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A comparative study of two forms of *Nitella acuminata* (A.Br. ex Wallam.) var. *acuminata* with regard to chromosome morphology and antheridial-filament cell size.

Md Mehboob Hossain, Sri Narayan Ojha and Samit Ray*

Department of Botany, Visva-Bharati, Santiniketan – 731 235, West Bengal. *Communicating author: ray_samit@rediffmail.com

Abstract

On the basis of recognizable morphological variations as well as sporadic variation in certain morphological features, *Nitella acuminata* is divided into three varieties and eight forms. In this study two populations of *N. acuminata* var. *acuminata* were collected and they have been identified as *N. acuminata* var. *acuminata* f. *stellaris* (P1*) and N. acuminata* var*. acuminata* f. *capitulifera* (P2). Apart from detail morphology which includes oospore dimension, two aspects of cytology - chromosome morphology and antheridial filament cell dimension have been studied in these two forms. The mitotically dividing cells of antheridial filament show n=18 chromosomes in both these forms. Though TF% for these two forms are to be very close, there is a big difference in S% value. These values indicate more asymmetry of the karyotype of the form *capitulifera*. Comparing the karyotype formula it has been indicated that there is minor variation in the structural details among these two forms. PCA with the cytological parameters clearly place these two forms in two distinct patches in the graph. They can be separated on the basis of F% profile, breadth of antheridial filament cells in anaphase and mature cells. More population must be studied to establish a clean correlation between cytological features and morphological variations.

Keywords: Chromosome, karyotype, *Nitella*, cell dimension

Introduction

Cytotaxonomical study of various species of *Nitella* collected from different states of India, have been conducted since long (Imahori and Sinha, 1964; Sarma and Khan, 1964, 1965, 1967b; Mukherjee and Noor, 1973; Chatterjee, 1975, 1979; Bhatnagar, 1989; Ramjee and Bhatnagar, 1978a, b; Pundhir *et.al* 1993). Most of these works did not involve pretreatment schedule to reveal chromosome morphology. Significant works in this regard have been done from West Bengal with the application of pretreatment schedule which resulted in perfect revelation of chromosome morphology from both vegetative cell and antheridial filament cells (Ray and Chatterjee, 1988, 1994; Ray, 1998). A systematic study of chromosome morphology of various *Nitella* species, worked out from antheridial filament cells, have been undertaken in the district of Birbhum, located in the State of West Bengal. In earlier studies (Mandal and Ray, 2001; Chakrabarty and Ray, 2016) species, forms and variety *of N. hyalina, N.furcata, N.translucens, N.pseudoflabellata* have been worked out with regard to chromosome morphology and ploidy level. *Nitella acuminata,* a well defined species morphologically, belonging to the tribe Nitelleae (Wood, 1965) is being worked out cytologically for the first time from this region of West Bengal. The taxon *N. acuminata* var. *acuminata* has been divided into 3 varieties under *N. acuminata* and 8 forms under *N. acuminata* var. *acuminata* on the basis of sporadic variation in some morphological features like – presence or absence of head, head axially or terminal; length of dactyl for 3 varieties and nature of head, axis diameter, length of branchlet, no. of oogonia at a node, dactyl length, colour of oospore for 8 forms. In this study chromosome morphology has been worked out from antheridial filament cells and also measured dimension of these cells at different stages of cell division along with morphological variations between two populations representing two forms of *N. acuminata* var. *acuminata.* The chromosome data have been analysed with regard to symmetry of the karyotype and interpretation of relative advanced or primitive nature of the species.

Materials and methods

a. *Geographical location*

Populations of *Nitella acuminata* were collected from the district of Birbhum of the state of West Bengal. Specimen collected from Nilnirjon, Dubrajpur, Birbhum is Population I and specimen collected from

Biprotikuri, Birbhum is Polulation II. The geographic coordinates of population I is 23º49'15" N & 87º24'02" E and for population II is 23º44'28" N & 87º45'09" E.

b. *Morphological identification*

The taxonomic identification was done following Wood (1965).

c. *Oospore Dimensions*

In oospore dimension following parameters were studied.

