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J. Nat. Prod. Plant Resour., 2013, 3 (4):17-23
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ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Some potential rice field BGA isolates from Sonitpur district Assam , North east India

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ABSTRACT

An effort was made to study the rice field cyanobacterial biodiversity and to screen them based on nitrogen fixing biofertilizer potential from 3 different subdivisions viz –Tezpur, Biswanath chariali, Gahpur of Sonitpur district of Assam(India). During the study period in both winter and summer altogether 115 no of soil samples were collected from paddy fields of low lying riverine belt of the river Brahmaputra. The distributional pattern showed that out of the 63 isolates 43 different BGA strains belonged to 15 genera. Among them the genera Nostoc, Anabaena, Aulosira, Calothrix, Wetiellopsis and Aphanocapsa were the dominant rice field BGA in the district. The highest amount of N₂ content was recorded in Anabaena Spiroids (4.21%) and the lowest amount was recorded in the strain Aphanotheceae Nagelii (0.65%) overall and the mean amount of N₂ content among all the genera was 2.57%. The present communication deals and concludes that the highly potential indigenous Cyanobacteria which are widely distributed can be exploited in the yield of rice cultivation in the district. Extension work is also utmost necessary to popularize the use of the indigenous Cyanobacteria as Bio-fertilizer.

Key words- Cyanobacterial N₂ Fixation , Sonitpur district, paddyfield, Assam.

INTRODUCTION

Cyanobacteria are the largest and most diverse group of Gram-negative, oxygenic photosynthetic prokaryotes that are distributed in all possible biotopes of the world. Due to their occurrence in diverse habitats, these organisms are excellent materials for investigation by ecologists, physiologists, biochemists, microbiologists and biotechnologists. Cyanobacterial diversity is reflected in the G+C content of the group which ranges from 35 to 71 %. Sometimes they store extra nitrogen as polymers of arginine or aspartic acid in cyanophycin granules [1]. They occupy a central position in global nutrient cycling especially due to their inherent capacity to fix atmospheric CO₂ and N₂ through Rubisco and nitrogenase enzymes respectively (Sinha *et al.*, 1995; 1997). [2][3]. Recently soil algalization with living di-nitrogen fixing cyanobacteria has received an increasing attention in the temperate countries also because of its implications in reducing environmental pollution, removing soil compaction and turf treatment (Roger and Kulasooriya 1981).[4] Most rice-N originating from cyanobacteria is made available to the crop through mineralization after their death. Possible beneficial effects of cyanobacteria on rice include (1) competition with weeds, (2) increased soil organic matter content, (3) excretion of organic acids that increase P availability to rice, (4) inhibition of sulfide injury in sulfate-reduction-prone soils by O₂ production, (5) increased water temperature, and (6) possible production of plant growth regulators (PGR) (Roger, 1996).[5] Keeping the vast use of rice field

cyanobacteria in view an approach was made to study distributional pattern and screening out the N₂ –fixing potential BGA isolates in Sonitpur district of Assam.

Description of Study site --- Assam, the gateway of North East and Biodiversity heaven of India which is situated between 20° N and 28° N latitude and 90° E and 96° E longitude It has an area of over 78,438 sq km. In Assam among all the productive sectors, agriculture makes the highest contribution to its domestic sectors. Sonitpur district is situated in the Northern plain agro –climatic zone of the state of Assam. The district falls between 26.28 to 27.08 degrees North latitude and 92.19 to 93.47 degree east longitude. The geographical area of the district comprises of three agricultural sub-divisions, viz. Tezpur, Biswanath chariali and Gahpur. Meteorological data of the study sites were collected from district agricultural and meteorological office. The overall geographical and climatic condition is suitable to flourish cyanobacterial vegetation.

Table[I].Climatic and agricultural scenario of Sonitpur district at a glance.

