ORIGINAL RESEARCH PAPER

Botany

MOLECULAR CHARACTERIZATION AND CONSTRUCTION OF PHYLOGENETIC TREE OF FEW CHAROPHYTE TAXA FOUND IN RANCHI

KEY WORDS: *Charophyta, Chara, Nitella,* RAPD

Ichha Purak Department Of Botany, Ranchi Womnen's College, Ranchi

Deepak Kumar Aakriti Biotechnology, Ranchi

Sushma Das Guru Department Of Botany, Ranchi Womnen's College, Ranchi

Rahul Anand* Aakriti Biotechnology, Ranchi *Corresponding Author

ABSTRACT

Charophyta are fresh water algae found in shallow ponds and lakes. The present investigation aims to characterize the five Charophyte taxa (*Chara fibrosa form tylacantha, Chara corallina form corallina, Chara braunii form braunii, Chara braunii form kurjii and Nitella hyalina form hyalina*) found in the water bodies of Ranchi city, on the basis of RAPD marker based analysis. The genomic DNA was successfully isolated by CTAB methodology. As the PCR based markers depend on purity of DNA, the extracted DNA was repeatedly purified. The purified DNA was further subjected to PCR amplification and RAPD analysis. Genetic analysis was made by using 3 Bangalore Genei arbitrary primers viz. OPG02, OPG03 and OPA09.
The genetic similarity was evaluated on the basis of presence or absence of DNA amplification bands. With the help of scorable bands range of molecular sizes was recorded in amplified products and phylogenetic tree was constructed based on PHYLIP (Version 6.a3, UPGMA).
After analyzing the results it was found that the species *N. hyalina form hyalina, Chara braunii* form *braunii* and *Chara braunii* form *kurjii* fell in the same group with Primer OPG03. But, *N. hyalina form hyalina*, *C. corallina, Chara braunii* did not form a group with OPA09 Primer. *C. fibrosa* did not give amplification with any of the primers.

INTRODUCTION:

Charophytes are highly diversified macroalgae including six genera *Chara, Nitella, Tolypella, Lychnothamnus, Lamprothamnium* and *Nitellopsis* of which *Chara* and *Nitella* are most common. These are found submerged in fresh water ponds, lakes, and slow running water. These are also called as 'stone wort' due to a cover of calcium carbonate on their surface. Thallus is generally 20-30 cm or more in height.

Each genus has similar structure with few distinct characteristics. The body consists of a long central axis punctuated by nodal cells, each giving rise to a whorl of secondary branches. Some species and forms are totally ecorticated (no secondary thickening) where as some taxa belonging to Chara are corticated

Their identification and classification is done on morphological variation of thallus structure as per Monographs by Pal, Kundu and Sundaralingam (1962) and Wood and Imahori (1965).

In last few decades attempts have been made to resolve the biodiversity of Charophytes on morphological and cytotaxonomical grounds (Bhatnagar, 1988, and Sinha and Purak, 1995). Bhatnagar *et al*, (1996) tried to resolve charophycean biodiversity and trace the phylogeny of this group on the basis of the Giemsa C-banding technique. Sundaralingam (2002), while reviewing over all investigations on Charophyta emphasized that biodiversity of this group can only be explored through molecular characterization.

A number of genetic based studies have been done recently on some of the species of the *Chara* in different parts of the world (O'Reilly CL *et al.*, 2007) and in India as well (Abrol D and Bhatnagar SK, 2006) using RAPD technique to analyze genetic relatedness and genetic distance between various taxonomic forms. First protocol for genomic DNA isolation from Indian charophytes has been reported (Bhatnagar *et al.*, 2005).

Studies have been performed to analyze biosystematics of selected *Chara* species (Charophyte) using amplified fragment length polymorphism(AFLP) to detect genetic diversity among *Chara hispida ,Chara intermedia* and *Chara tomentosa* (Mannschreck et al., 2002). The results showed high degree of polymorphism. Urbaniak J and Combik C 2017 studied the morphology of Chara *tenuispina* and *Chara globularis* , as well as their phylogenetic relationships, based on three cpDNA gene sequences. Result of analysis supported the taxonomic interpretation that *C. tenuispina* is a distinct species rather than variety of *C. globularis*

The present investigation aims to isolate high quality genomic DNA from each *sample* and to characterize the five Charophyte taxa (*Chara fibrosa form tylacantha, Chara corallina form corallina, Chara braunii form braunii, Chara braunii form kurjii and Nitella hyalina form hyalina*) on the basis of RAPD marker based analysis by using three random primers. Out of these five taxa only *Chara fibrosa* form *tylacantha* have cortication on the central main axis, rest four are totally ecorticated (Table No-1)

Table No-1 Taxonomic details of Charophyte taxa investigated for molecular characterization :RAPD analysis