a) Width of fossa
b) Number of Striae
c) LPA= length of the polar axis of the gyrogonite.
d) LED= largest equatorial diameter of the gyrogonite.
e) AND= distance from the apical pole to the LED as calculated along the polar axis.
f) ISI= isopolarity index i.e 100X(LPA/LED)
g) ANI= anisopolarity index i.e 100X(AND/LPA)

d. *Ecological parameters*

The pH, conductivity, inorganic phosphate, acidity, Alkalinity, Free CO_2, Dissolved O_2 are measured following standard methods (APHA-AWWA-WPCE-1976).

e. *Cytological methods*

Combination of Lacto-propiono staining and pretreatment as established by Ray and Chatterjee (1989) has been applied here. Metaphase stage of mitotically dividing cells of antheridial filament were considered for karyotype study.

i. Dimension of antheridial filament cells at different stages of division

Dimension of antheridial filament cells such as length, breadth, nuclear volume were measured by the Micrometer software. In the antheridial filament prophase, metaphase, anaphase and mature cells with antherozoids were undertaken for five each readings which were averaged.

ii. Chromosomal study

Karyotype features such as total chromosome length, short arm length, long arm length were measured by the Micrometer software. Some karyotype asymmetry indices such as symmetry index (S% - Arabbeigi *et. al.* 2011) where Symmetry index is the ratio of length of the lowest chromosome in a complement to the length of the largest chromosome, total form percentage (TF% - Huziwara 1962), centromeric index (F% - Levan et. al. 1964) were calculated. Chromosomes were further categorised according to their length (Khan and Sarma, 1967a).

iii. Principal Component Analysis (PCA biplot)

PCA was carried out for cell dimension (table no. 2) of each population and for chromosomal length (table no. 4 & 5) by PCA software.

Results and Discussion

Earlier, chromosomal variations at the population level within same species were undertaken in *Chara corallina and Chara setosa* (Ray and Chatterjee, 1987; Ray and Mukhopadhayay, 2003). It was demonstrated

that though chromosome number was n=42 in all the three populations of *Chara corallina* and n=28 in two population of *Chara setosa* there were morphological variations among the chromosomes that was reflected in karyotype formula and karyogram. These variations were not reflected in phenotype. Intra-specific chromosomal variation in three forms of *Chara zeylanica* was shown by Ray and Chatterjee (1992).

Morphological and cytological features of populations of *Nitella acuminata* are being reported for the first time from this part of the State of West Bengal. Following Wood (1965) these two populations of *N. acuminata* have been identified as *N. acuminata* var. *acuminata* f. *stellaris* (Population I) and *N. acuminata* var. *acuminata* f. *capitulifera* (Polulation II) on the basis of different axis diameter, formation or absence of head and oospore dimension, colour.

The sites of occurrence of these two populations are separated by 60 kms. However, there are no major differences in the physico-chemical parameters (Table no. 1) of the water bodies in which these populations were growing. Concentration of CO_2 and acidity is little higher at site II than site I. Under such similar ecological conditions not much morphological variations are expected.

Table 1: Data related to Physico-chemical parameters

Name of taxa	Co_2 mg/L	D.O mg/L	Alkalinity mg/L	Acidity mg/L	pH	Conductivity	Inorganic Phosphate mg/L
Population I f. *stellaris*	8.80	6.08	67	15	7.56	0.135	0.09
Population II f. *capitulifera*	13.2	6.89	50	25	7.59	0.153	0.16

These two forms of *N.acuminata* var. *acuminata* have chromosome number n=18 confirming previous counts of this species by Sarma and Khan (1964), Hotchkiss (1965), Wood (1965), Sinha and Noor (1967), Ramjee and Bhatnagar (1978b) and Ray and Chatterjee (1983). Tindall (1969) studied in great detail the *N. acuminata* complex of specimens collected from South-western U.S.A. and Northern Mexico. He obtained a chromosome count of n=9 and n=18 in different populations of *N. acuminata* showing certain differences in morphological characters. This variation of ploidy level reported has not been found here. Though both the forms show n=18 chromosomes, chromosome morphology and karyogram details of these two forms show some differences (Fig.1 & 2). The range of chromosome length in f. *capitulifera* (population II) is shorter (0.63 – 2.33 µm) than that of f. *stellaris* (population I - 1.47 – 3.22 µm). Correspondingly, it has been found that the nuclear volume of population II having smaller chromosomes, is also much smaller (Table 2). From karyotype formula (Fig.2) it is evident that there is gross similarity between the two populations except that there is only one submedian chromosome in the medium size category in f. *stellaris* which is absent in f. *capitulifera*. The difference in the degree of symmetry of the karyotype is reflected in the value of S%, f. *stellaris* with S% value of 45.65 has more symmetrical karyotype.

