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CYTOLOGICAL STUDIES OF *NYMPHAEA* SPECIES AVAILABLE IN BANGLADESH

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Abstract

The present investigation was undertaken in order to study the somatic karyotypes of four species of *Nymphaea* available in Bangladesh. Karyotypes of four species of *Nymphaea* revealed that most of the chromosomes were metacentric indicating their primitive nature and chromosome counts revealed a polyploid series of tetraploid, pentaploid and hexaploid based on X=14. Findings of the present study indicated that *Nymphaea pubescens* is a hexaploid (6X=84, white flower) which might have originated by chromosome doubling of *Nymphaea daubeniana* (3X=42) while *Nymphaea rubra* and *Nymphaea nouchali* (Type-1) are tetraploid (4X=56) species.

Key words: *Nymphaea*, karyotype

Introduction

Nymphaea species are aquatic herbs with perennial rhizomes or rootstocks anchored with mud. In Bangladesh, the common Bengali name of *Nymphaea* is "Shapla". In addition to many natural hybrids, a large number of artificially raised varieties (Gray 1900, Ames 1900 and Nult 1967) have been increasing the list of *Nymphaeas*. Gupta (1980) investigated the genus *Nymphaea* cytologically and commented on their evolutionary relationship. His findings revealed a polyploid series ranging from diploid to octaploid levels based on X=14. He had noticed various abnormalities in the mitotic and meiotic configurations. *Nymphaea* species with white flower is the national flower of Bangladesh. Cytomorphological report on this species along with other species is very much scanty in the country. The present study was thus undertaken on six different types of *Nymphaea* available in Bangladesh from cytomorphological point of view.

Materials and methods

Four species of *Nymphaea* comprising six different types were used as plant material in this study. The species were *Nymphaea pubescens*, *Nymphaea alba* (it might be cultivated somewhere else, but it was found to be grown wild in different places at Rajshahi, Bangladesh from where it was used as experimented material), *Nymphaea rubra* and *Nymphaea nouchali* (Type 1, 2 and 3) and they were collected from different parts of Rajshahi district. A thorough morphological study was made on these species (Table 1). They were then differentiated into species and types with the help of available literature and the data obtained from this study. To count the chromosome number as well as to study chromosome morphology the root tips of different species were fixed 1:3 acetoalcohol and preserved in 70% ethanol just after pre-treatment with the saturated solution of para-dichlorobenzene (PDB). Chromosome staining was performed following the schedule of Haque *et al.* (1976). Photomicrographs were made from the metaphase plates and the chromosomes were measured from cameralucida drawings.

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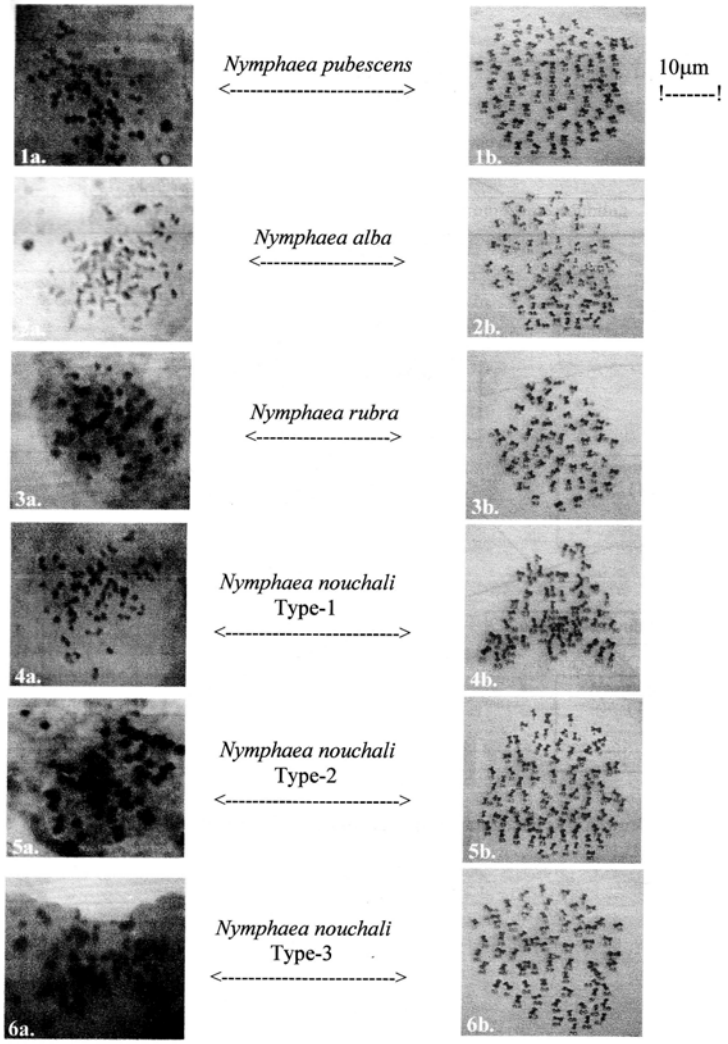
Table 1. Morphological characters in four species of *Nymphaea.*