Annual average maximum and minimum temperature	28-30 ⁰ C(July – August) and 19-20 ⁰ C(December- January)
Annual average relative humidity	69.8-80.7%
Annual Average rainfall	1355-2348mm.
Total Geographical Area	5.32 lakh hectares (5324 sq . km
Cropping intensity	162.9%
Summer rice productivity	2250kg/Ha
Fertilizer consumption	20.55 kg/Ha.
No's. Agricultural Sub-divisions	3 nos.
Normal P ^H agricultural soils	5.6 – 6.6

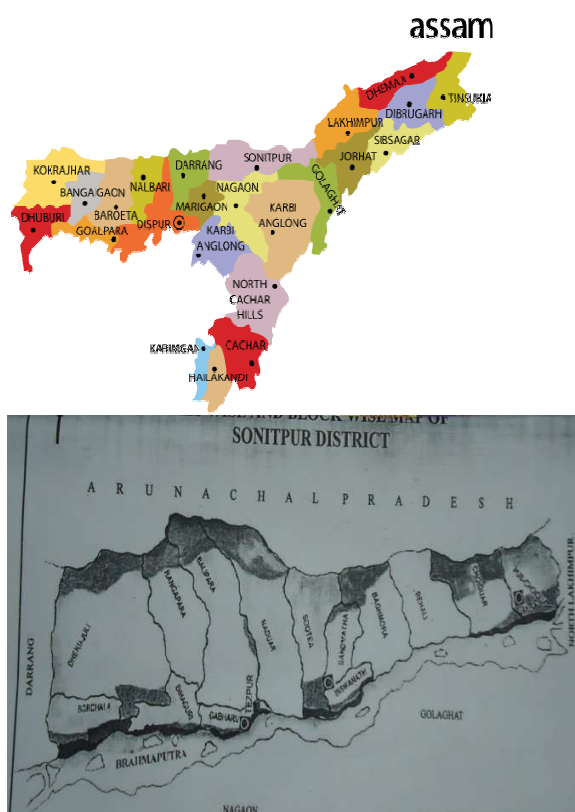


Figure [L.] Map location showing the sonitpur district of Assam along with different sampling stations. Materials and Methods

Selection of sampling sites Collection, and isolation of BGA strains

For the convenience of study each subdivision of the district viz- Tezpur, Biswanath chariali and Gahpur is divided into agricultural divisional circle (ADO circle) again each ADO circle is divided into VLEW eleka (Village level extension workers eleka). Each VLEW eleka consist of many small areas of rice fields. Sampling collecting stations were paddy fields of low lying riverine belt of the river Brahmaputra. Representative and randomized soil samples from the selected rice fields of each VLEW eleka were collected. After collection and serial dilution of the soil sample, standard plating and streaking techniques were used for isolation of cyanobacterial strain. Temporary slides were prepared from each plate for preliminary observation.

Identification purification and maintenance of cyanobacterial strains

After preliminary observation isolated BGA strains were inoculated to flasks containing sterilized BG₀₁₁ (nitrogen free media) and Foggs (1949) media depending on suitable for various genera [6] [7]. These were subsequently cultured and incubated at 25 +/- 2 °C, 14/10 hour light / dark cycle with illumination of 20 μEm⁻² sec⁻¹ by cool white fluorescent tubes (Phillips, India) and under aseptic condition culture flasks were maintained. Unialgal BGA strains were then viewed and photographed under digi eye 210/330 microscope with micro measurement and image analysis software (capturepro). After two to three weeks of growth samples were transferred for total N₂ estimation. The growth pattern and morphological examinations of the cyanobacterial strains were carried out at different stages of growth and BGA strains were identified on the basis of size and shape of vegetative cells, heterocystous / nonheterocystous, filamentous / colonial, sheathed / unsheathed and presence of akinetes, using the keys of Desikachary (1959) and Anand [8].

Estimation of Total nitrogen content

The capacity of these cyanobacteria to grow on nitrogen free media gave evidence that nitrogen was present in their body. This nitrogen was harnessed by the BGA from the air. The algal mass for each isolate after growth for 30 days was separated from the medium by filtration through whatman No 41 Paper and nitrogen content was determined by microkjeldahl digestion and distillation method (Jackson 1958).

RESULTS AND DISCUSSION

Altogether 115 Nos. of soil samples were collected to isolate the BGA strains. The result showed that 43 different BGA strains had been identified out of the 63 isolates which belongs to 15 genera. From the observation highest nos of heterocystous strains were included in the genera *Nostoc* (8), followed by *Anabaena* (6) *Aulosira* (5), which speculates around 5 – 10 % of the cells develop into heterocysts when these cyanobacteria are deprived of both nitrate and ammonia their preferred nitrogen sources. This distributional pattern showed that the genera *Nostoc*, *Anabaena*, *Aulosira*, *Calothrix* and *Wetiollopsis* and *Aphanocapsa* were the dominant rice field Cyanobacteria in District. The maximum nos. of strains were recorded in *Nostoc*, (8) > *Anabaena* (6) > *Aulosira* (5) and *Aphanocapsa* > *Calothrix* (3) and *Oscillatoria* (3). It showed the frequency distribution of the isolates in terms of total nos. of strains was such *Nostoc* (23.25%) > *Anabaena* (18.60%) > *Aulosira* (13.95%) and *Aphanocapsa* (13.95%) *Wetiollopsis* (11.62%) > *Rivularia* (9.30%) and *Hapalosiphon* (9.30%).

