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Chromosomal variations and cytotaxonomical considerations in two populations of *Nitella hyalina* (Charophyceae, Characeae) from West Bengal, India

Rajat Chakrabarty and Samit Ray*

Department of Botany, Visva-Bharati, Santiniketan – 731 235, West Bengal, India.

*Corresponding author – ray_samit@rediffmail.com

Abstract

Chromosome number and morphological variation of two populations of *N. hyalina* (DC.) Ag. have been studied. Chromosome morphology was worked out from well spreadout metaphase plates from dividing antheridial filament cells. Population I collected from the bed of Ajoy river (district Burdwan) shows n=18 chromosomes. Population II collected from the bed of the river Kopai (district Birbhum) shows n=21 chromosomes. The karyotype formula for population I is L (Sm_7+m_1+St_0) + M (Sm_4+m_2+St_0) + S (Sm_2+m_2+St_0). The karyotype formula for population II is L (Sm_1 + m_2+ St_0) + M (Sm_{12}+ m_3+ St_0) + S (Sm_1+m_2+St_0). Total form percent (TF%) calculated for population I and II are 38.21 and 38.25 respectively. These two populations do not show any variation in morphological features but variation in chromosome number and morphology is evident. Occurrence of different ploidy level and symmetrical nature of karyotype for *N. hyalina*, as compared to other species of *Nitella*, is established.

Key words: Charophyceae, Characeae, Chromosome, Cytotaxonomy, *Nitella hyalina*, West Bengal.

Introduction

Cytological data with reference to chromosome number, morphology and karyotype pattern provide much information in tracing origin, affinities and interrelationship of plants including algae (Ehrendorfer *et al.*, 1968; Sharma, 1969; Stebbins 1971). Investigation on higher plants with regard to karyotype and karyogram has helped in establishing evolutionary status for a taxon and relationship between different taxonomic groups. However, any definite systematic conclusion in algae on the basis of chromosome data is very difficult because it is not easy to reveal the exact chromosome morphology.

In the members of the order Charales centromeric position and chromosome size variation could be studied with perfection because of excellent metaphase chromosome preparations achieved by the application of proper pretreatment and staining schedule (Dyer, 1963; Sawa, 1965; Ray and Chatterjee, 1989; Ray, 1998). Kanahori (1974) refined techniques for examination of chromosomes from vegetative shoot apices of Charophytes in a study of *Nitella hyalina*. To get a clear picture of chromosome profile from karyotype of different species four different criteria are taken into account viz. (i) variation in absolute chromosome size, (ii) variation in the position of centromere, (iii) variation in relative chromosome size, (iv) variation in basic number. Morphological characters primarily considered to describe and distinguish various taxa, under Charales, are simultaneously considered with cytological data to draw a clear demarcating line between different species and intraspecific taxa. This approach helps to ascertain the validity of the evolutionary status of various taxa, established on the basis of morphological characters only (Chatterjee, 1976 a, b, 1979 a, b; Ray, 1998; Ray and Chatterjee 1988a, b, 1989, 1992, 1994; Mandal and Ray, 2000). Chakraborty *et al.* (2009) has established definite correlation through CCA ordination study in *Chara fibrosa* complex between morphological features and chromosome number.

Cytology and cytotaxonomy of different species of *Nitella* have extensively been studied from different parts of India (Sarma and Khan, 1964, 1965; Khan and Sarma, 1967; Ramjee and Sarma, 1971; Ramjee and Bhatnagar, 1978 a, b; Subramanyam and Chowdhury, 1992; Pundhir *et al.*, 1993). All these studies were based on cytological data obtained from unpretreated materials. A large number of species and interspecific taxa belonging to *Nitella* have been studied from West Bengal and significant cytotaxonomic conclusions have been drawn (Ray and Chatterjee, 1983, 1988b, 1994; Ray, 1998; Mandal and Ray, 2001). Using the refined technique (combination of pretreatment and lacto-propiono orcein staining) the chromosomes were worked out both from vegetative apices as well as

