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CYTOLOGICAL STUDIES ON THE GENUS *RHIZOCLONIUM* KUETZ.[1]

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ABSTRACT

The paper deals with the nuclear cytology of nine taxa of *Rhizoclonium* (two belonging to *R. hookeri* and seven belonging to *R. heiroglyphicum*). The chromosome numbers determined for two taxa of *R. hookeri* are 46 and 22 and the seven taxa of *R. heiroglyphicum* revealed 12,22,24,32,34 and 36 (two with the last number) chromosome. Chromosome numbers for the two taxa of *R. hookeri* and four of *R. heiroglyphicum* are recorded for the first time. The cytology of the taxa in relation to their morphology has been discussed. Further, the results presented here show that aneuploidy has played a major role in the speciation of the genus.

INTRODUCTION

Order Cladophorales is one of the very extensively studied group from a karyological point of view. Studies on the genus *Rhizoclonium* were initially made by Peterschika (1932) and Geitler (1936). Subsequently, significant contributions were made by Indian workers who reported a chromosome number of 2n=24 in *R. heiroglyphicum* (Ag.) Kütz. (Sinha, 1958; Balakrishnan, 1961; Sinha and Noor, 1967). Similar reports were made in other species like *R. casparyi* Harv. *R. arenosum* (Carm.) Kütz. (Akhaury, 1971; Sinha and Ahmed, 1974) and *R. arenosum* var. *occidentale* Kütz. (Sinha and Ahmed, 1974). Studies of Patel (1961, 1971) revealed a diploid chromosome number of 22 and 36 in *R. tortuosum* Kütz. Diploid chromosome count of 18 and 36 also exist in literature for *R. riparium* (Sinha, 1958) and the latter number was reported by Sinha and Ahmed (1974) in *R. casparyi* as well. The present paper deals with a cytological study of nine taxa belonging to two species of *Rhizoclonium* i. e., *R. hookeri* (two forms) and *R. heiroglyphicum* (seven forms) from Punjab.

Recent cytological investigations on *Rhizoclonium* revealed haploid chromosome numbers of 12, 16, 18 and 30 for *R. pachydermum* Kjellman, *R. arboveum* Zeller, *R. dimorphum* Witter, *et.* Nordst. and *R. crassipelitum* West & West respectively (Prasad and Vijay Kumari 1980).

MATERIALS AND METHODS

The algae under investigation were collected from Patiala city and Punjab University campus. These were washed in running tap water to remove extraneous matter and epiphytic growth. Subsequently, these were fixed in acetic alcohol (1 : 1) fixative for 24 hours. Godward's iron-alum acetocarmine method

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(1948) was employed for cytological preparations. Squashing and light tapping procedures were carried with vegetative filaments for bringing chromosomes in one plane and for better spreading. All the algal samples fixed in 4% formaldehyde and are deposited in the Museum of the Department. The departmental accession numbers are given in parenthesis.

RESULTS

The following is a brief taxonomic description of the taxa alongwith the cytological features:

R. hookeri Kützing Form-I

Filaments coarse, branched; cells of the main axis long, 61.6-77.0 μm diameter; 4-14 times longer than the diameter; wall 3.9 μm thick, cells of the branch 385.0-446.6 μm long, 30.8-46.2 μm broad, not partitioned off from the main filament by a septa.

Habitat : A ditch in front of Bikram College of Commerce, Patiala (PUN 290).

Interphase nucleus nearly spherical, 6-8 μm in diameter, 10-18 nuclei per cell. During prophase, the nuclear size increases almost double the size of interphase nucleus. The chromosomes appear as rod-shaped structures varying in length from 1.5-8.00 μm and breadth 1.5 μm. The chromosome number is found to be 46 (Fig. 1).

R. hookeri Kützing Form-II

Filaments slightly coarse, branched; cells long, 53.9-92.4 μm diameter; 2-11 times longer than diameter; wall 6.85-8.00 μm thick; branches pleuricellular, not partitioned off from the cell, cells of the branches 46.2-61.6 μm in diameter, 3-9 times longer than diameter.