Characters	*Nymphaea pubescens*	*Nymphaea alba*	*Nymphaea rubra*	*N. nouchali Type-1*	*N. nouchali Type-2*	*N. nouchali Type-3*
Habit	Rooted free floating	Rooted free floating	Rooted free floating	Rooted free floating	Rooted free floating	Rooted free floating
Root pocket	Present	Present	Present	Present	Present	Present
Leaf colour	Light green and upper surface waxy and glabrous	Light green and upper surface waxy and glabrous	Reddish green and upper surface waxy and glabrous	Greenish and upper surface waxy and glabrous	Greenish and upper surface waxy and glabrous	Deep green and upper surface waxy and glabrous
Leaf size and shape	Rounded and heart shaped, margin dentate	Rounded and heart shaped, margin dentate	Rounded and heart shaped, margin dentate	Rounded and heart shaped, margin crenate	Rounded and heart shaped, margin crenate	Rounded and heart shaped, margin crenate
Leaf length × breadth (cm.)	26.33×20.27	27.35×21.57	29.40×27.50	24.90×21.20	23.43×20.90	25.22×21.10
Flower colour	White	White and pinkish	Red	Light pink	Light blue	White
Flowering time	Bloom at night Opening time: 8-8.30pm Closing time: 8-9am	Bloom at night Opening time: 8-8.30pm Closing time: 8-9am	Bloom at night Opening time: 8-8.30pm Closing time: 8-9am	Bloom at day Opening time: 8-9am Closing time 3-4pm	Bloom at day Opening time: 8-9am Closing time 3-4pm	Bloom at day Opening time: 8-9am Closing time 3-4pm
Largest sepal length × Breadth (cm.)	6.87×2.90	6.18×2.46	7.96×2.46	4.0×2.05	4.02×2.10	4.50×2.30
Largest petal length × breadth (cm.)	5.07×2.10	5.90×2.80	6.12×1.80	4.45×1.48	4.50×1.55	4.80×1.60
Smallest petal length × breadth (cm.)	4.40×1.28	4.75×1.50	4.48×1.22	4.15×1.22	4.20×1.13	4.45×1.25
Petiole length (cm.)	145.80	148.06	140.86	109.62	110.80	115.75
Peduncle length (cm.)	108.12	110.15	116.80	74.40	75.60	76.20
Rhizomes length × breadth (cm.)	12.30×6.20	14.40×7.80	15.10×7.90	13.15×6.80	16.20×8.10	15.70×7.48
Diameter of rhizome (cm)	14.60	16.70	22.12	18.75	19.40	18.90
Fruit size and shape	Rounded hairy	Rounded hairy	Absent	Flat rounded and covered by calyx	Flat rounded and covered by calyx	Flat rounded and covered by calyx
Fruit length × breadth (cm.)	5.5×6.4	5.0×6.2	Absent	4.6×5.2	4.8×5.4	4.2×5.0
Diameter of fruit (cm)	16.70	15.80	Absent	10.30	12.20	10.50

