CARYOLOGIA Vol. 56, no. 3: 353-358, 2003

New reports of chromosome number and genome size in eight mangroves from coastal Orissa

SATYANARAYAN JENA, PRAGNYA SAHOO, ANATH BANDHU DAS*

Cytogenetics Laboratory, Regional Plant Resource Centre, Bhubaneswar 751015, Orissa, India.

Abstract - Detailed karyotype analysis and cytophotometric estimation of 4C DNA amount as well as inter-phase nuclear volume (INV) were carried out in eight mangrove associates found in Bhitarkanika mangrove forest of coastal Orissa, India. Somatic chromosome number of *Sarcolobus carinatus* (2*n*=22), *Lumnitzera racemosa* (2*n*=24), *Tylophora tenuis* (2*n*=24), *Sphaeranthus indicus* (2*n*=30), *Cerbera manghas* (2*n*=40), *Sesuvium portulacastrum* (2*n*=48), *Syzygium cumini* (2*n*=66), and *Hibiscus tiliaceous* (2*n*=86) were recorded for the first time. Karyotype analysis revealed numerical and structural alterations of somatic chromosomes in different species. Significant variations of 4C DNA content was noted among the species ranged from 5.31pg in *T. tenuis* to 27.83pg in *C. manghas*. Genome size varied about five fold among the taxon of different families from 1301 Mbp in *T. tenuis* to 6818 Mbp in *C. manghas.* Chromosome length, volume and INV showed significant correlation between them. ANOVA analysis confirmed the variation in nuclear DNA content in the interspecific as well as intergeneric level.

Key words: Karyotype; genome size; *Cerbera*; *Hibiscus*; *Sarcolobus*; *Sesuvium*; *Sphaeranthus*; *Syzygium*; *Tylophora*.

INTRODUCTION

Mangroves, the native flora of estuarine and inter-tidal regions of tropical and sub-tropical coasts, constitute a unique and dynamic ecosystem. True mangroves and other terrestrial as well as marsh-land species are capable of growing in high salt mangrove habitats. Though mangrove associates play an inconspicuous role in the basic structure of mangrove forest, they may give clues to evolutionary pathway by which the highly specialized adaptive syndrome of mangrove has been achieved (TOMLINSON 1986). Out of a total 90 mangrove species (CHAPMAN 1976) from tropical and sub-tropical world, 55 mangroves species are reported from Indian mangals. Orissa coasts have the highest mangrove diversity than any other mangals of the Indian sub-continent. *Sesuvium portulacastrum* of Aizoaceae is a eu-halophytic herbs, reported first from South-East Asia coast and East Africa (MEPHAM and MEPHAM 1984) whereas *Cerbera manghas* of Apocynaceae and *Hibiscus tiliaceous* of Malvaceae, *Syzygium cumini* of Myrtaceae are three tropical tree mangrove associates, observed nearer to the source of fresh water flow of Mahanadi delta (BANERJEE 1990). *Tylophora tenuis* and *Sarcolobus carinatus* of Asclepiadaceae are two twinning creepers found in the mangal region with an occurrence of *Sphaeranthus indus* away somewhat to the sea level.

Comparative studies on chromosome numbers are the basic tenets in establishing evolutionary relationships in any taxonomic groups. Determination of chromosome number and karyotype analysis is the preliminary requisite to access the genomic status of genetically diverse