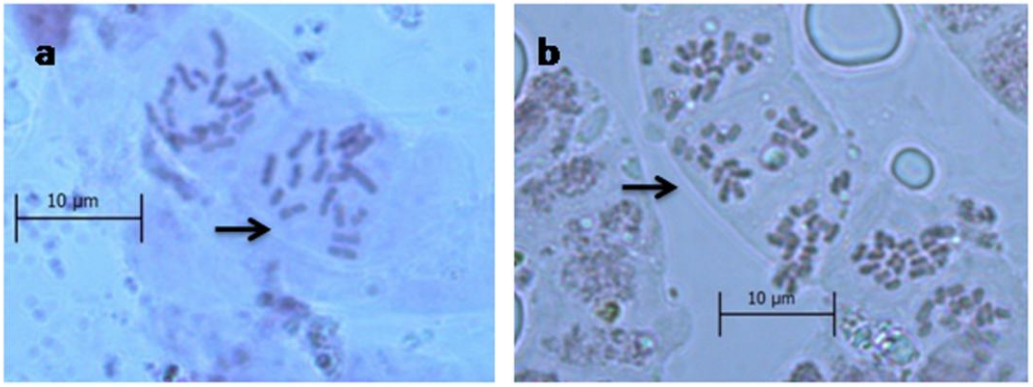

Fig. 1 Metaphase plate in antheridial filament cells of *N. acuminata* showing n=18 chromosemes of (a) f. *stellaris* , (b) f. *capitulifera*.

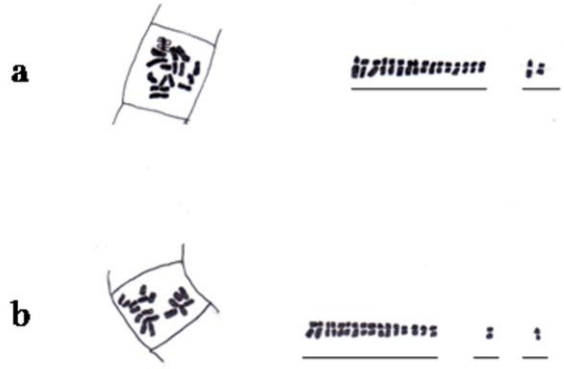

Fig. 2: f. *stellaris* (a) karyotype and karyogram showing 16 submedian and 2 median chromosomes. f. *capitulifera*(b) karyotype and karyogram showing 16 submedian, 1 median and 1 subterminal chromosomes.

Table 2: Data related to Cell Dimensions of antheridial filament cells

Name of taxa		Prophase			Metaphase		Anaphase		Mature cell	
		Length (µm)	Breadth (µm)	Nuclear volume (μm^2)	Length (µm)	Breadth (µm)	Length (µm)	Breadth (µm)	Length (µm)	Breadth (µm)
Population I f. *stellaris*	Mean	**6.27**	**12.74**	**184.40**	**10.83**	**13.20**	**10.88**	**12.41**	**5.70**	**12.06**
	SD	0.778	0.74724	40.6777	0.427	0.81564	0.2891	0.88537	0.4341	0.64643
Population II f. *capitulifera*	Mean	**9.42**	**11.95**	**57.06**	**14.25**	**11.58**	**13.02**	**12.52**	**6.25**	**12.15**
	SD	1.2402	0.24754	5.13518	0.4515	0.27889	0.3493	0.44162	0.1887	0.46137

The detail karyotype worked out by Ray and Chatterjee (1983), of a population *of N. acuminata* var. *acuminata* f. *acuminata* from West Bengal showed ten sub median, two median and six subterminal chromosomes. The longest chromosome was being 3.22 µm (submedian category) and shortest 1.38 µm (also of submedian categoty) in length. The karyotype formula reported was L (0) + M (Sm_2 +m_0+st_0) + S (Sm_{10}+st_4+m_2). TF% calculated was 35.35%, value close to that of f. *stellaris.* A close similarity is found between the chromosome profile of f. *stellaris* and that worked out by Ray and Chatterjee (1983) where chromosome length is similar (1.47 – 3.22 µm). In f. *capitulifera* the range of chromosome length is shorter (0.63 – 2.33 µm) as compared to both f. *stellaris* and f. *acuminata*. TF% of f. *stellaris* is 38.29% and for is 40.68% which are quite similar to the previous work done. Nearly similar values of TF% of these three forms validate their inclusion in the same species.