Habitat : Nursery of Botanic Gardens, Punjabi University, Patiala (PUN 291).

Interphase nuclei are nearly spherical and vary in diameter from 5.3-7.6 μm. During prophase, nuclear size increases to two to three times the size of interphase nucleus. Number of nuclei per cell varies from 30-72. At metaphase the chromosomes get condensed to the maximum degree being 1.5-7.6 μm long, 0.76-1.53 μm broad. The chromosome number is found to be 22 (Fig. 2).

R. heiroglyphicum (Ag.) Kützing Form I

Filaments coarse, unbranched, not much curved, 17.3-30.8 μm in diameter, 1.5-6 times longer than the diameter; inflated at some places; cells measure 1.9-3.9 μm thick.

Habitat : Stagnant water of a tube well from Sheikhupura (PUN 292).

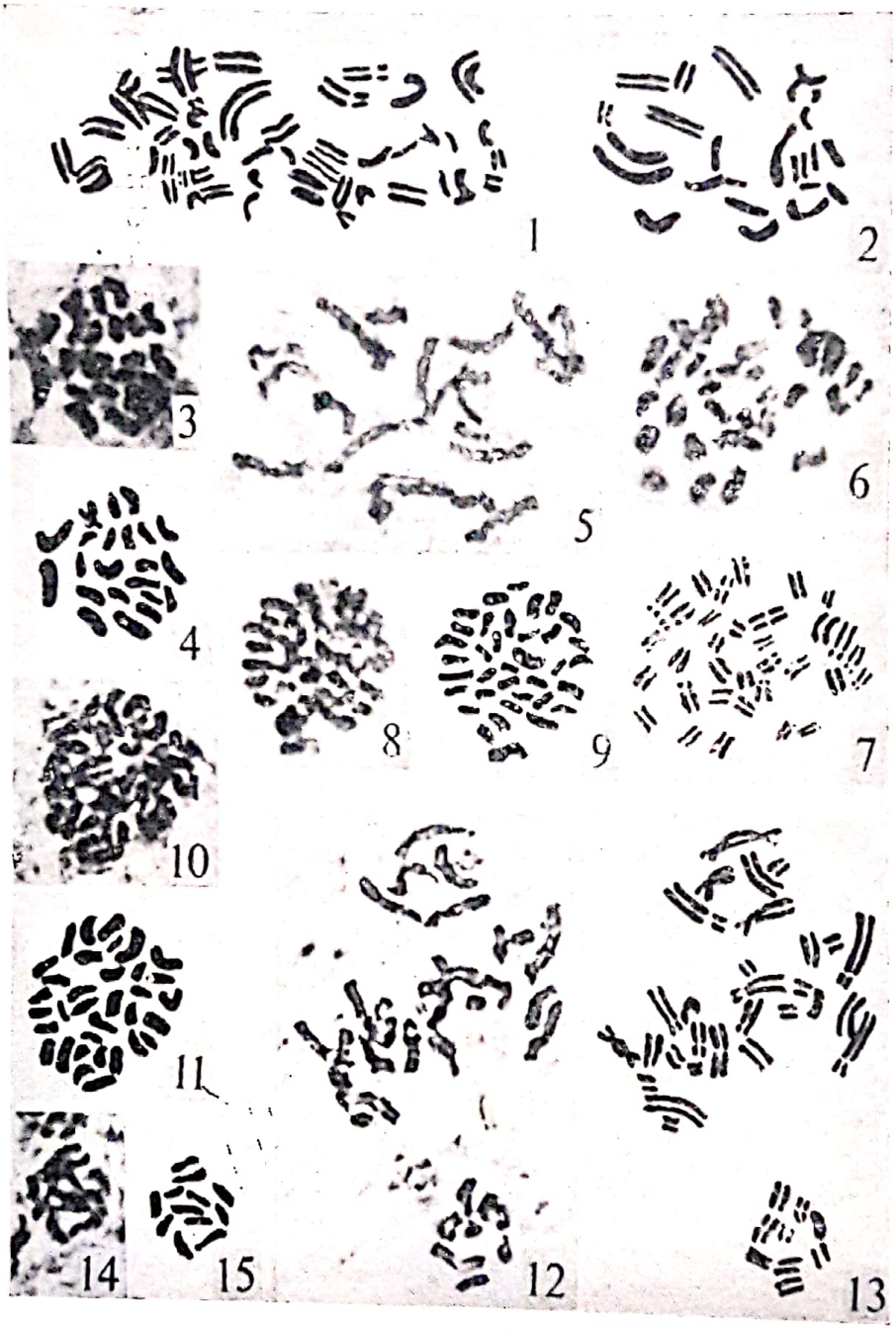
Interphase nuclei vary in diameter from 6.1-7.6 μm and number of nuclei per cell vaies from 2-4. During prophase, nuclear size increases almost double the size of the interphase nucleus. The chromosomes appear as thick rod-shaped structures varying in length from 1.5-3.0 μm and breadth 1.1-1.5 μm. The chromosome number is 24 (Figs. 3 & 4).