* Corresponding author: e-mail: a_b_das@hotmail.com; metaphase@rediffmail.com

mangroves as chromosome number is the raw material on which evolutionary forces have been acting, leading to origin and evolution of the entire biological diversity. Past reports in chromosome number of mangrove associates like *Acanthus* (DAS *et al.* 1996), *Hibiscus tiliaceous* (YOUNGMAN 1927; SKOTTSBERG 1955), *Sesuvium portulacastrum* (SHARMA and BHATTACHARYA 1956; RAGHAVAN and SRINIVASAN 1940) have though considerably advanced our knowledge of the prevailing genetic diversity, it is still inadequate to attempt in depth assessment of the gene pool of Bhitarkanika, Orissaa. The numerical variation of diploid chromosome numbers $2n$=40 to 96 in *H. tiliaceous* as well as $2n$=36 and 48 in *S. portulacastrum* were reported and controversy still remains with their aneuploid occurance in different ecotypes. There are no chromosome number report, so far, available in *Cerbera manghas, Lumnitzera racemosa, Sarcolobus carinatus, Sphaeranthus indicus, Syzygium cumini* and *Tylophora tenuis.* Besides, nuclear DNA amount (C-value) and genomic size are important biodiversity characters of fundamental significance and with many uses (BENNETT *et al.* 2000). However, the availability of C-value data varied widely between true and associates mangroves, being scarce in associates (approximately 3%). The DNA C-value has many diverse nucleotypic consequences (BENNETT *et al.* 2000) and genomic obesity (BENNETZEN and KELLOGG 1997) and the importance is being realized. Comparison of nuclear DNA amounts provide a useful data in many cytotaxonomic and evolutionary studies (PRICE 1976; RAINA 1990). A scanty report on nuclear DNA content available, so far, for mangrove associates except *Acanthus* and *Thespesia*, *Dolichondrone* and *Suaeda* (DAS *et al.* 1996; JENA *et al.* 2002). The present investigation has been made on 8 mangrove associates, found in Bhitarkanika mangrove forest of Orissa for the first time in order to record the numerical and structural details of the somatic chromosome, their karyotype, interphase nuclear volume and estimation of 4C DNA content of these species as part of the gene pool conservation strategy of the mangroves of coastal Orissa.

MATERIAL AND METHODS

Seeds of 8 mangrove species were collected from the Bhitarkanika mangrove forest, Orissa, India. The voucher specimens were identified and kept in the herbaria of the Regional Plant Resource Centre. Seeds were grown in the experimental mangrove nursery of the Centre. Healthy young root-tips were pretreated in half saturated paradichloro-benzene and aesculine mixture for 4h at 14°C followed by overnight fixation in propionic acid:ethanol (1:3). Chromosome staining was made in 2% propionic orcein after cold hydrolysis in 5N HCl for 7 min. Root-tips were squashed in 45% propionic acid and well scattered ten metaphase plates were selected for karyotype analysis for each species. The total chromosome length was

Table 1 – Somatic chromosome number, chromosome size, genome size of different species of mangroves of coastal Orissa.

Family/Taxon	$2n$	Karyotype formulae	TCL (µm±SE)	TCV (µm³±SE)	4C DNA content (pg±SE)	Genome size in Mbp	INV (µm³±SE)	TF% (±SE)
Apocynaceae								
Cerbera manghas	40	38C+2D	47.74±1.12	88.78±0.98	27.83±0.02	6818	578.58±4.04	43.94±0.23
Malvaceae								
Hibiscus tiliaceous	86	2A+74C+10D	114.20±1.34	95.13±0.56	18.72±0.03	4586	1341.97±6.23	43.73±0.35
Combretaceae								
Lumnitzera racemosa	24	14C+10D	56.91±2.13	24.10±0.34	22.51±0.09	5514	235.08±1.20	38.08±0.56
Asclepiadaceae								
Sarcolobus carinatus	22	20C+2D	40.62±1.68	35.04±0.78	11.05±0.04	2707	481.28±2.23	44.32±0.67
Aizoaceae								
Sesuvium portulacastrum	48	4A+32C+12D	142.62±1.99	195.34±1.05	18.22±0.01	4463	1214.32±8.93	38.71±0.85
Asteraceae								
Sphaeranthus indicus	30	2B+8C+20D	88.50±2.03	91.10±1.02	14.32±0.02	3508	1766.37±6.75	34.04±0.25
Myrtaceae								
Syzygium cumini	66	56C+10D	45.43±1.11	18.56±0.23	8.49±0.05	2080	381.53±2.13	43.83±0.34
Asclepiadaceae								
Tylophora tenuis	24	2A+20C+2D	38.10±1.45	15.68±0.12	5.31±0.01	1300	314.40±2.56	42.98±0.56

$2n$=Somatic chromosome number; TCL=Total chromosome length; TCV=Total chromosome volume; INV=Inter phase nuclear volume; TF%=Total form percentage.

ascertained by adding the length of all chromosomes in the karyotype and the total chromosome volume of a karyotype was calculated by applying the formula $\pi r^2 h$, where r and h represents the radius and length of the chromosome, respectively. The form % (F%) of individual chromosomes was calculated following the method of LEVAN *et al*. (1964). Total F% of karyotype was the average of F% of a karyotype. Mean values of total genomic chromosome length and total chromosome volume with standard error were calculated.