S N	Name of Taxa (After Wood and Imahori,1965)	Identifying Features
1	*Chara fibrosa form tylacantha*	**Axis 2-corticated**,stipulodes in one tier alternating with branchlets, Gametangia restricted to branchlet nodes, branchlets totally ecorticated,monoecious, Corunula - 1 tier of 5 cells
2	*Chara Corallina form corallina*	**Axis and branchlets ecorticated**, Presence of gametangia both at branchlet nodes and base of the whorl, monoecious, Corunula- 1 tier of 5 cells
3	*Chara braunii form braunii*	**Axes and branchlets ecorticated**, presence of one pair of gametangia at each branchlet node, monoecious, stipulodes in one tier at nodes of central axis, Monoecious, Corunula- 1 tier of 5 cells
4	*Chara braunii form kurjii*	**Axes and branchlets ecorticated**, presence of two pairs of gametangia at each branchlet node, monoecious, stipulodes in one tier at nodes of central axis, Monoecious, Corunula- 1 tier of 5 cells
5	*Nitella hyalina form hyalina*	Main axix repeatedly furcated, **totally ecorticated**, At each furcation antheridia terminal and oogonium lateral, monoeciuos, corunula-2 tiers of 5 cells each

Volume-7 | Issue-10 | October-2018 | PRINT ISSN No 2250-1991

MATERIALS AND METHODS:

Source of Sample: The materials of Chara and Nitella were collected from local water bodies of Ranchi in the month of February 2017 and kept in a container in tap water. Identification up to form level were worked out with the help of 'Monograph of the Characeae' by R D Wood and K Imahori (1965)

DNA Extraction: Genomic DNA from decolorized and epiphyte free samples was isolated by CTAB based extraction method with some changes. The DNA was checked by agarose gel electrophoresis in 0.8% agarose gel from decolorized and epiphyte free Charophyte taxa.

DNA Purification: The obtained DNA was purified by RNase treatment at 37°C for 1 hour, followed by Phenol-Chloroform-Isoamylalcohol (25:24:1 ratio) based purification.

DNA Quantification: The DNA was quantified by taking the ratio of O.D. at 260 nm and 280 nm using spectrophotometer.

RAPD – Polymerase Chain Reaction: PCR reactions were done in a 20 µl reaction volume with 60ng template DNA and run on programme with initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 18°C for 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes.

Table No-2 Components of RAPD-PCR

Components	Concentration/Volume
Nuclease free water	10.3 µl
Assay Buffer	2 µl
MgCl2	1.2 µl
dNTPs	2 µl
Primer	10 pM (2 µl)
Template DNA	60 ng (1.5 µl)
Enzyme	1 µl

Primers: Following random decamers (Bangalore Genei Pvt Ltd.) were used as Primers for the PCR

Table No-3: List of primers used and their G-C ratio

Sl. No	Primers	Sequence (5'-3')	G-C ratio
1.	OPG02	GGCACTGAGG	70%
3.	OPG03	GAGCCCTCCA	70%
2.	OPA09	GGGTAACGCC	70%

Table No- 4 Details of RAPD-PCR Programme

Temperature	Duration	Cycles
94°C	5 minutes	35 cycles
94°C	30 seconds	
18°C	60 seconds	
72°C	1 minute	
72°C	10 minutes	

Analysis: The genetic similarity was evaluated on the basis of presence or absence of DNA amplification bands. With the help of scorable bands range of molecular sizes was recorded in amplified products and phylogenetic tree was constructed based on PHYLIP (Version 6.a3, UPGMA).

PHYLIP (the *PHY*Logeny Inference Package) was used for analyzing the banding pattern. It is a package of programs for inferring phylogenies (evolutionary trees).

The data are read into the program from a text file with name 'infile', which the user can prepare using any word processor or text editor Output is written onto special files with names like "outfile" and "outtree". PHYLIP is probably the most widely-distributed phylogeny package. It is the sixth most frequently cited phylogeny package, after MrBayes, PAUP*, RAxML, Phyml, and MEGA.

RESULTS:

DNA Isolation: Different concentrations of CTAB were used, ranging from 2% to 10% in the Lysis buffer to optimize the minimum concentration of CTAB required. 6% CTAB worked the best and gave a good quantity DNA (Figure 1).

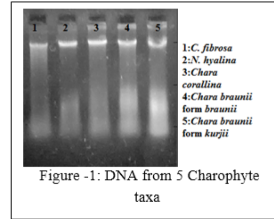

Figure -1: DNA from 5 Charophyte taxa

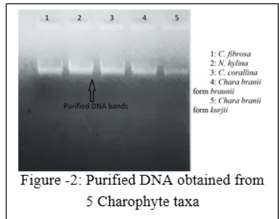

Figure -2: Purified DNA obtained from 5 Charophyte taxa

DNA Purification and estimation: The obtained genomic DNA was purified by Phenol: Chloroform: Isoamyl alchohol (25:24:1) method. Quantity of DNA was estimated by spectrophotometer and found out to 150ng/µl for *C. fibrosa,* 175ng/µl for *N. hyalina*, 180ng/µl for *C.corallina* and *C. braunii* form *braunii*; and 190ng/µl for *C. braunii* form *kurjii.*

RAPD-PCR Amplification : *C. fibrosa* did not give amplification with any of the primers