R. heiroglyphicum (Ag.) Kützing Form-II

Filamants thin, coarse, not much curved, 38.5-46.2 μm diameter, 3-8 times longer than diameter, cells inflated in the middle; cell wall 6.0-9.7 μm thick; branches small, rhizoidal.

Habitat : Stagnant water of a tube well from Sheikhupura (PUN 293).

Interphase nuclei are nearly spherical, have a diameter of 6.12 μm. Number of nuclei vary from 20-50. During prophase, the size of the nucleus increases to nearly double the size of interphase nucleus. The chromosomes are thick, elongated strctures having a length of 4.7-9.18 μm and breadth 1.53 μm. A chromosome count of 22 is noticed (Fig. 5).



Figs. 1-15. Fig. 1. Line drawing of metaphase plate of *R. hookeri* Form I showing 46 chromosomes. (X11650). Fig. 2. Line drawing of metaphase plate of *R. hookeri* Form II with 22 chromosomes. (X2300). Fig. 3. Metaphase plate of *R. heiroglyphicum* Form I, with 24 chromosomes. Fig. 4. Line drawing of Fig. 3. Fig. 5. Metaphase plate of *R. heiroglyphicum* Form II with 22 chromosomes. Fig. 6. Metaphase plate of *R. heiroglyphicum* Form III with 32 chromosomes. Fig. 7. Line drawing of Fig. 6. Fig. 8. Metaphase plate of *R. heiroglyphicum* Form IV with 36 chromosomes. Fig. 9. Line drawing of Fig. 8. Fig. 10. Metaphase plate of *R. heiroglyphicum* Form V with 36 chromosomes. Fig. 11. Line drawing of Fig. 10. Fig. 12. Metaphase plate of *R. heiroglyphicum* Form VI with 34 chromosomes. Fig. 13. Line drawing of Fig. 12. Fig. 14. Metaphase plate of *R. heiroglyphicum* Form VII with 12 chromosomes. Fig. 15. Line drawing of Fig. 14. (Figs. 3 to 15 X 1960).

R. heiroglyphicum (Ag.) Kützing Form-III

Filaments thin, coarse, unbranched, curved, attached to the substratum by hold fast, 26.9-30.8 μm in diameter, 1-6 times longer than the diameter; slighly inflated, cell wall 1.9 μm thick.

Habitat : Near 'Chungi', Patiala-Rajpura road (PUN 294).

Interphase nuclei are nearly spherical and have a diameter of 7.6 μm Number of nuclei vearies from 2-4 per cell. Nuclear size increases to nearly double the size of interphase nucleus during prophase. The chromosomes pappear as thick, elongated structures varying in length from 2.4-3.2 μm and in breadth from 0.8-1.6 μm. The chromosome number is 32 (Figs. 6 & 7).