For scoring of interphase nuclear volume (INV), the root-tips of about 2-2.5mm length were fixed in 1:3 acetic:ethanol for 24h at 25°C, hydrolysed in 1N HCL at 4°C for 15min. Root-tips were put into Schiff's reagent for 1h at 20°C after though washing and kept in the dark for staining. Squash preparation was done in 45% acetic acid and the scoring was made as per our earlier method (DAS and MALLICK 1993).

For Feulgen cytophotometric estimation of nuclear DNA content, ten fixed root-tips from each species were hydrolysed in 1N HCl for 12 min at 60°C, washed in distilled water and stained in Schiff's reagent for 2h at 14°C; each root-tip squash was made in 45% acetic acid separately. Ten scorings were made from each slide and 4C DNA was estimated from metaphase chromosomes using Nikon Optiphot microspectrophotometer following the method of SHARMA and SHARMA (1980) with monochromatic light at 550nm. *In situ* DNA were obtained on the basis of optical density which were converted to picograms (pg) using VANT HOF'S (1965) 4C nuclear DNA values for *Allium cepa* cv. Deshi (67.1pg) as standard. The 1C DNA amount in Mbp was calculated from 4C DNA content in pico grams (pg) of each plant species where 1pg=980 Mpb (CAVALIER-SMITH 1985). To find out the significant differences of different cytochemical parameters among different species, if any, ANOVA test (SOKOL and ROHLF 1973) was performed. The correlation coefficient analysis between different chromosomal parameters were done to find out the relationship between different genomic characteristics.

RESULTS

Karyotype analysis

Somatic chromosome number varied from 2*n*=22 in *S. carinatus* to 2n= 86 in *H. tiliaceous* (Table 1, Figs. 1-8). The chromosome size varied within the karyogram of each species, from 0.61µm to 1.63µm in *C. manghas*; 0.90µm to 1.80µm in *H. tiliaceous*; 1.16µm to2.45µm in *T. tenuis*; 2.31µm to 3.86µm in *S. indicus*; 2.06µm to 4.63µm in *S. portulacastrum*; 0.90µm to 1.93µm in *S. cumini*; 1.41µm to 2.57µm in *S. carinatus*; 0.87 to 1.59µm in *L. racemosa*. On the basis of size and position of the primary and secondary constriction, a general description of the representative types is given below.

Type A included large sized chromosome with one primary and one secondary constriction. The relative position of the two constrictions were medium to nearly median and nearly sub- terminal respectively.

Type B comprised medium to long sized chromosome with secondary constrictions, the primary constriction was median to sub-median in position, the secondary constriction being on the long arm of the chromosome.

Type C includes medium to long size chromosomes with nearly median to median primary constriction.

Type D contained small size chromosomes with sub-median to nearly sub-median primary constriction.

The detailed description of the somatic complements and different chromosomal characteristics of 8 species revealed species-specific variation in the genomic behaviour (Table 1). Type C and D chromosomes were found common in all the studied species. Type A chromosomes was observed in *H. tiliaceous, S. portulacastrum* and *T. tenuis*; type B was found only in *S. indicus.* Total genomic chromosome length varied from 19.045µm in *T. tenuis* to 71.31µm in *S. portulacastrum.* The maximum genomic chromosome volume was 97.67µm^3 in *S. portulacastrum* and minimum was 7.84µm^3 in *T. tenuis*. The genomic chromosome volume of *H. tiliaceous, S. indicus, C. manghas, S. carinatus, L. racemosa, S. cumini* was 47.56, 45.55, 44.39, 17.52, 12.05,9.28µm^3 respectively. Analysis of TF% value showed symmetric karyotype in all the studied species except in *S. indicus* of Asteraceae having average submedian chromosomes in its karyotype. Significant interspecific variation of different cytological parameters was observed and correlation between the total length and volume of the chromosome was noted.

INV and 4C nuclear DNA content

Interphase nuclear volume (INV) differed significantly among the studied species. INV was comparatively less in *T. tenuis* (314.40µm^3) where as highest was observed in *S. indicus* (1766.37µm^3).The frequency distribution of the interphase nucleus showed a prominent peak around the mean in *S. indicus* followed by 2 oth-

er peaks around the mean in *H. tiliaceous* and in *S. portulacastrum* whereas 4 other species showed minor peaks (data not shown). The nuclear DNA amount differed significantly among the species from 5.31pg in *T. tenuis* to 27.83pg in *C. manghas.* Moderate correlation was observed between the INV and chromosome length (r=0.765) as well as chromosome volume (r=0.685) and chromosome length and volume (r=0.886). No such significant correlations were observed between 4C DNA content and other chromosomal parameters. The calculated genome size also varied from 1301 Mbp in *T. tenuis* to 6818 Mbp in *C. manghas* (Table 1). The average nuclear DNA content also significantly correlated with average chromosome volume and length.