antheridial filament cells. Ray and Chatterjee (1983) published the chromosome number and Karyogram of *N. acuminata* var. *acuminata f. acuminata* A. BR. EX. WALLM. New chromosome number (n=18) and karyogram, worked out from vegetative cells, of *N. stuartii* A. BR. was reported from West Bengal (Ray and Chatterjee, 1988b). All these cytological works have been worked out with technique involving pretreatment and Lacto-propiono orcein staining (Ray and Chatterjee, 1989). Cytotaxonomic work based on karyotype analysis at the subspecific level in *N. furcata* complex was done for the first time by Ray and Chatterjee (1994). All these works involved correlation of morphological and cytological variations and the significance of chromosome data in characterization of the taxa of *Nitella*. Mandal and Ray (2001) reported for the first time the cytology of *N. hyalina* var. *hyalina f. hyalina* and *f. formosa* (T. F. A.) R.D.W. from West Bengal having chromosome number n=18 which is similar to earlier reports from India and abroad (Guerlesquin, 1963; Hotchkiss, 1965; Sarma and Khan, 1965; Kanahori, 1974; Peshkov *et al.,* 1974). Considering the variations found in cytological profile of various taxa under *N. hyalina* as reported by earlier workers, a further study of *N. hyalina* populations has been undertaken in this investigation.

Material and methods

(a) *Geographical location:* Populations *N. hyalina* were collected from the districts of Burdwan and Birbhum of the State of West Bengal having similar weather condition. Both the locations of collection have Vindiyan alluvial type of soil**.** Within each location four permanent water bodies with charophyte population were identified.

(b) *Morphological identification:* The species was identified following Wood (1965).

(c) *Cytological Methods:* Combination of Lacto-propiono orcein 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 in most of the cases. The young shoot tips were collected from the natural aquatic habitats or from soil-water culture established in the net house. The tip portions were observed under dissecting microscope for detection of correct stage of antheridium for cytological study.

i) *Pretreatment:* Thoroughly washed tip portions were pretreated in a chilled mixture of 0.1% aqueous solution of Colchicine and 0.002M aqueous solution of 8-hydroxyquinoline. Then the apical portion with antheridia was put into the chilled pretreatment chemical. The antheridia were allowed to stay there at 16^{o}C-18^{o}C for 2-2½ hrs.

ii) *Fixation:* After pretreatment the material was fixed in freshly prepared 1:3 mixture of Propionic acid and Absolute alcohol for 2 hours.

iv) *Treatment with 0.1N HCl:* After fixation, the shoot tip along with the antheridia was softened in 0.1N HCl at 60^{o}C for 3-4 minutes.

v) *Washing in water and 45% propionic acid:* The material was washed in distilled water to remove the HCl. The water was decanted and the material was washed with 45% Propionic acid and kept in the propionic acid for 3-5 minutes.

vi) *Staining:* The material was stained in 2% Lacto-propiono Orcein (Dyer, 1963) overnight.

vii) *Squashing:* After staining, the material was put into 45% propionic acid. The antheridial filaments were teased out from the antheridia under a binocular dissecting stereomicroscope and the final squashing of the antheridial filaments were done in 45% propionic acid under a cover slip.

Chromosome morphology was designated according to Levan *et al.* (1964) on the basis of centromeric index value. Total form percent (TF %) was calculated for each taxon using the formula suggested by Huziwara (1962). Chromosomes were further categorized according to their length (Khan and Sarma, 1967a).

Results

Morphological features

Plants monoecious; upper whorls crowded and covered with mucus; internodes 2 – 4 times as long as branchlets; branchlets heteroclemous; fertile branchlets 8 in a whorl; 2 – 3 furcate; sterile similar to fertile; dactyls 2-celled, 4 – 6; basal cell tapering, end cell conical, acute; gametangia conjoined at all branchlet furcations; oogonia solitary; oospore

reddish brown, striae thin, ridges prominent. Two populations resembled each other with regard to taxonomically significant morphological features.

