Detailed Investigations on Cytotaxonomical Perspectives in *Nitella hyalina* Complex (Charales: Charophyceae)

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Summary Present paper deals with the detailed morphological, cytological and cytotaxonomical studies on 3 cytotypes of *N. hyalina* f. *hyalina*, collected from Mudasarilova tank (Visakhapatnam), Sakhya Sagar dam (Shivpuri) and Tighra dam (Gwalior). The cytological preparations reveal the occurrence of chromosome counts of $n=15$, 18 and 21, respectively. Count of 3 chromosomes numbers in 3 different cytotypes clearly established polyploidy in this taxon, if $x=3$ considered as the ancestral number. It was found that polyploidy is certainly associated with the morphological diversifications. As the chromosome numbers increased certain additional characters appeared, while, with reduction in chromosome numbers these additional characters have not been observed.

Key words Cytology, Cytotaxonomy, *Nitella hyalina* f. *hyalina*, Chromosome counts, Polyploidy

The tribe Nitelleae of the order Charales (Class Charophyceae) constitute an interesting group of green algae, showing some unique characteristic features like uncorticated plant body, monopodially divided branchlet furcations and lateral oogonia with 10-celled coronula in 2-tiers. Systematics of this group was widely described by Faridi (1956), Wood (1965), Corillion and Guerlesquin (1972), Caceres (1975), Compere (1982), Fu-shan and Hualong (1982), Comelles (1984), Hotchkiss and Imahori (1987), Pundhir *et al.* (1994) and others. Although large number of species of this group were studied for their cytology by several workers such as Gillet (1959), Sawa (1965), Khan and Sarma (1967a), Kanahori (1971), Mukherjee and Noor (1973), Ray and Chatterjee (1988), Pundhir and Gautam (1993) and Pundhir *et al.* (1993), very few attempts have been made to analyse them cytosystematically. In the present investigation, detailed cytotaxonomical studies were carried out on 3 cytotypes of *Nitella hyalina* (DeCandolle) Agardh f. *hyalina* found at 3 different ploidy level exhibited interesting correlations between cytotaxonomical and morphological characters.

Materials and methods

Living specimens of *Nitella hyalina* f. *hyalina* were collected from Mudasarilova tank (Visakhapatnam), Sakhya Sagar dam (Shivpuri) and Tighra dam (Gwalior), during Dec. 1991 to Jan. 1992. Young growing fertile upper healthy tips were fixed in absolute alcohol glacial acetic acid (3 : 1) for cytological studies. Plants were further preserved in 4% formalin for morphological observations. Identification was made following Wood (1965). Godward's (1948) iron-alum acetocarmine technique was employed for squashing spermatogenous antheridial filaments. Chromosome numbers were determined at metaphase when the chromosomes are found to be highly condensed and well spread. Camera lucida drawings and corresponding photographs were taken from temporary preparations. Slides were made permanent using propionic acid-butanol series and mounted in euperal. Voucher specimens and permanent slides are deposited in Cytology Laboratory, D.S. College, Aligarh (U.P.), India.

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Table 1. Comparative morphology of *Nitella hyalina* (DeCandolle) Agardh f. *hyalina*