DISCUSSION

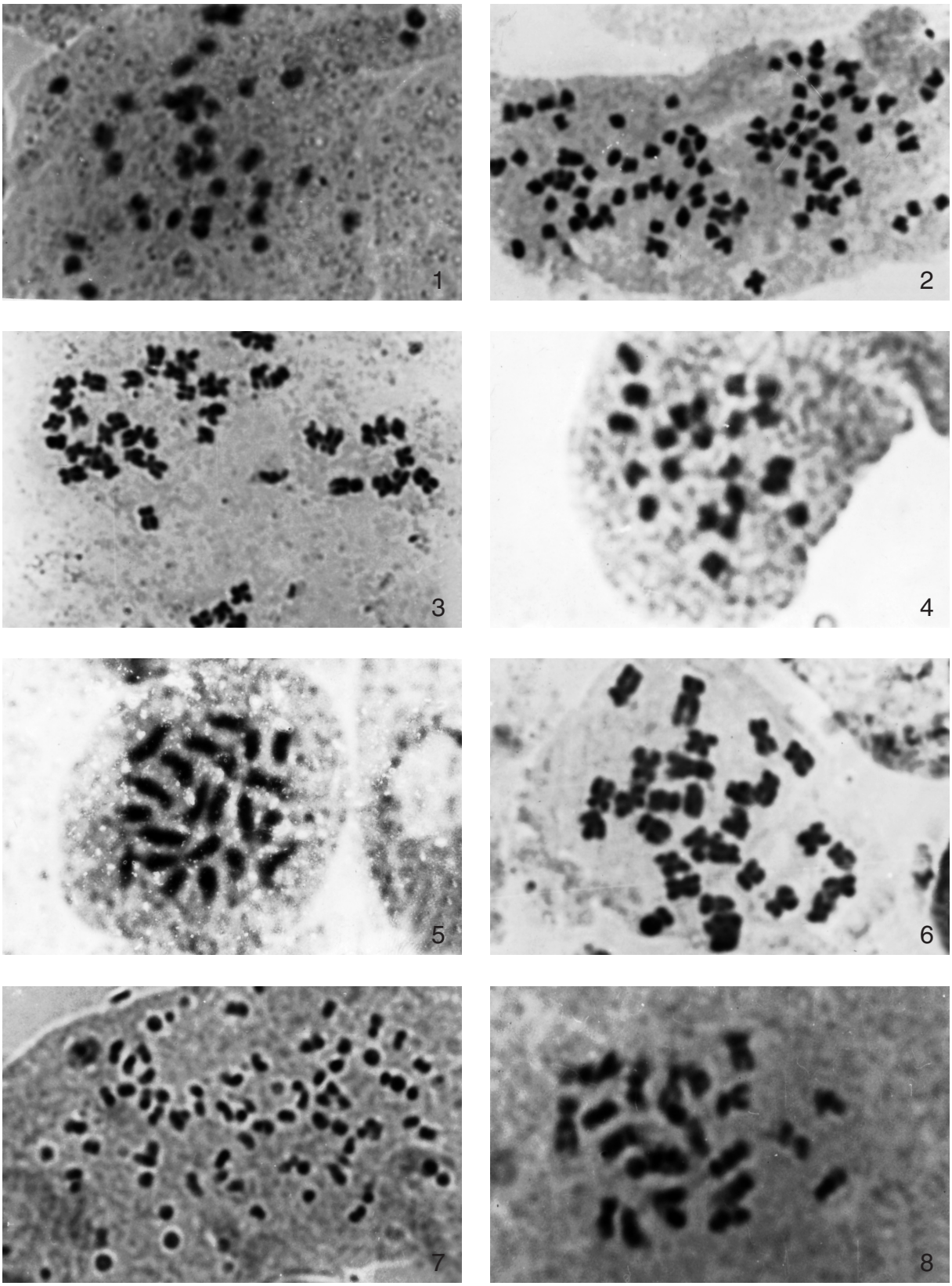
Karyotype, chromosome length, volume and TF%