Results and discussion

A thorough morphological study was made on four species of *Nymphaea*. The results of this study are presented in Table 1. The microphotographs of metaphase plates of four species of *Nymphaea* are presented in Figures 1a-6a and the Camera Lucida drawings used for measuring the chromosome are also shown in Figures



Figs. 1-6(a-b). Somatic metaphase chromosomes in six taxa of *Nymphaea* (Ca. 750x).
1a-6a. Photomicrographs of somatic metaphase chromosomes in six taxa of *Nymphaea*;
1b-6b. Cameralucida drawings of somatic metaphase chromosomes in four species of *Nymphaea*.

The average value ($\overline{X}$) for chromosome length, total chromatin length, total frequency, arm ratios, etc. are given in Table-2. In *Nymphaea pubescens*, *Nymphaea alba*, *Nymphaea nouchali* (Type 1, 2 and 3) and *Nymphaea rubra* the longest mean absolute lengths of chromosome sets were 3.43, 2.97, 2.06, 3.99, 3.02, & 3.38 µm and shortest mean lengths of chromosome sets were 2.34, 1.44, 1.56, 1.82, 1.70 and 1.44 µm, while total chromatin lengths were 115.26, 71.26, 72.66, 117.06, 86.86 & 66.48 µm, respectively. In all of the *Nymphaea* species TF % were found to be the same.

Out of nearly 40 species belonging to this genus, 22 species have been investigated cytologically so far and revealed a basic number of X=14. Similarly euploid forms have been reported by Lohammar (1942) in *Nymphaea candida,* Love and Love (1942) and Ehrenberg (1945) in *Nymphaea alba* while Wood (1959) recorted aneuploidy in *Nymphaea tetragona*. In the present study the chromosome number of all the specimens were found to be euploidy in nature, viz. *Nymphaea pubescens* had 2n=84, *Nymphaea alba* had 2n=70, *Nymphaea rubra* had 2n=56, *Nymphaea nouchali* (Type-1) had 2n=56, *Nymphaea nouchali* (Type-2) had 2n=84, and *Nymphaea nouchali* (Type-3) had 2n= 70. In the present study different chromosome number of genus *Nymphaea also* indicated a dominant role in speciation with basic chromosome number X=14. Gupta (1978) studied nineteen taxa of both Indian wild and American cultivated forms of *Nymphaea and* reported diploid, triploid, tetraploid and hexaploid series based on X=14. Gupta (1980) made the chromosome count in the genus *Nymphaea* and also reported diploid to octaploid based on X=14. Thus the present findings agreed well with the previous reports of Guignard (1898) as well as of others. However, a number of workers (Langlet and Sodenberg 1927, Lohammar 1942, Love and Love 1942, Ehrenberg 1945, Harada 1952, Heslop-Harrison 1955, Janaki Ammal 1956, Raghavan and Arora 1958, Wood 1959, Mitra and Datta 1967, Sen and Bhaduri 1971, Gupta 1972, 1976, 1978a, 1978b, 1979 and 1980) reported the six different types of *Nymphaea* (Figure 7), which were studied in the present investigation. However, in *Nymphaea rubra* 2n=56 (4x) chromosomes and *Nymphaea nouchali* (Type-1) 2n=56 (4x) chromosomes were found which may be evolved by the doubling of chromosome (2n=28) from ancestral species.