R. heiroglyphicum (Ag.) Kützing Form-IV

Filaments thin, unbranched, curved at places, attached to the substratum by means of hold fast, 23.1-30.8 μm in diameter, 1-4 times longer than the diameter, Cells long, inflated, cell wall 1.9 μm thick.

Habitat : Baradari Gardens, Patiala (PUN 295).

Nuclei are nearly spherical and vary in diameter from 4.59-9.18 μm. Number of nuclei varies from 1-4 per cell. During prophase, the nuclear size icreases to double the size of interphase nucleus. Chromosomes appear as rod-shaped structures during metaphase, have a length of 1.53-4.59 μm and breadth 0.76-1.3 μm. The chromosome number is 36 (Figs. 8 & 9).

R. heiroglyphicum (Ag.) Kützing Form-V

Filaments dark green, curved, 77 μm in diameter, cells sometimes inflated in the middle; cell wall 3.9-7.8 μm thick; branches small.

Habitat : A ditch at the back of Department of Botany, Punjabi University Patiala (PUN 296).

Nuclei are nearly spherical, vary in diameter from 6-8 μm. Number of nuclei varies from 40-56 per cell. During prophase, nuclear size increases to almost double the interphase nucleus. The chromosomes appear as thick, elongated structures during metaphase, varying in length from 1.91-6.65 μm and in breadth 2.29-3.06 μm. The chromosome number is found to be 36 (Figs. 10 & 11).

R. heiroglyphicum (Ag.) Kützing Form-VI

Filaments thin, unbranched, curved, 19.25-25.55 μm in diameter, 2-6 times longer than the diameter; cells inflated at some places; cell wall 3.9-4.5 μm thick.

Habitat : Nursery of Botanic Gardens Punjabi University, Patiala (PUN 297).

Interphase nuclei are almost spherical and have diameter of nearly 7.65 μm. Nuclei vary in number from 1-2 per cell. During prophase, the size of nucleus increases to double the size of interphase nucleus. The chromosomes appear as thick, short structures with a length of 1.53-6.12 μm, bradth 1.53-2.29 μm. The chromosome number is noted to be 34 (Figs. 12 & 13).

R. heiroglyphicum (Ag.) Kützing Form-VII

Filaments thin, coarse, unbranched, curved, 19.25-25.55 μm in diameter; slightly inflated; cell wall 1.95 μm thick.

Habitat : Baradari Gardens, Patiala (PUN 298).

Interphase nuclei are nearly spherical and have a diameter of 3.85 μm. Number of nuclei varies from 4-16 per cell. Nuclear size increases to 1½ to nearly 2 times times the size of interphase nucleus during prophase. The chromosomes appear as thick, rod-shaped elongtated structures varying in length from 1.53-3.82 μm and in breadth 0.76 μm. The chromosome number is 12 (Figs. 14 & 15).

DISCUSSION

Chromosome nmubers of the taxa in the present study range from 12-46 and of the six taxa constitute new records. Since no swarmer formation has been detected in these members, it cannot be conclusively said whether these represent haploid number. Table-I shows the chromosome numbers of these presently studied forms and gives a comparative account of the earlier work with identical numbers. Two forms of *R. hookeri* revealed a chromosome number of 46 and 22 and seven forms of *R. heiroglyphicum* were found to possess 12, 22, 24, 32, 34 and 36 chromosomes (two forms with latter number). Of these, forms with 12, 22, 32 and 34 chromosomes constitute first report while 24 and 36 were earlier reported by Sinha (1958), Balakrishnan (1961), Sinha and Noor (1967), Sinha and Akhaury (1970) and Prasad *et al.*, (1973).