A critical analysis of karyotype in 8 mangrove associates revealed numerical and structural alterations of chromosomes. The maximum average size of the chromosome was 2.97μm in *S. portulacastrum* and the minimum was 0.69μm in *S. cumini.* Chromosome number of *C. manghas* (2*n*=40), *H. tiliaceous* (2*n*=86), *L. racemosa* (2*n*=24), *S. carinatus* (2*n*=22), *S. portulacastrum* (2*n*=48), *S. indicus* (2*n*=30), *S. cumini* (2*n*=66) and *T. tenuis* (2*n*=24) was reported for the first time. Although, there were a several reports on somatic chromosome numbers in *H. tiliaceous* like 2*n*=40 (HSU 1968) and 2*n*=96 (YOUNGMAN 1927), we report 2*n*=86 for the first time. Again, in *S. cumini* we conform 2*n*=66 number of chromosome in their root tip cells as reported earlier (MEHRA and KHOSLA 1972) besides the other reported numbers like 2*n*=22 (MEHRA and KHOSLA 1972). We also report 2*n*=30 in *S. indicus* for the first time, a new cytotype in Bhitarkanika with aneuploid number, in contrast to its earlier report of 2*n*=20 (CHATTERJEE and SHARMA 1968). Type C and D chromosomes without any secondary constrictions were observed in *C. manghas*, *L. racemosa, S. carinatus, S. cumini* out of 8 species studied. Type A, C and D chromosomes were common in *H. tiliaceous, S. portulacastrum, T. tenuis*. Only *S. indicus* has the B, C and D type chromosomes. The dose variation of C and D chromosomes among the species was more prominent in *C. manghas, S. carinatus* and *T. tenuis* where most of the chromosomes are median constricted. Interestingly, *S. indicus* had mostly of sub-median constricted chromosomes in its karyotype. The TF% of around 34% to 44% in the karyotype suggest involvement of more nearly median chromosomes in the karyotype architecture in the mangrove associates belong to different families. The structural alterations in the chromosome morphology among the species might be due to duplication of chromosomes or translocation between the chromosomes with or without secondary constrictions at a very early stage of evolution.

The total chromosome length and volume in metaphase complement also differed significantly in different species. The chromosome lengths were directly proportional to their respective chromosome volume. The minimum average chromosome length (0.69μm) and volume (0.289μm^3) was observed in *S. cumini*. The total chromosome length and volume in all the three species also showed positive correlation (r=0.886). The variations in the chromosome length or chromosome volume could be due to species-specific differential condensation and spiralization of chromosomes.

Nuclear DNA amount in relation to genomic chromosome volume and INV

A detailed analysis revealed significant variations in the total chromosome volume per chromosome that was 0.28μm^3 in *C. cumini* (2*n*=66), 0.65μm^3 in *T. tenuis* (2*n*=24), 1.01μm^3 in *L. racemosa* (2*n*=24), 1.11μm^3 in *H. tiliaceous* (2*n*=86), 1.59μm^3 in *S. carinatus* (2*n*=22), 2.22μm^3 in *C.* manghas (2*n*=40), 3.04μm^3 in *S. indicus* (2*n*=30) and 4.07μm^3 in *S. portulacastrum* (2*n*=48). Interphase nuclear volume showed significant correlation with chromosome length (0.760), chromosome volume (0.690) where as no such correlation was obtained with 4C DNA content (0.460). These characters were independent and differential interaction of genomic characteristics lead to the variation in DNA content. Critical investigation on 4C DNA amount showed significant variations among the species. The maximum 4C DNA amount (27.83pg) in *C. manghas* and the minimum (5.31pg) were noted in *T. tenuis*. Average DNA amount per chromosome also varied markedly; the chromosome volume have no any significant correlation with 4C DNA amount (r=0.430). The genome size (1C DNA in Mbp) also varied significantly from 1301 Mbp in *T. tenuis* to 6818Mbp in *C. manghas.* The variation of DNA amount as well as genome length in all



Figs. 1-8 – Somatic metaphase plates of different species of mangroves (×3216). Fig. 1 – *Cerbera manghas* (2*n*=40). Fig. 2 – *Hibiscus tiliaceous* (2*n*=86). Fig. 3 – *Lumnitzera racemosa* (2*n*=24). Fig. 4 – *Sarcolobus carinatus* (2*n*=22). Fig. 5 – *Sesuvium portulacastrum* (2*n*=48). Fig. 6 – *Sphaeranthus indicus* (2*n*=30). Fig. 7 – *Syzygium cumini* (2*n*=66). Fig. 8 – *Tylophora tenuis* (2*n*=24).

the species was due to varying number of chromosomes as well as differential amount of repetitive DNA sequences in the genome. Though the DNA values in these mangrove associates, were reported for the first time, such type of variations were noticed in several other species (PRICE 1976). The variability of DNA amount might be attributed to the loss or addition of high repeats in micro- and macro-environment during evolution of new species (PRICE 1976) for stable structural gene function during macro-evolution for maintaining genetic heritability.