Plant parts	Tighra plants	Mudasarilova plants	Sakhya Sagar plants	Wood (1965)
Plant sex	Monoecious	Monoecious	Monoecious	Monoecious
Plants height	Upto 8.0 cm, small to medium in size	Upto 14 cm, small to medium in size	Upto 4.0 cm, small size	Medium size
Mucus presence	Upper whorls covered with mucus	Upper whorls densely covered with mucus	Whole plant body densely covered with mucus	Upper whorls often crowded and covered with mucus
Axes	Slender	Slender	Slender	Slender
Axial diameter	390–420 μm	270–295 μm	285–375 μm	220–500 μm
Internodes	1–4 times as long as the branchlets	1–8 times as long as the branchlets	Shorter to 2 times as long as the branchlets	2–4 times as long as the branchlets
Branchlets	Heteroclemous, fertiles 8 in a whorl	Heteroclemous, fertiles 8 in a whorl	Heteroclemous, fertiles 8 in a whorl	Heteroclemous, fertiles 8 in a whorl
Furcations	2–4 furcated	3–4 furcated	2–3 furcated	2–3(–4) furcated
Primary rays	2/3–3/4 times of branchlets	1/2–3/4 times of branchlets	3/5–3/4 times of branchlets	1/2–3/5 times of branchlets
Secondary rays	7–8 (of which 1–2 may unfurcated) with percurrent central axis which developed into branchlets	8 of which all are furcated	8–10 of which 2–4 unfurcated with one central antheridium instead of percurrent central axis	7–10 of which 1–3 are simple
Tertiary rays	5–7 of which 3–5 unfurcated	6–8 of which 3 are simple	6 of which 4–5 unfurcated	4–7 of which 1–2 may unfurcate
Quaternary rays	5–6	6 of which 1–2 furcated	6	4–5
Quinary rays	3–5	4–6	—	—
Dactyls	3–5, 2-celled; end cell conical, acute, very much reduced, mucronate	6, 2-celled; end cell conical, confluent	6, 2-celled; end cell conical, acute, mucronate, sometimes confluent	4–6, 2-celled; end cell conical, acute, confluent
Heads	Terminal with dense mucus	Not formed, but upper whorls densely covered with mucus	Formed with dense mucus	Not formed, but upper whorls may become crowded and often enveloped in mucus
Gametangia	Stalked, conjoined or sejoined at 2–4 nodes	Sessile, conjoined at all branchlet furcations	Stalked, conjoined at 2 lowest branchlet nodes	Conjoined at all branchlet furcations
Oogonia	Solitary, 270–420 μm long (excl. coronula), 225–345 μm wide, convolutions 8	Solitary, 300–415 μm long (excl. coronula), 285–330 μm wide, convolutions 8	Solitary, 270–420 μm long (excl. coronula), 200–330 μm wide, convolutions 8–9	Solitary (rarely geminate), 425–565 μm long (excl. coronula), 375–400 μm wide, convolutions 8
Coronula	Unequal, 30–45 μm high, 45–60 μm wide	Unequal, 45–60 μm high, 45–60 μm wide	Unequal, 30–45 μm high, 45–60 μm wide	45–60 μm high, 60 μm wide
Oospores	Dark brown, 285–330 μm long, 240–270 μm wide, striae 6–7 with prominent ridges, membrane reticulate	Light brown to dark brown, 405–450 μm long, 300–345 μm wide, striae 6–7 with prominent ridges, membrane granulate	—	Reddish brown, 300–350 μm long, 280–320 μm wide, striae 6–7 with prominent ridges, membrane finely granulate
Antheridia	285–345 μm in diameter	270–360 μm in diameter	270–345 μm in diameter	350–425 μm in diameter

Table 2. Comparative cytology and cytotaxonomy of *Nitella hyalina* (DeCandolle) Agardh f. *hyalina*

Characters	Tighra plants	Mudasarilova plants	Sakhya Sagar plants
Chromosome numbers	15	18	21
Cell's length (μm)	13.0–15.0	13.0–18.0	13.5–19.5
Cell's breadth (μm)	11.2–13.5	12.6–15.2	10.5–12.0
Chromocentres	2	1–2	2
Nucleus diameter (μm)	6.0–13.5	12.0–15.0	9.0–10.5
Nucleolus number	1	1–2	1
Nucleolus diameter (μm)	3.0–4.5	4.2–4.5	4.5–6.0
Chromosomes length (μm)	2.62–6.00	1.37–3.12	1.00–5.87
Chromosomes breadth (μm)	0.75–1.00	0.62–0.87	0.62–0.87
Metacentric chromosomes	—	2	2
Submetacentric chromosomes	12	11	12
Subtelocentric chromosomes	1	1	1
Telocentric chromosomes	2	4	6

Results and discussion

Morphological observations of all the 3 cytotypes are summarized in Table 1. Detailed comparative cytology and cytotaxonomy has been given in Table 2.

Indian specimens belonging to *Nitella hyalina* f. *hyalina* are highly conspicuous having heteroclemous branchlets and arthrodactylous dactyls covered with thick layer of mucus which provided to these, a characteristic hyaline appearance (Table 1). They possess 2 rows of accessory branchlets in contrast to another heteroclemous species, *N. stuartii* (Wood 1965) which is having only 1 row of accessory branchlets and anarthrodactylous dactyls.