Cytological features

Population - I of *N. hyalina* collected from Ajay river bed (District of Burdwan) shows n=18 chromosomes (Fig.1a, 2a). The chromosome length varies from 2.50 to 5.63 µm and the total chromosome length is 76.91 µm. There are 13 submedian (Sm), 5 median (m) chromosomes (Fig.2b). The Karyotype formula is L ($Sm_7+m_1+St_0$) + M ($Sm_4+m_2+St_0$) + S ($Sm_2+m_2+St_0$), where L, M and S indicate long, medium, and short chromosomes and zero represents the absence of a particular type of chromosome. The population – II from Kopai (District of Birbhum) is found to have n=21chromosomes (Fig.1b, 2c) and the length of the chromosomes varies from 2.50 - 6.88 µm. The total length of the chromosome set was 80.07 µm. There are 14 Sm, 7 m chromosomes (Fig.2d). The karyotype formula constructed for this population is L (Sm_1 + m_2+ St_0) + M (Sm_{12}+ m_3+ St_0) + S ($Sm_1+m_2+St_0$). The TF% values were 38.21 and 38.25 for the population with n=18 and for the population with n=21 respectively.

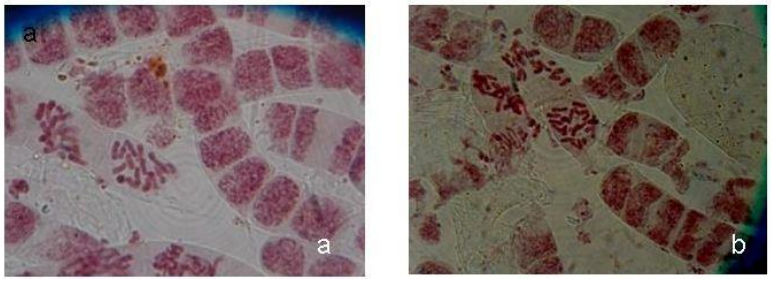

Fig. 1: Metaphase plate in antheridial filament cells of *N. hyalina* showing (a) n = 18 chromosomes in population I, (b) n = 21 chromosomes in population II.

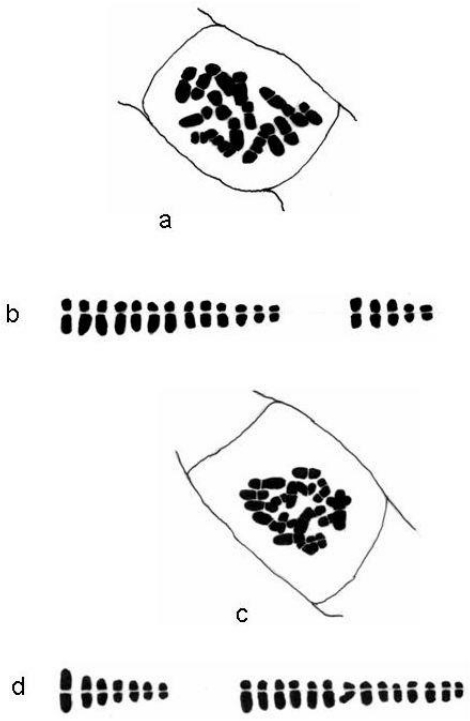

Fig. 2: Population I (a) karyotype, (b) karyogram showing 13 submedian and 5 median chromosomes. Population II (c) karyotype, (d) karyogram showing 14 submedian and 7 median chromosomes.

Table-1: Chromosome number of various populations of *N. hyalina* from West Bengal.