This observation is in close conformity of with the findings Mitra 1951, Sardesh Pandey and Goyale 1981, Singh et al 1997 and Venkataraman (1975) opined that *Nostoc* was the dominant BGA strain in the rice fields of Kerala, Tamilnadu, West Bengal. The other genera were restricted into some circles only which can be said that these were not widely distributed (among the 43 strains) in the Circles. This variations of distribution can also be supported with reference to the findings of Anand and Hopper, 1995, Dprycanta 1997, Saikia and Bordoloi 1994 and Singh et al 1997. This restricted may be due to some environmental factor, high pH, value and modern agricultural practices. Some regional difference could be noticed in the distribution of many Cyanobacteria.

Graph [I] – Total amount of Nitrogen content of different cyanobacterial strains encountered in Sonitpur District.

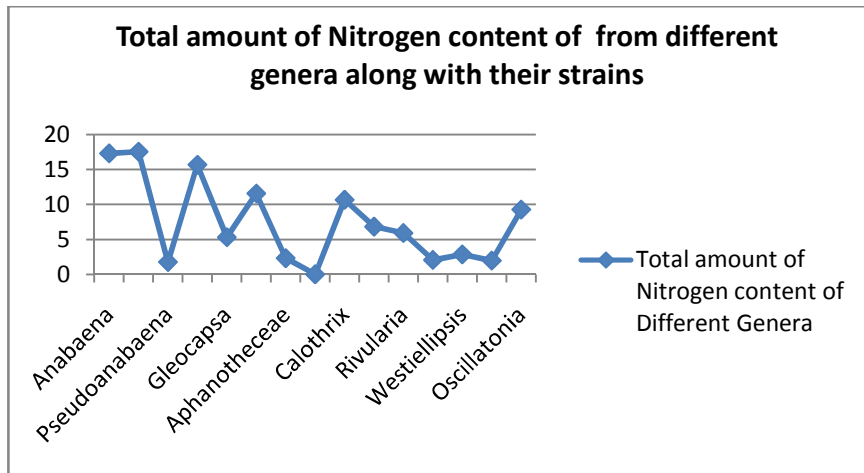


Table [II.] Total number of BGA isolates of different genera isolated from 15 agricultural circles of Tezpur, Biswanath chariali and Gahpur subdivision of Sonitpur District

Nos showing the different Agricultural Circle under sonitpur district

Genera	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total no of isolates	Incidence of BGA in circles
1.Nostoc	+	+	+	-	+	+	+	--	--	+	+	-	++	-	-	10	9
2.Anabaena	+	+	--	-	-	+	+	+	-	+	+	+	-	-	-	8	8
3.Pseudoanabaena	-	-	-	+	-	-	-	+	-	+	+	-	-	-	-	2	2
4.Aulosira	-	+	+	+	-	-	+	-	+	-	+	-	-	-	-	6	6
5.Gleocapsa	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	3	3
6.Aphanocapsa	-	-	++	-	-	+	-	+	+	-	+	-	-	-	-	6	5
7.Aphanothece	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	2	2
8.Tolypothrix	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	1	1
9.Calothrix	+	-	-	+	-	+	-	+	+	+	-	-	-	-	-	6	6
10.Rivularia	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	4	4
11.Lyngbya	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	1	1
12.Hapalosiphon	-	-	-	+	-	-	-	+	-	+	-	-	-	-	+	4	4
13.Westiellopsis	-	-	-	+	+	-	+	-	-	-	+	-	-	-	+	5	5
14.scytonema	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	1	1
15.Oscillatoria	-	-	-	-	-	+	-	-	-	-	-	-	-	+++	-	4	2
Total no of genera isolates in each ADO circle	4	3	5	7	4	5	6	6	3	5	5	1	2	5	2	63	

Table-[III]: Maximum and Minimum amount of N₂ content of the Cyanobacteria no. of genera and species and mean amount of N₂ content over the genera and within the genus from Tezpur ,Gahpur, and Biswanath chariali subdivision.