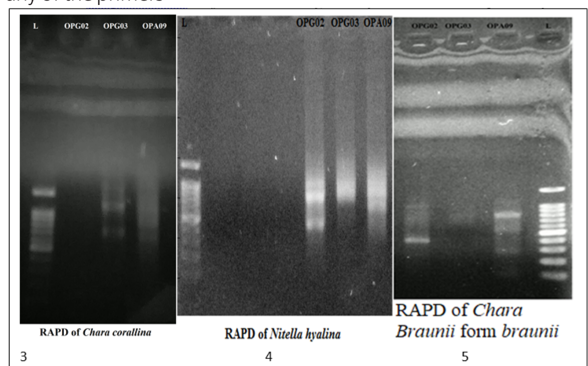

Figure: 3-5 RAPD-PCR amplification patterns of individual DNA samples of Charophyta taxa collected from water bodies of Ranchi with primers OPG03 and OPA09

Table No-5 Proportion of polymorphic bands recorded with different primers in Charophyta taxa

Primer	No of monomorphic bands	No of polymorphic bands	Ratio of polymorphic bands
OPG03	6	8	4:3
OPA09	3	9	3:1

Analysis outcome:

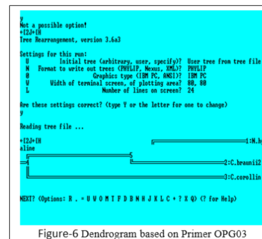
Figure-6 Dendrogram based on Primer OPG03

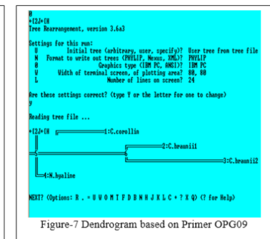
Figure-7 Dendrogram based on Primer OPG09

CONCLUSION:

After analyzing the results it was found that the species *N. hyalina form hyalina, Chara braunii* form *braunii* and *Chara braunii* form *kurjii* fell in the same group with Primer OPG03. But, *N. hyalina* form *hyalina, C. corallina, Chara braunii* did not form a group with OPA09 Primer (Figure 6-8).

Chara fibrosa form *tylacantha* did not show amplification with any of the two primers OPG03 and OPA09, probably due to cortication on the central axis which may hinder the DNA quality suitable for amplification. The rest of the four ecorticated taxa showed amplification by annealing with primers at the complementary nucleotide sequences scattered.

After analyzing the results it was found that out of the three

primers used, OPA09 gave closest phylogenetic neighbouring branch of *Chara braunii* form *braunii, Chara braunii* form *kurjii*. These two species are distant relative of *N. hyalina* and *C. corallina* as per the genetic information indicated by neighbouring branch. OPG03 and OPA09 gave provided similar profile in neighboring tree (Phylip, version 3.6a3 Program).

The profile displayed in phylogenetic tree based on 8 and 10 RAPD based DNA markers using primer OPG03 and OPA09 respectively.

REFERENCES:

1. Abrol D and Bhatnagar SK (2006) Biodiversity of few Indian charophyte taxa based on molecular characterization and construction of phylogenetic tree. African Journal of Biotechnology. 5 (17): 1511-1518.
2. Abrol, D and Bhatnagar, S. K, (2006) Molecular characterization of five species of the genus Chara (Division Charophyta) from India. Jour. Biol. Res. Vol 6: 213-220
3. Bhatnagar SK (1988) A karyological approach to phylogeny and interrelationships of Charophyta. Cytologia. 53: 723-729.
4. Bhatnagar SK, Abrol D, Kumar S (2005) First protocol for genomic DNA isolation in Indian Charophyta. Jour. Biol. Res. (Greece) 3: 109- 111
5. Mannschreck B, Fink T and Melzer A (2002) Biosystematics of selected Chara species (Charophyta) using amplified fragment length polymorphism. Phycologia . 41(6), 657-666
6. O'Reilly CL, Cowan RS, Hawkins JA (2007) Amplified fragment length polymorphism genetic fingerprinting challenges the taxonomic status of the near-endemic species Chara curta Nolte ex Kütz. (Characeae). Botanical Journal of the Linnean Society. 155: 467–476.
7. Pal BP,Kundu BC, Sundaralingam VS and Venkataraman GS (1962) Charophyta, ICAR, New Delhi.
8. Sinha S and Purak I (1995) Taxonomy of Charophya-trends and perspectives –a review Review article in Book Recent trends in Algal taxonomy Vol I APC Publications 179-220.
9. Sundaralingam VS (2002) Charophyta - A review. In: A. ANAND (ed.) - Algological research in India. pp. 97-111 Bishen Singh Mahendra Pal Singh, Dehra Dun. XXIV.
10. Urbaniak J and Combik C (2017) Taxonomic status of Chara tenuspina A. Br. (Streptophyta: Charales) based on LM morphology, matK, atpB and rbcL of cpDNA sequences. Fottea, Olomouc, 17(1): 20–33
11. Wood RD and Imahori K (1965) A revision of Characeae, Monograph of the Characeae. Cramer, Weinhelm Vol.1.J.