KARYOTYPE VARIATION AND GENOMIC CHARACTERIZATION IN FIVE MONOCOTYLEDONOUS MANGROVE ASSOCIATE FROM ORISSA COAST

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Chromosome number and karyotype analysis of five monocot mangrove associates from Dhamra and Paradeep coast of Orissa revealed 2n=48 in *Porteresia coarctata*; 2n=22 in *Crinum defixum* and *Cryptocoryne ciliata* and 2n=20 in *Asparagus racemosus* var. *javanica* and *Cyperus cephalotes*. Total chromosome length varied from 42.44μm in *A. racemosus* var. *javanica* to 93.86μm in *C. defixum*. Significant variation of chromosome volumes were also recorded among the species. The 4C DNA content varied significantly from 47.15pg in *C. defix*um to 6.31pg in *C. cephalotes*. A positive significant correlation between the chromosome volume and 4C nuclear DNA content among five different species was revealed. The genome size was around 1,545mbp in *C. cephalotes* to 4,074mbp in *P. coarctata* with a highest size (11,551mbp) in *C. defixum* that suggests the high repetitive DNA sequences in the *Crinum* with highest chromosome length and volume of this species

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Key words. genome size, karyotype, somatic chromosome, *Crinum, Cryptocoryne, Asparagus, Cyperus, Porteresia.*

گوناگونی کاریوتیپ[1] و ویژگی ژنوم در پنج مانگرو تک‌لپه از ساحل اوریسا، هند

اس ینا و آنات باندهو داس

شمارش کروموزومی و تجزیه کاریوتیپ تعداد پنج مانگرو تک‌لپه از دارما و ساحل پارادیپ در اوریسا (هندوستان) نشان داد که در گونه *Porteresia coarctata* ۲n=۴۸، در گونه‌های *Crinum defixum* و *Cryptocaryne ciliata* ۲n=۲۲، در گونه‌های *Asparagus racemosus* var. *javanica* و *Cyperus cephalotes* ۲n=۲۰ می‌باشد. طول کل کروموزومی از ۴۲/۴۴ در گونه *Asparagus racemosus* var. *javanica* تا ۹۳/۸۶ در گونه *Crinum defixum* تغییر می‌کند. حجم کروموزومی در گونه‌های مختلف تغییرات زیادی را نشان می‌دهد.

محتوای 4C DNA از ۴۷/۱۵ pg در گونه *Crinum defixum* تا ۶/۳۱ pg در گونه *Cyperus cephalotes* تغییر می‌یابد. همبستگی معنی‌دار مثبتی بین حجم کروموزومی و محتوی 4C DNA هسته در بین پنج گونه مشاهده گردید. اندازه ژنوم در حدود ۱۵۴۵ mbp در گونه *Cyperus cephalotes* تا ۴۰۷۴ mbp در گونه *Porteresia coarctata* است و حداکثر آن ۱۱۵۵۱ mbp در گونه *Crinum defixum* می‌باشد. این موضوع بیانگر توالی DNA بالا با بیشترین طول و حجم کروموزومی در جنس *Crinum* است.

Introduction

Mangroves form extensive, unique communities in tropical coastal areas and tidal low lands, covering 60-75% of tropical shorelines. Mangrove species under monocots is a type of low-lying coastal vegetation, their adoption along with other mangroves is very much interesting. Tidal rivers and sea water inundate the flat low land and lagoons, which are the habitats of monocotyledonous halophytes. Jagatap & al. (1993) have reported that the Orissa coasts have the highest mangrove diversity than any other mangals of the Indian sub-continent. The monocot mangroves includes families like *Asparagaceae, Arecaceae, Araceae, Amaryllidaceae, Cyperaceae, Pandanaceae, Poaceae*. In true sense, the monocot mangroves flora play very dominant and important role in the estuarine mouths, sea-land interphase. *Crinum defixum* of *Amaryllidaceae*, *Cryptocornye ciliata* of *Arecaceae* and *Cyperus cephalotes* of *Cyperaceae* are found on the river bank or near cricks which is far away from sea mouth. *Porteresia coarctata* a member of *Poaceae* is always found in the high saline zone in contrast to *Asparagus racemosus* var *javanica* of *Asparagaceae* which is found only on the upland of the mangrove areas containing less salinity

Although, the Indian sub-continent harbours a fairly high proportion of World's mangrove plant gene pool, no adequate attempt has been made in depth for assessments of genetic diversity in the existing gene pool. Comparative studies on chromosome numbers are the basic tenets in establishing evolutionary relationships in any taxonomic groups, therefore, various facets of chromosomal research are gaining importance for the analysis of genomes in different taxa including mangroves. The chromosome number is considered as the raw material on which evolutionary forces have been acting, leading to origin and evolution of the entire biological diversity (Grant 1971). The detailed karyotype study of monocot mangroves is not well known. Besides these, nuclear DNA amount (C-value) and genome size are important biodiversity characters of fundamental significance and with many uses (Bennett & al. 2000). It was reported earlier from our group that the availability of C-value data varied widely between true and associates mangroves (Das et al. 1996, 2001, Jena et al. 2002). It is important that DNA C-value has many diverse nucleotypic consequences (Bennett et al. 2000) and genomic obesity that increase unless or until selection acts on one or more of these (Bennett et al. 1998). Data on nuclear DNA content were used to establish interrelationship among the genomic constituents in some mangrove taxa at intergeneric and family level (Das et al. 1996, 2001; Jena et al. 2002).