Sl. No.	Genera	No. of species in each genus	N ₂ content		Mean amount of N ₂ content in percentage
			Maximum in percentage	Minimum in percentage	
1	Overall	43	4.21	0.65	2.57
2	Anabaena	6	4.21	1.43	2.37
3	Nostoc	8	2.83	1.75	2.42
4	Pseudoanabaena	1	1.75		
5	Aulosira	5	4.05	2.05	2.72
6	Gleocapsa	2	2.85	2.45	2.60
7	Aphanocapsa	5	3.20	1.65	2.31
8	Aphanotheceae	2	1.65	0.65	1.15
9	Microchactae	1			
10	Calothrix	3	3.85	2.55	3.55
11	Hapalosiphon	2	3.65	3.15	3.40
12	Rivularia	2	3.05	2.85	2.95
13	Lyngbya	1	2.05		
14	Westiellopsis	1	2.85		
15	Scytonema	1	1.95		
16	Oscillatoria	3	3.25	2.85	3.15
	Total -	43			

Figure [II] Cyanobacteria isolated from paddy field Tezpur Subdivision .

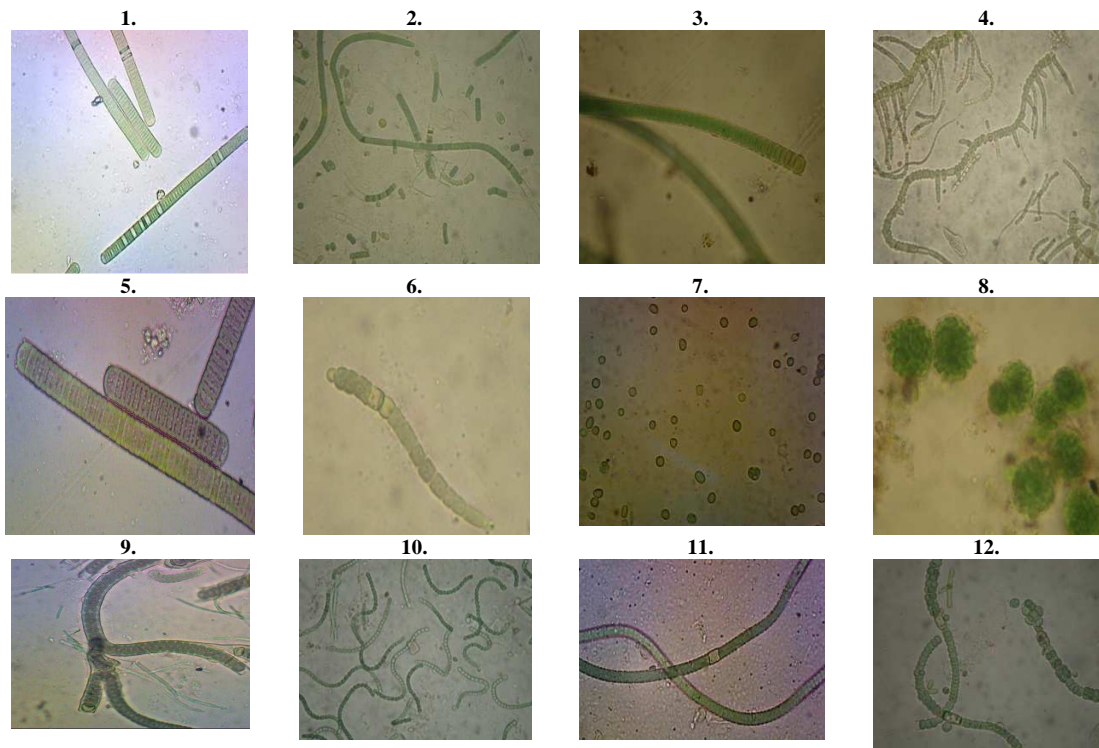


Figure II 1.*Oscillatoria margaritiferra* 2.*Phormidium corium* 3.*Oscillatoria curviceps*
4.*Hephalosiphon welwüschii*. 5.*Oscillatoria princeps* 6.*Rivularia aquatica* 7.*Aphanothece stagnina* 8.*Chroococcus limneticus* 9.*Plectonema*
sp. 10.*Nostoc punctiforme* 11.*Scytonema mirabile* 12. *Westiellopsis prolifica*.