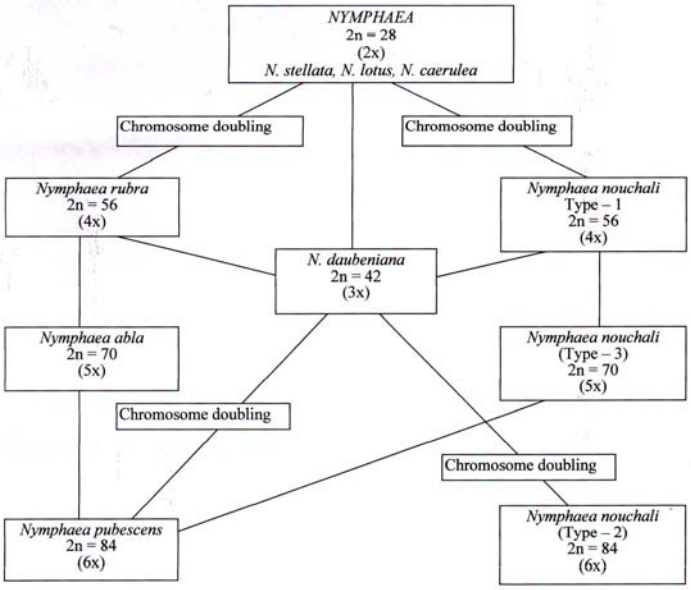


Fig. 7. Presumed phylogenetic relationships among seven species of *Nymphaea* based on karyotype and ploidy level.

Table 2. Analysis of the metaphase chromosome sets (mean values) in different species of *Nymphaea*. (First row represent absolute length in µm, second row gives arm ratios, third row gives relative chromosome length and fourth row TCL%).

Chromosome sets	*N. nouchali* Type-3 5x=70 TF% = 50% TCL=68.68(µ)	*N. alba* 5x=70 TF% = 50% TCL=71.16(µ)	*N. pubescens* 6x = 84 TF% =50% TCL=115.26(µ)	*N. nouchali* Type-2 6x = 84 TF% =50% TCL=117.06(µ)	*N. nouchali* Type-1 4x=56 TF%=50% TCL=72.66(µ)	*N. rubra* 4x =56 TF% = 50% TCL=66.48(µ)
I	3.20 1.00 96.79 3.47	2.97 1.00 9.07 4.17	3.43 1.00 84.81 3.36	3.99 1.00 89.84 3.41	2.06 1.00 98.08 4.21	3.38 1.00 86.67 5.09
II	2.79 1.00 89.36 3.21	2.79 1.00 92.93 3.91	2.95 1.00 73.10 2.56	3.38 1.00 83.66 2.89	2.86 1.00 91.67 3.94	2.74 1.00 70.26 4.12
III	2.74 1.00 87.82 3.15	2.60 1.00 86.87 3.65	2.86 1.00 70.79 2.48	2.95 1.00 72.94 2.52	2.74 1.00 87.82 3.77	2.60 1.00 66.67 3.91
IV	2.74 1.00 67.82 3.15	2.60 1.00 86.87 3.65	2.74 1.00 66.82 2.38	2.86 1.00 70.79 2.44	2.60 1.00 83.33 3.58	2.60 1.00 66.67 3.91
V	2.74 1.00 87.82 3.15	2.60 1.00 86.87 3.65	2.74 1.00 67.82 2.38	2.78 1.00 68.81 2.37	2.60 1.00 83.33 3.58	2.60 1.00 66.67 3.91
VI	2.66 1.00 85.13 3.05	2.44 1.00 81.63 3.43	2.74 1.00 67.82 2.38	2.74 1.00 67.82 2.38	2.60 1.00 83.58 3.58	2.60 1.00 67.67 3.91
VII	2.60 1.00 83.33 2.99	1.82 1.00 60.67 2.55	2.74 1.00 67.82 2.38	2.74 1.00 67.82 2.34	2.60 1.00 83.33 3.58	2.60 1.00 66.67 3.91

Table 2. Cont. Analysis of the metaphase chromosome sets (mean values) in different species of *Nymphaea*. (First row represent absolute length in µm, second row gives arm ratios, third row gives relative chromosome length and fourth row TCL%).