Three cytotypes of *N. hyalina* f. *hyalina* collected from Tighra dam, Mudasarilova tank and Sakhya Sagar dam were studied for their cytological features and revealed the occurrence of $n=15$ (Figs. 1, 2), $n=18$ (Figs. 3, 4) and $n=21$ (Figs. 5, 6) chromosomes, respectively. The count of $n=15$ in Tighra plants confirms the finding of Mukherjee (1978) and count of $n=21$ in Sakhya Sagar dam plants confirms the report of Bhatnagar (1988). The chromosome number $n=18$ determined for Mudasarilova plants is in agreement with the counts of Sato (1959), Guerlesquin (1961, 1963), Hotchkiss (1965), Sarma and Khan (1965), Khan and Sarma (1967b), Ramjee and Sarma (1971) and Ramjee and Bhatnagar (1978). The reports of $n=12$ (Sato 1959, Sinha and Verma 1970, Subramanian and Ganesan 1983), $n=12-14$ (Stewart 1937), $n=14$ (Sato 1959), $n=16$ (Gillet 1959) and $n=33$ (Das and Majumdar 1984) could not be confirmed in the present study. The count of different chromosome numbers ($n=15, 18, 21$) during present study alongwith other counts ($n=12, 33$) (Sato 1959, Sinha and Verma 1970, Subramanian and Ganesan 1983, Das and Majumdar 1984) are fit in euploid series of $n=6, 9, 12, 15, 18, 21, 24, 27, 33, 36, 48$ and therefore, uphold the view of $x=3$ as the proposed basic chromosome number for this genus (Sarma and Khan 1964, 1965, Khan and Sarma 1967b). The count of $n=14$ by Stewart (1937) and Sato (1959) might be counting error or may be represent the heteroploidy in this taxon.

Gillet (1959) suggested $x=6$ as the basic chromosome number for *Nitella* which was supported by Hotchkiss (1963). Guerlesquin (1961) has, however, suggested 6 and 7 as the basic chromosome numbers for this genus. For arthrodactylous forms, an additional basic chromosome number $x=9$ was suggested by Hotchkiss (1963). But the occurrence of $n=9$ chromosome number in an anarthrodactylous form *N. mirabilis* reported by Ramjee and Bhatnagar (1978) contradicted the above generalization.

It is interesting to note that the cytological features other than chromosomal morphology and numbers, are, almost alike in the 3 populations of *N. hyalina* f. *hyalina*. The chromosomes are fairly

Figs. 1–6. Chromosome numbers at metaphase plate in *Nitella hyalina* f. *hyalina* ($\times 2400$). 1) Chromosome number in Tighra plants ($n=15$). 2) Reconstruction of Fig. 1. 3) Chromosome number of Mudasarilova plants ($n=18$). 4) Reconstruction of Fig. 3. 5) Chromosome number in Sakhya Sagar dam plants ($n=21$). 6) Reconstruction of Fig. 5.

small to large in the plants of Sakhya Sagar dam and Tighra dam but they are small to medium in Mudasarilova plants. The diameter of nucleolus is small in lowest chromosome number ($n=15$) while large in highest chromosome number ($n=21$) consisting plants. It shows some relationship between number of chromosomes and size of nucleolus (Table 2).

Karyotypic analysis shows that karyotype of Mudasarilova plant (Fig. 8) resembles the karyotype of Sakhya Sagar dam plant (Fig. 9) while the karyotype of Tighra plant (Fig. 7) differs with the karyotypes of these plants in lacking median chromosomes. It might be due to reduction in chromosome number from 18 or 21 to 15. The origin of 15 chromosomal form leads the suspicion and might be through the hybridization between $n=12$ and $n=18$ chromosomal form of its polyploid races.

Polyploidy is certainly associated with the phenotypic variations. It is evidenced in the present observations on *N. hyalina* f. *hyalina*. Cytotype with 21 chromosomes shows stalked gametangia and confluent to mucronate dactyls, whereas, the material showing 15 chromosomes was found to have stalked gametangia, mucronate dactyls and reticulate oospore wall ornamentation in contrast to the Mudasarilova plants which possess sessile gametangia, confluent dactyls and granulate

oospore wall ornamentation characterised by 18 chromosomes. Rest of the characters in all the 3 cytotypes agree with the Wood (1965) description (Table 1).

A study of oospores wall ornamentation for various species of the genus *Nitella* was conducted by John and Moore (1978). They have been described the oospore wall ornamentation of the species *N. hyalina* as "finely punctate or granulate and often obscurely reticulate under light microscope. Under SEM it consists of anastomosing fibrils with loosely arranged fossa. They are occasionally flattened. These fibrils extend up onto the striae where they are more dense and often laterally fused. The 6 to 8 striae are thin but prominent and without a flange." It has to be mentioned here that these studies are based on herbarium and 4% formalin liquid preserved material deposited in British Museum (Natural History), London. They were not subjected for cytomorphological studies and are arranged according to Braun (1882) on gross morphological features. There is possibility that they are randomly collected and might be mixed cytotypes of reticulate, granulate and finely granulate oospore wall ornamentation consisting plants bearing $n=15$, 18 and 21 chromosomes, respectively, recorded during the present study.

Figs. 7–9. Karyotypic organisation in *Nitella hyalina* f. *hyalina*. 7) Karyotype of Tighra plants. 8) Karyotype of Mudasarilova plants. 9) Karyotype of Sakhya Sagar dam plants.

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