Table 3: Data related to Oospore dimension

Name of the taxa		LPA (µm)	LED (µm)	AND (µm)	ISI	ANI	No of straie	Width of fossa (µm)
Population I f. *stellaris*	Mean	**242**	**205**	**103**	**119**	**52**	**6-7**	**49**
	SD	10.09	27.69	4.91				2.45
Population II	Mean	**262**	**233**	**135**				**44**

f. *capitulifera*	SD	8.09	1.97	7.82	**112**	**52**	**6-7**	6.69

The karyotype data also resembles the karyotype worked out of the vegetative apical cells by Ray and Chatterjee (1983). Differences are always found in various populations of the same species with regard to morphological features. Here we find chromosomal variations also between different populations.

Table 4: The detailed karyotype of *N. acuminata* var. *acuminata* f. *stellaris*

Chromosome, n=18, Longest Chromosome: 3.22 µm, Shortest Chromosome: 1.47 µm
TF% - 38.29; S% - 45.65, Karyotype formula: L (0) + M (Sm_1+m_0+st_0) + S (Sm_{15}+ m_2+ St_0)

Ch. No.	Length of short arm(µm)	Length of long arm(µm)	Total length (µm)	F%
1	1.12	1.30	2.52	44.44
2	1.12	1.12	2.24	50.00
3	0.778	0.927	1.70	45.76
4	0.859	1.19	2.05	41.90
5	0.852	1.52	2.37	35.94
6	0.989	1.25	2.23	44.34
7	0.735	0.735	1.47	50.00
8	0.799	2.04	2.84	28.13
9	0.792	2.04	2.83	27.98
10	0.739	1.05	1.79	41.28
11	0.979	1.29	2.27	43.12
12	0.876	1.06	1.94	45.15
13	0.679	1.03	1.71	39.70
14	0.960	2.262	3.22	29.81
15	0.671	1.24	1.91	35.13
16	0.960	1.181	2.14	44.85
17	0.792	1.53	2.32	34.13
18	0.869	1.47	2.34	37.13

Additionally parameter of cell dimension has also been studied in these two populations. PCA biplot was constructed with cell dimension data of both the forms from antheridial filament cells. It was noted that cell length forms a cluster and does not vary much across the stages of mitosis. In contrast, the cell breadth varies across the mitotic stages and is more between the first two stages (prophase and metaphase) versus last two stages (anaphase and mature cells)**.** The PCA result shows the highest variability (58% - Fig. 3) that is accounted for by anaphase breadth and mature cell breadth. Thus, dimension of antheridial filament cells of two populations is able to segregate the two forms. The PCA biplot on the basis of chromosome length (short arm, long arm and F

%) of both the forms constitute two distinct patches and the highest variability (>62.3% - Fig. 4) is accounted for by the value of F%. Variation in F% profile indicates difference in type of chromosome.

Study of more intra specific populations from different agro-climatic zones is required to establish structural alteration of chromosomes amongst themselves..