Forms I & II of *R. hookeri* are aquatic

TABLE I

PRESENT CHROMOSOME COUNTS IN THE GENUS *Rhizoclonium* TOGETHER WITH CORRESPONDING CHROMOSOME NUMBERS OF EARLIER REPORTS

Chromosome numbers of presently studies taxa		Earlier reports		
Name of the Species	Chromosome number 2n?	Name of the Species	Chromosome number 2n	Authrcs
R. hookeri Kuetz Form I	46		Not reported	
R. hookeri Kuetz. Form II	22		—do—	
R. heiroglyphicum (Ag.) Kuetz. Form I	24	*R. heiroglyphicum* (Ag.) Kuetz.	24	Geitler, 1936, Sinha, 1958; Balakrishnan, 1961 Sinha & Noer 1967; Prasad *et al.*, 1973; Patel, 1961
		R. casparyi Harv.	24	Verma, 1969
		R. arenosum (Carm.) Kuetz.	24	Akhaury, 1971 Sinha & Ahmed, 1974
		R. arenosum var. *occidentale*	24	Sinha & Ahmed, 1974
R. heiroglyphicum (Ag.) Kuetz. Form II	22	*R. tortuosum*	22	Patel, 1961
R. heiroglyphicum (Ag.) Kuetz. Form III	32		Not reported	
R. heiroglyphicum (Ag.) Kuetz. Form IV	36	*R. heiroglyphicum* (Ag.) Kuetz.	36	Prasad *et al.*, 1973
		R. riparium (Roth.) Harv.	36	Sinha, 1958
R. heiroglyphicum (Ag.) Kuetz. Form V	36	*R. casparyi* Harv.	36	Sinha & Ahmed, 1974
R. heiroglyphicum (Ag.) Kuetz. From VI	34		Not reported	
R. heiroglyphicum (Ag.) Kuetz. Form VII	12		—do—	

and have thicker walls ranging from 3.9-7.8 um. These have chromosome numbers 46 and 22 respectively. Longest chromosome in Form I is 8.0 μm while in Form II 7.6 μm. The smallest has a length of 1.5 μm in both the forms. These two forms have been worked out for the first time.

Various forms of *R. heiroglyphicum* are also aquatic and have thicker walls. The thickness varies from 1.9-7.8 μm. Earlier reports of the thickness of the wall range from 1.5 to 2.0 (Collins, 1923; Patel, 1961). However, Prasad *et al.*, (1973) reported a thickness of 1.3 to 3.5 μm in two forms of *R. heiroglyphicum*. The number of nuclei observed range from 1-56 per cell. Patel (1961) and Balakrishnan (1961) observed the variation of nuclei from 1-24 per cell.

In all the forms studied the interphase nuclear size varied from 4.5 to 9.1 μm. However, in other forms studied by earlier workers the size of the resting nucleus was found to measure upto 4 μm (Patel, 1961; Balakrishnan, 1961) and 8 μm (Prasad *et al.*, 1973). During prophase the size of the nucleus almost increased to double the original in all the forms. There is a significant variation in the size of the longest chromosome among the forms studied. The longest chromosome ranges from 3.0-9.1 μm and the smallest from 1.53-3.06 μm.

The base number of the order Cladophorales was suggested to be 6 (Sinha and Ahmed, 1971). The chromosome numbers known in various species of *Rhizoclonium* can be put into euploid series with 2n 24 and 36 (Geitler, 1936; Sinha, 1958; Balakrishnan, 1961; Sinha and Noor, 1967, and Prasad *et al.*, 1973). Form VII of *R. heiroglyphicum* with chromosome number 12 is the lowest recorded so far. New chromosome numbers in this genus with counts of 12, 22, 24, 32, 34 and 36 are also recorded in other forms of *R. heiroglyphicum*. Forms of *R. heiroglyphicum* with numbers 22, 32 and 34 do not fit into the euploid series. Two forms of *R. hookeri* have been worked out for the first time with the numbers 22 and 46. These observations strongly suggest towards the aneuploid trends in the speciation of the genus. However,, it is interesting to note that Prasad and Vijay Kumari (1980) reported a haploid number of 14 chromosomes for *R. hookeri* which differs from the earlier report of n=18 by Sinha (1958).

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