Chromosome sets	*N. nouchali* Type-3 5x=70 TF% = 50% TCL=68.68(µ)	*N. alba* 5x=70 TF% = 50% TCL=71.16(µ)	*N. pubescens* 6x = 84 TF% =50% TCL=115.26(µ)	*N. nouchali* Type-2 6x = 84 TF% =50% TCL=117.06(µ)	*N. nouchali* Type-1 4x=56 TF%=50% TCL=72.66(µ)	*N. rubra* 4x =56 TF% = 50% TCL=66.48(µ)
VIII	2.60 1.00 83.33 2.99	1.82 1.00 60.67 2.55	2.74 1.00 67.82 2.38	2.74 1.00 67.82 2.34	2.60 1.00 83.33 3.58	2.60 1.00 66.67 3.91
IX	2.60 1.00 83.33 2.99	1.67 1.00 55.74 2.35	2.74 1.00 67.82 2.38	2.74 1.00 67.82 2.34	2.60 1.00 83.33 3.58	2.60 1.00 66.67 3.91
X	2.60 1.00 83.33 2.99	1.56 1.00 52.00 2.19	2.60 1.00 64.36 2.26	2.74 1.00 67.82 2.34	2.21 1.00 70.83 3.04	2.21 1.00 56.57 3.33
XI	2.44 1.00 78.33 2.81	1.46 1.00 48.08 2.05	2.60 1.00 64.36 2.26	2.60 1.00 64.36 2.22	1.82 1.00 58.33 2.50	1.82 1.00 46.47 2.74
XII	1.82 1.00 58.33 2.10	1.44 1.00 48.00 2.02	2.60 1.00 64.36 2.26	2.60 1.00 64.36 2.22	1.82 1.00 58.33 2.50	1.82 1.00 46.47 2.74
XIII	1.75 1.00 56.03 2.03	1.44 1.00 48.00 2.02	2.60 1.00 64.36 2.26	2.34 1.00 57.92 2.00	1.69 1.00 54.18 2..32	1.63 1.00 41.66 2.46
XIV	1.70 1.00 54.49 1.98	1.44 1.00 48.00 2.02	2.34 1.00 57.92 2.03	1.82 1.00 45.05 1.55	1.56 1.00 50.00 2.14	1.44 1.00 36.92 2.17

In *Nymphaea daubeniana* 2n=42 (3x) chromosomes were reported by Gupta (1978). This species might be evolved by the crossing of *Nymphaea rubra* 2n=56 and *Nymphaea stellata* or *Nymphaea lotus* or *Nymphaea caerulea* (2n=28). *Nymphaea daubeniana* might have also evolved by the crossing of *Nymphaea nouchali* (Type-1) 2n=56 (4x) and *Nymphaea stellata* or *Nymphaea lotus* or *Nymphaea cerulea* 2n=28. Similarly in the present study *Nymphaea pubescens* 2n=84 (6x) and *Nymphaea nouchali* (Type-2) 2n=84 (6x) have been evolved by the chromosome doubling of *Nymphaea daubeniana* 2n=42 (3x). In case of *Nymphaea alba* 2n=70 (5x) might be appeared by the crossing of *Nymphaea rubra* 2n=56 (4x) and *Nymphaea pubescens* 2n=84 (6x). Likely *Nymphaea nouchali* (Type-3) 2n=70 (5x) might also be originated by the crossing of *Nymphaea pubescens* 2n=84 (6x) and *Nymphaea nouchali* (Type-1) 2n=56 (4x). None of the members in the present study was found as aneuploid. Anyhow, most of the species hybridize freely among themselves naturally. However, the cytomorphological findings in the present study indicated that *Nymphaea pubescens* (6x=84, white flower) might have originated by chromosome doubling of *Nymphaea daubeniana* (3x=42).

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