Sl. No.	Name of Taxon	Location	Chromosome No.	Reference
1	*Nitella hyalina*	Medinipur	n=18	Ray, 1988
2	*N. hyaline*	Birbhum	n=18	Mandal and Ray, 2001
3	*N. hyalina*	Birbhum	n = 18	Present author
4	*N. hyaline*	Birbhum	n = 21	Present author
5	*N. hyalina*	Burdwan	n = 18	Present author

Discussion

In addition to the morphological features (vegetative and reproductive), several other parameters have been taken into consideration for the identification of the species of *Nitella*. One of the most important parameters considered is the chromosome number, morphology and karyogram (Mandal and Ray, 2001). Ray and Chatterjee (1988b, 1992, 1994) clearly indicated the importance of chromosome number, morphology and karyotype in the classification of various interspecific taxa belonging to *C. fibrosa* species complex, *C. zeylanica* and *N. furcata*. Standardization of cytological technique by Ray and Chatterjee (1989) and adoption of staining technique of Dyer (1963) have helped to ascertain the chromosome number and morphology with precision in charalean species. As the various populations of *N. hyalina* were being studied with the help of revised cytological procedure two populations with different ploidy levels were found from this geographic region at two locations having similar soil type but separated by a distance of 10 km. Cosmopolitan taxa like *N. acuminata* and *N. hyalina* show wide ranging chromosome number n=9, 12, 15, 18, 21 (Ray, 2005).

Nitella hyalina belongs to section Decandollea (Wood, 1965). *Nitella hyalina* var. *hyalina* f. *hyalina* is a monoecious species with heteroclemous branching. This taxon was studied cytologically in India by Sarma and Khan (1964), Ramjee and Sarma (1971), Ramjee and Bhatnagar (1978), Ray (1998) and Chromosome number reported in every case is n= 18. A chromosome number of n=18 was found in representatives of this taxon in Sweden (Walther, 1929), Japan (Sato, 1959; Kanahori, 1974), France (Guerlesquin, 1963), New Caledonia, US, and Mexico (Hotchkiss, 1964). It is interesting to note that Kanahari (1974) studied two clones of *N. hyalina* (n=18) occurring in Japan and noted differences in chromosome length and arm ratio of the two clones studied from somatic cells.

In the present investigation, 2 populations of *N. hyalina* were studied. Owing to the clarity of the primary constriction, the categorization of the chromosome was possible. The TF% value of 38.21 (n=18) and 38.25 (n=21) for these two populations of *N. hyalina* var. *hyalina* f. *hyalina* reveals a relative symmetrical karyotype and thus is evidence of relatively primitive status of this heteroclemous taxon among the species of *Nitella*. It is pertinent to mention here that *N. stuartii* another heteroclemous taxon shows TF%=38.21 (Ray and Chatterjee, 1988b) while *N. acuminata* a homoclemous taxon show TF%= 32.2 (Ray and Chatterjee, 1983). Primitive nature of *N. hyalina* as compared to homoeclemous taxa like *N. acuminata* is thus evident. The earlier findings that n=18 is a deep seated number and wide spread among the species of *Nitella* and that there is common occurrence of euploidy in this species complex is confirmed. Additionally, this part of India is added in the list of regions where populations of *N. hyalina* with varied ploidy level are found.

Bibliography

Chakraborty, R., S.N. Ojha and S. Ray, 2009. Morphological and Chromosomal variation in various populations of *Chara fibrosa* from West Bengal, India and an analysis of Physico-chemical parameters of the aquatic habitat. *Perspectives in Cytology and Genetics* **14:** 157-164.

Chatterjee, P. 1976a. Cytotaxonomical studies of West Bengal Charophyta: Karyotype analysis in *Chara braunii*. *Hydrobiologia* **40**: 171-174.

Chatterjee, P. 1976b. Cytotaxonomic studies on *Chara socotrensis* Nordst. In Kuhn f. *Nuda* (Pal) R.D.W. from West Bengal, India. *Cytologia* **41**: 659-663.

Chatterjee, P. 1979a. Karyological investigation of *Chara wallichi* A. Br. from West Bengal. *Cell and Chromosome Newsletter* **2**: 21-23.

Chatterjee, P. 1979b. *Chara fibrosa* var. *Fibrosa* f. *Longicorollata:* A new record for India and its cytology. *Curr. Sci.* **48**: 545-547.