Figure III Cyanobacteria isolated from paddy field Biswanath chariali subdivision

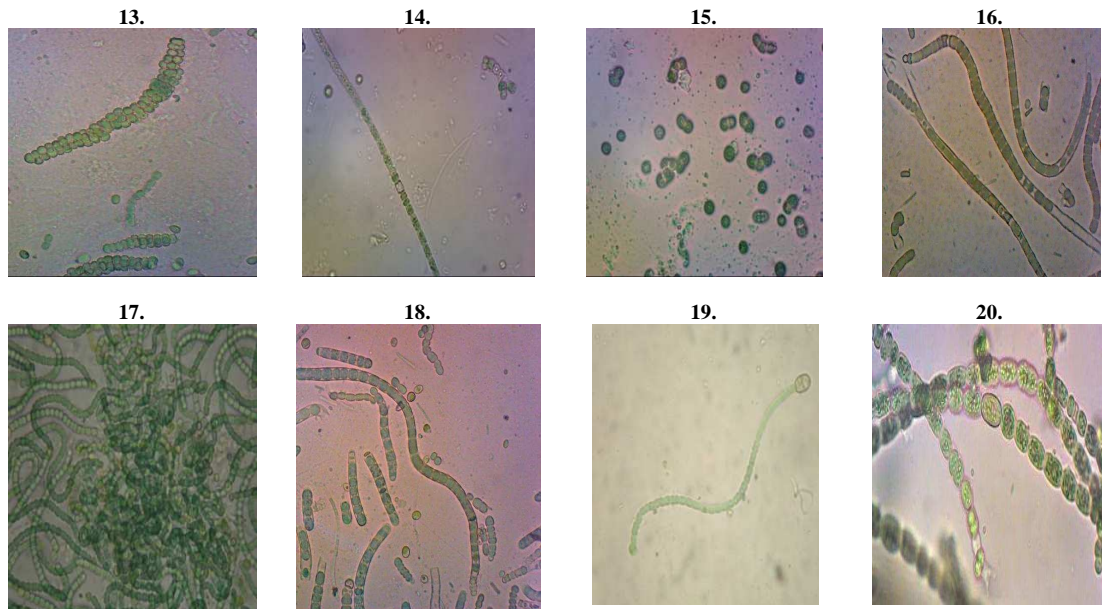


Figure [III] 13. *Westiellopsis prolifica* Janet. 14. *Anabeana volzii* 15. *Gleotheca rupestris* 16. *Calothrix Castelli* 17. *Nostoc commune*. 18. *Calothrix javanica*. 19. *Nostoc mucrosum* 20. *Anabeana macrospora*.

It is seen that only few genera showed the higher mean amount of N₂ content than the overall. These are Hapalosiphon (3.40%), Calothrix (3.55%), Rivularia (2.95%), Aulosira (2.72%), Gleocapsa (2.60%) and Oscillatoria (3.15%). This showed that the genera Calothrix, Hapalosiphon, Rivularia and Gleocapsa Aulosira were the dominant N₂ fixing Cyanobacteria in the rice field soils of the sonitpur district . Singh and Biseye (1986) carried out same investigation at CRRI and found Nostoc, Aulosira, Anabaena, Aphanotheceae, and Gleocapsa the most dominant Cyanobacterial strains in terms of N₂ Centent. The reports of Singh 1961, Singh 1983 in case of *Aulosira*, *Anabaena*, *Calothrix* ,*Nostoc* and *Tolypothrix* found the same result. These reports as discussed above are in almost close conformity with the findings of the present investigation.

Figure[IV] Cyanobacteria isolated from paddy field Gahpur subdivision.