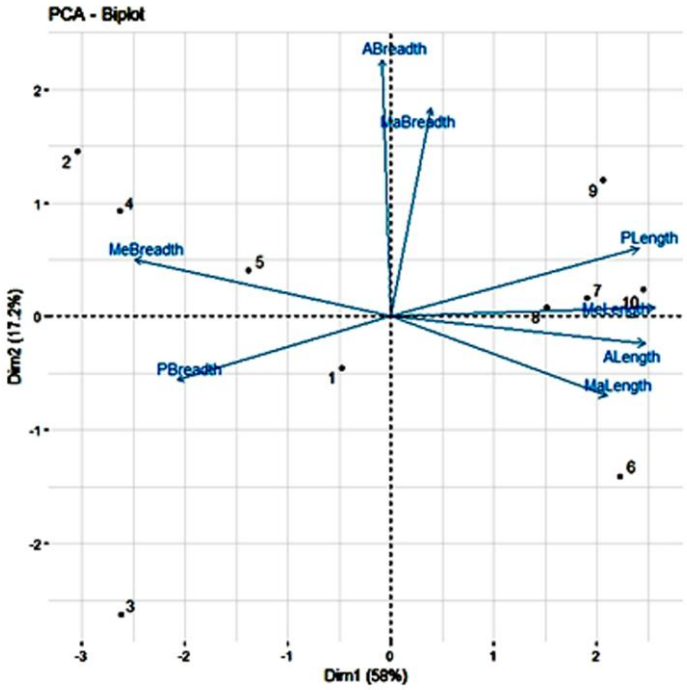

Fig. 3: PCA output for cell dimension features analised in f. *stellaris* (1-5)and f. *capitulifera* (6-10). P= prophase, Me=Metaphase, A= Anaphase, Ma=Mature cells

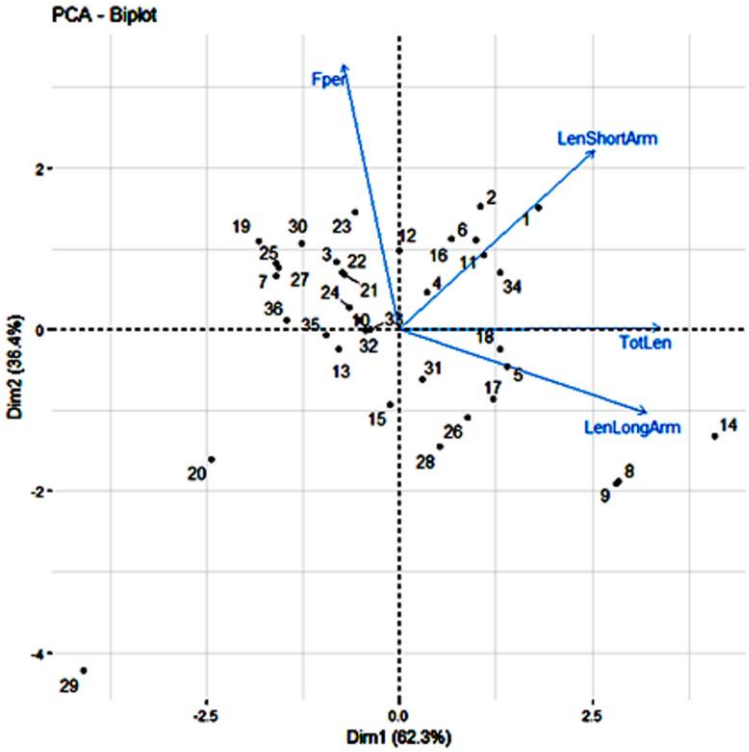

Fig 4: PCA output for chromosome length analised in f. *stellaris* (1-18)and f. *capitulifera* (19-36). Len= Length, F= F%

Table 5: The detailed karyotype of *N. acuminata* var. *acuminata* f. *capitulifera*

Chromosome, n=18, Longest Chromosome: 2.33 µm, Shortest Chromosome: 0.63 µm, TF% - 40.68; S% - 27.03
Karyotype formula: L (0) + M (0) + S (Sm_{16}+ m_1+ St_1)

Ch. No.	Length of short arm (µm)	Length of long arm (µm)	Total length (µm)	F %
1	0.701	0.701	1.40	50.00
2	0.407	0.789	1.20	33.91
3	0.773	0.960	1.73	44.68
4	0.773	0.951	1.72	44.94
5	0.863	0.911	1.77	48.75
6	0.741	1.01	1.75	42.34
7	0.692	0.778	1.47	47.07
8	0.739	1.48	2.22	33.28
9	0.692	0.792	1.48	46.75

10	0.671	1.44	2.11	31.80
11	0.112	0.519	0.63	17.70
12	0.751	0.815	1.57	47.83
13	0.741	1.29	2.04	36.32
14	0.734	1.08	1.81	40.55
15	0.741	1.09	1.83	40.49
16	0.974	1.36	2.33	41.80
17	0.679	0.979	1.66	40.90
18	0.646	0.863	1.51	42.78

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