Dyer, A.F. 1963. The use of Lacto-propiono orcein in rapid squash method for chromosome preparation. *Stain Technology* **38**: 85-90.

Ehrendorfer, F., F. Krendl, E. Habeler and E. Sauer, 1968. Chromosome numbers and evolution in primitive angiosperms. *Taxon* **17**: 337-353.

Guerlesquin, M. 1963. Contribution á l'étude chromosomique des Charophycées d' Europe occidentale, et d' Afrique du Nord II. *Revue Générale de Botanique* **70**: 335-370.

Hotchkiss, A.T. 1964. Chromosome numbers in Characeae from the South Pacific. *Pacific Science* **19**: 31-37.

Huziwara, Y. 1962. Karyotype analysis in some genera of Compositae VIII. Further studies on the chromosomes of *Aster*. *American Journal of Botany* **49**: 116-119.

Kanahori, T. 1974. The karyotype of two species of the Characeae. *Chromosome Information Service* **16**: 29.

Khan, M. and Y.S.R.K. Sarma, 1967. Studies on cytotaxonomy of Indian Charophyta-II. *Nitella*. *Phykos* **6**: 48-81.

Khan, M. and Y.S.R.K. Sarma, 1984. Cytogeography and cytosystematics of Charophyta.- In: *Systematics of the green algae* Ed. Irvine, D.E.G. and D.M. John, Academic Press, London, pp 303-330.

Labh, L. and B.N. Verma, 1986. New Counts for *Chara fibrosa* complex. *Cytologia* **51**: 185-191.

Levan, A., K. Fredga, and A. Sandberg, 1964. Nomenclature for centromeric position on chromosomes. *Hereditus* **52:** 201-220.

Mandal, D. K. and S. Ray, 2001. Karyotype analysis and cytotaxonomic study in the genus *Nitella* (Charophyceae). *Phytomorphology* **51(1):** 27-32.

Meiers, S.T., V.W. Proctor, and R.L. Chapman, 1999. Phylogeny and Biogeography of *Chara* inferred from 18S r-DNA sequences. *Australian Journal of Botany*. **47:** 347-360.

Mukherjee, S. 1978. Polyploid chromosome numbers in the genus *Nitella*. *Curr. Sci.* **47:** 386.

Mukherjee, S. and M.N. Noor, 1973. A first report of karyological studies in *Nitella wattii* Groves from India. *Science and Culure* **39:** 459-460.

Noor, M.N. and S. Mukherjee, 1977. Some new records of chromosome numbers in Indian Charophyta. *Cytologia,* **42:** 227-232.

Peshkov, M.A., T.L. Mirasaidov and L.A. Tikhomirova, 1974. Investigation of the chromosome numbers and tentative karyotypes of some *Chara* algae of the Uzbekistan. *Izvestia Akad. Nauk. USSR. Ser. Biol*. **5:** 672-687.

Proctor, V.W., C.R. Malone and V.L. de Vlaming, 1967. Conspecificity of some Charophytes. *Journal of Phycology,* **3:** 208-211.

Proctor, V.W., D.G Griffin III. and A.T. Hotchkiss, 1971. A synopsis of the genus *Chara,* series *Gymnobasalia* (subsection *Willdenowia* R.D.W.). *American Journal of Botany* **58:** 894-901.

Pundhir, H.S., A. Gautam and Vidyavati, 1993. Comparative study and new chromosome counts for *Nitella hyalina* var. *hyalina* f. *Indica* (A.Br.) R.D.W. from India. *Phykos* **32(1, 2):** 169-174.

Ramjee and S.K. Bhatnagar, 1978a. Significance of new chromosome number in *Nitella mirabilis* Nordst. Ex Groves em R.D.W. *Hydrobiologia* **57:** 99-101.

Ramjee and S.K. Bhatnagar, 1978b. Cytotaxonomic studies on *Nitella furcata* subsp. *Flagellifera* f. *Patula* Gr. Ex Allen and comparison with other taxa of *N. Furcata complex. Caryologia* **31:** 457-462.