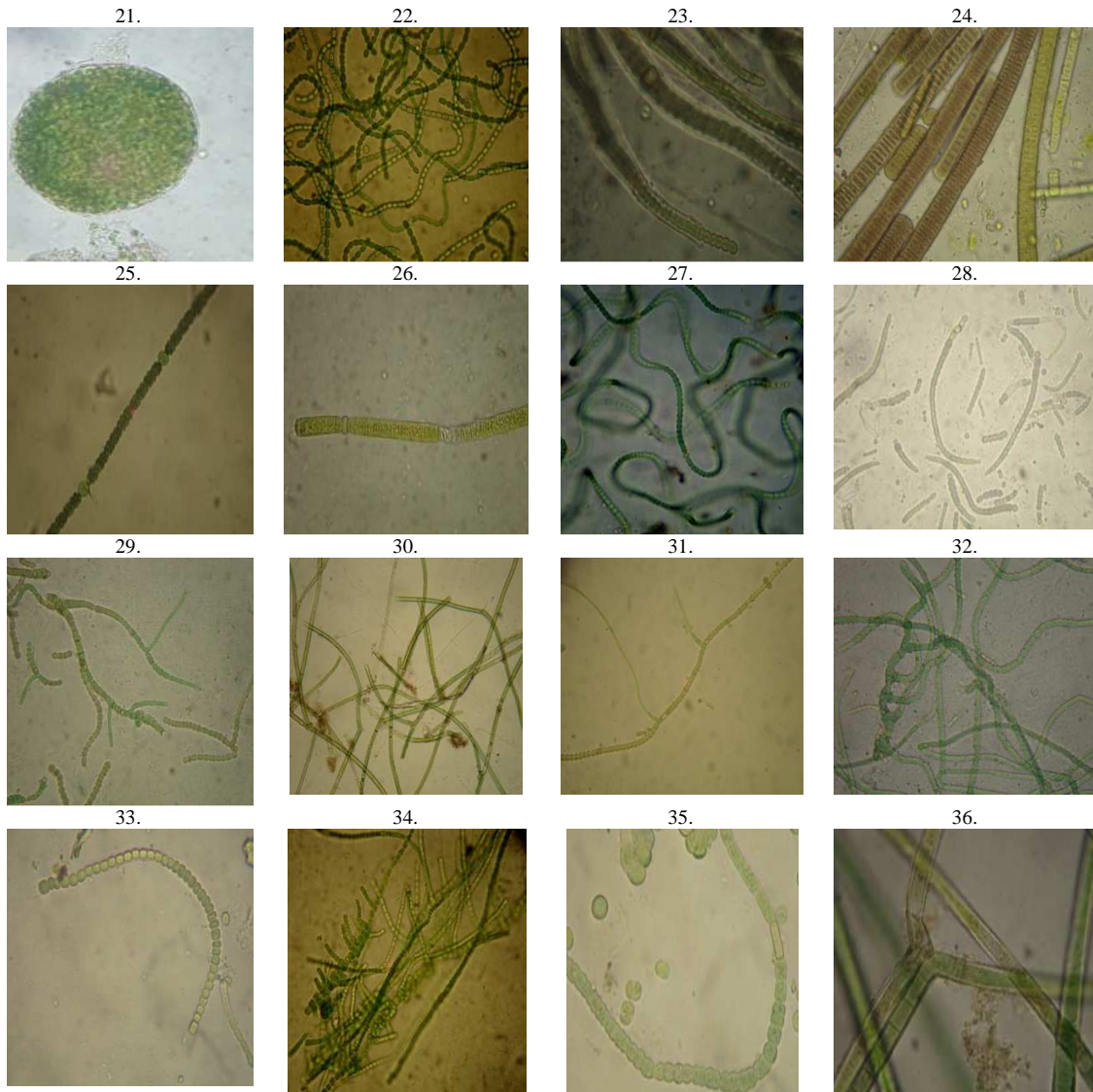


Figure- [IV] 21. *Aphanocapsa grevillei* 22. *Nostoc poludosum* 23. *Calothrix marchica* 24. *Oscillatoria Tenuis*. 25. *Anabaena oscillarioides* 26. *Oscillatoria simplissima* 27. *Nostoc phariecum* 28. *Calothrix lineari*. 29. *Hapalosiphon welwitschii*. 30. *Aulosira prolifica* 31. *Hapalosiphon hiberinicus* 32. *Mastigocladus laminosus*. 33. *Anabaena gilatinicola* 34. *Hapalosiphon luteoluss* 35. *Anabaena variabilis* 36. *Scytonema spsm*.

CONCLUSION

The highly potential indigenous Cyanobacteria which are widely distributed in the districts can be exploited in the yield of rice cultivation. As N₂ is the most important nutrient among all crop nutrients and the production of chemical N₂ is a tedious and energy extensive process, these potential Cyanobacteria can be exploited in place of chemical N₂ fertilizer with a low cost technology. Reassures are to be taken in this regard and this is the need of the hour in the prevailing energy crisis and environmental degradation in the Globe.

Acknowledgement - The authors take this opportunity to express their sincere thanks to UGC for granting financial assistance, along with the fund of other instrumental facilities to the Department of Botany ADP college Nagaon . We are also thankful to all the persons who helped us in the investigation and while working on the project.

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