Acknowledgements – The authors are grateful to Department of Environment and Forests, Government of India for financial assistance [F. No. 3-7/2000-CS(M)], for preparation of chromosome Atlas of Indian mangroves and Director, Regional Plant Resource Centre for other facilities.

REFERENCES

BANARJEE L.K. and RAO T.A., 1990 – *Mangroves of Orissa coast and their ecology*. Bishen Singh Mahenda Pal Singh, pp. 1-5. Derha Dun, India.

BENNETT M.D., BHANDAL P. and LEITCH I. J., 2000 – *Nuclear DNA amounts in angiosperms and their modern uses-807 new estimates*. Annals of Botany, 86: 859-905.

CAVALIER-SMITH T., 1985 –*The evolution of genome size*. John Wiley and Sons, Chichester.

CHAPMAN V.J., 1976 – *Mangroves biogeography*. In: V.J. Chapman (Ed.), "Wet Coastal ecosystem", pp. 1-29. Elesvier Scientific publishing Company, Amsterdam.

CHATTERJEE T. and SHARMA A.K., 1968 – *Cytological studies on different genera of three tribes of Compositae*. Bull. Bot. Soc. Bengal, 22: 101-104.

DAS A.B., BASAK U.C. and DAS P., 1996 – *Karyotype analysis and 4C nuclear DNA estimation in three species of Acanthus, a mangrove associate from coastal Orissa.* Cytobios, 87: 151-159.

DAS A.B. and MALLICK R., 1993 – *Karyotype diversity and interspecific 4C DNA variation in Bupleurum.* Biologia Plantarum, 35: 355-363.

HSU C.-C.,1968 – *Preliminary chromosome studies on the vascular plants of Taiwan (II)*. Taiwania, 14: 11-27.

JENA S., SAHOO P., MOHANTY S., DAS A.B. and DAS P., 2002 – *Karyotype variation and cytophotometric estimation of in situ DNA content in some minor and associate mangroves of India.* Cytologia, 67: 15-24.

LEVAN A., FREDYA K. and SANDBERG A., 1964 – *Nomenclature for centromeric position on chromosome.* Heridity, 52: 201-220.

MEHRA P. N. and KHOSLA P. K., 1972 – *Cytogenetical studies of east Himalayan Hamamelidaceae, Combretaceae and Myrtaceaea.* Silvae Genet., 21: 186-190.

MEPHAM R. and MEPHAM J.S., 1984 – *The Flora of Tidal Forests- a rationalization of the use of term 'mangrove'.* S. African J. Bot., 51: 77-79.

PRICE H. J., 1976 – *Evolution of DNA contents in plants.* Bot. Rev., 42: 27-52.

RAGHAVAN T.S., SRINIVASN V.K., 1940 – *Studies in Indian Aizoaceae.* Annal. Bot., 4: 651-661.

RAINA S.N., 1990 – *Genome organization and evolution in the genus Vicia*. In: Kawano S (Ed.), "Biological Approaches and evolutionary trends in plants", pp. 183-201. Academic Press, London.

SHARMA A.K., BHATTACHARYA N.K., 1956 – *Cytogenetics of some members of Portulacaceae and related families.* Caryologia, 8: 257-274.

SHARMA A.K. and SHARMA A. 1980 –*Chromosome techniques: theory and practice.* 3rd Edition, Butterworths, London.

SOKAL P.R. and ROHLF F.J., 1973 – *Introduction to biostatistics*. Freeman, San Francisco.

TOMLINSON P.B., 1986 – *The Botany of Mangroves*. Cambridge University Press, Cambridge.

VANT HOF J., 1965 – *Relationships between mitotic cycle duration, S period duration and average rate of DNA synthesis in the root meristem cells of several plants.* Expl. Cell. Res., 39: 48.

YOUNGMAN W., 1927 – *Studies in the cytology of the Hibisceae.* Ann. Bot., 41: 755-778.

Received January 15, 2003; accepted April 30, 2003