Ramjee and Y.S.R.K. Sarma, 1971. Some observations on the morphology and cytology of Indian Charophyta. *Hydrobiologia* **37:** 367-382.

Raven, J.A. 1987. Biochemistry, biophysics and physiology of Chlorophyll-b containing algae: implications for taxonomy and phylogeny. *Progress in Phycological Research* **3:** 1-22.

Ray, S. 1988. Cytomorphological investigation on Characeae of West Bengal and an analysis of the factors involved in their oospore germination. Ph.D. Thesis, Calcutta University.

Ray, S. 1998. Study of chromosome morphology from vegetative shoot apices of *Nitella hyalina* f. *Hyalina* (Charophyceae). *Phycologia* **37(1):** 64-66.

Ray, S. and P. Chatterjee, 1983. A new technique in karyological investigations of Characeae. *Cell and Chromosome Research* **6:** 27-29.

Ray, S. and P. Chatterjee, 1986a. Karyomorphological investigation of three populations of *Chara corallina* var. & f. *Corallina* in West Bengal. *Cytologia* **52:** 17-20.

Ray, S. and P. Chatterjee, 1988a. Cytotaxonomical study of some members of *C. Fibrosa* complex. *Cryptogamie; Algologie* **9(1):** 65-72.

Ray, S. and P. Chatterjee, 1988b. Cytotaxonomical study of West Bengal Charophyta: a new chromosome number count of *Nitella stuartii* and its Karyotype. *Acta Botanica Neerlandica,* **37(4):** 523-525.

Ray, S. and P. Chatterjee, 1989. A new chromosome number report of *Chara globularis* var. & f. *Leptosperma. Current Science* **58:** 312-313.

Ray, S. and P. Chatterjee, 1992. Cytotaxonomy of three forms of *Chara zeylanica* Klein ex Willd. Var. *Zeylanica* (Charophyta) from India. In: *Perspective in Cytology and Genetics* Ed. Manna G.K. and R.P. Roy. **7:** 827-834.

Ray, S. and P. Chatterjee, 1994. Cytotaxonomy of Charophytes in West Bengal, India: Karyotype analysis of three subspecies of *Nitella furcata. Act. Botanica Gallica* **141(1):** 43-48.

Sarma, Y.S.R.K. and M. Khan, 1964. Chromosome numbers in some Indian species of *Nitella*. *Chromosoma* **15:** 246-256.

Sarma, Y.S.R.K. and M. Khan, 1965. A preliminary report on the survey of chromosome numbers of Indian Charophyta. *The Nucleus* **8:** 33-38.

Sato, D. 1959. The protokaryotype and phylogeny in plants. *Scientific Papers of the College of General Education*. University of Tokyo. **9:** 303-327.

Sawa, T. 1965. Cytotaxonomy of the Characeae: Karyotype analysis of *Nitella opaca* and *Nitella flexilis*. *American Journal of Botany* **52:** 962-970.

Sharma, A.K. 1969. Evolution and taxonomy of monocotyledons. *Chromosomes today* **2:** 241-249.

Stebbins, G.L. 1971. Chromosomal evolution in higher plants. London. Edward Arnold (Pub.) Ltd., pp. 216.

Subramanyam, B.V.S. and Y.B.K. Chowdary, 1992. Karyology of *Nitella* from South India. *Cytologia* **57:** 209-212.

Sundaralingam, V.S. 1959. A systematic account of South Indian Charophyceae. *Proc. Indian Acad. Sci.* **49B:** 7-51.

Walther, E. 1929. Entwicklungs geschichtliche unde zytologische untersuchungen an einigen Nitellen. Inaugural dissert zurich Archiv der Julius Klaus-Stiftung für Vererbungs forschung. **4:** 23-121.

Wood, R.D. 1965. Monograph of the Characeae. In: *A Revision of the Characeae,* Vol. 1. Wood, R.D. and Imahori, K. Ed. J. Cramer, Weinheim, Germany, pp. 1-904.