. Mulligan, M. E., & Haselkorn, R. (1989). Nitrogen fixation (nif) genes of the cyanobacterium Anabaena species strain PCC 7120: the nifB-fdxN-nifS-nifU operon. *Journal of Biological Chemistry*, *264*(32), 19200-19207.
8. Tsygankov, A. A. (2007). Nitrogen-fixing cyanobacteria: a review. *Applied biochemistry and microbiology*, *43*, 250-259.
9. Joshi, A. K., Mishra, B., Chatrath, R., Ortiz Ferrara, G., & Singh, R. P. (2007). Wheat improvement in India: present status, emerging challenges and future prospects. *Euphytica*, *157*, 431-446.
10. Steindler, L., Huchon, D., Avni, A., & Ilan, M. (2005). 16S rRNA phylogeny of sponge-associated cyanobacteria. *Applied and Environmental Microbiology*, *71*(7), 4127-4131.