The somatic chromosome number reported for *C. defixum* range from 2n=19 to 60 (Tandon and Mathur 1966, Raina 1978). Similarly, the somatic chromosome number of 2n=22 and 2n=33 was reported in *C. ciliata* (Sarkar and Chatterjee 1979). In *P. coarctata*, Krishnaswamy and Chandrasekharan (1957) reported 2n=48 chromosome while in *C. cephalotes* 2n=42 (Rath and Patnaik 1978) was reported long before. A varied number of polyploid series of chromosomes was reported in *A. racemosus* var. *javanica* with 2n=20 (Sharma and Bhattacharya 1957, Sheidai and Inamdar 1997) besides its 2n=30 (Thombre 1959) chromosomes in roots. There are no reports, so far, on nuclear estimation in monocot mangrove associates. The present investigation has been made on five monocot mangrove associates, found in Dhamra and Paradeep coast, Orissa for the first time in order to present the somatic chromosomes number and karyotype details, interphase nuclear volume (INV) and estimation of 4C

DNA content of these species as a part of the ongoing database preparation on Indian mangroves. Such data may be used in planning of conservation strategies of endangered mangroves and their regeneration programme.

Materials and methods

Young plantlets of five monocotyledonous mangrove associates namely, *Asparagus racemosus* Willd. var. *javanica* Baker, *Crinum defixum* Ker-Gawl., *Cryptocoryne ciliata* (Roxb.) Fisch. ex Wydler, *Cyperus cephalotes* Vahl., *Porteresia coarctata* (Roxb.) Takeoka were collected from the Dhamra and Paradeep coast, Orissa, India and planted in the mangrove nursery of Regional Plant Resource Centre, Bhubaneswar. The voucher specimens were identified and kept in the herbaria of the Centre. Young healthy root-tips were pretreated in half saturated paradichloro-benzene and aesculine mixture for 4h at 18°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; well scattered ten metaphase plates were selected for karyotype analysis. The total chromosome length was determined following the method of Das and Mallick (1993a) The form percentage (F%) of individual chromosomes was calculated following the method of Levan & al. (1964). Total form % (TF%) 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. After a through washing the root-tips were put into Schiff's reagent for 1h at 20°C and kept in the dark for staining. Squash preparation was done in 45% acetic acid. Scoring was made as per our earlier method (Das and Mallick, 1993b).

For Feulgen cytophotometric estimation of 4C DNA, 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 scoring 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 Van't Hof's (1965) 4C nuclear DNA values for *Allium cepa* cv Deshi (67.1pg) as a standard. To find out the significant differences in the 4C DNA content among different species, if any, as well as other chromosomal and nuclear parameters, ANOVA test (Sokal and Rohlf, 1973) was performed. The correlation coefficient analysis between different chromosomal parameters were done to find out the relationship between different genomic characteristics.

Karyotype analysis

Karyotype analysis made in five monocot mangrove associates revealed 2n=20 in *A. racemo*sus var. *javanica* and *C. cephalotes*, 2n=22 in *C. defixum* and *C. ciliata* as well as 2n=48 in *P. coarctata* (Tables 1, Figs.1-5). On the basis of size and position of the primary and secondary constriction four types of chromosome (Type A, B, C, D) were present among the species. Only Type C (median constricted) and D (sub-median constricted) were present in *C. cephalotes* ; A, C, D types were observed in *P. coarctata*; B, C, D type were present in *C. ciliata* and all the four types of chromosome were present in *C.*

Table 1. Chromosome number, chromosome size, genome size of different species of monocot mangroves of coastal Orissa.

Species \ Family	Somatic chromosome number(2n)	Karyotype Formulae	Total chromosome length (µm±SE)	Total chromosome volume(µm^3±SE)	4C DNA content (pg±SE)	TF% (±SE)	INV (µm3±SE)
Amaryllidaceae							
Crinum defixum	22	2A+4B+10C+6D	93 86±1.66	335 39±2.16	47.15±1.12	35.32±0.45	4391 15±2.65
Araceae							
Cryptocoryne ciliata	22	2B+12C+8D	82 96±1.76	121.31±1.88	10 35±0.20	30 01±0.62	403.83±3.12
Cyperaceae							
Cyperus cephalotes	20	18C+2D	45 50±0.98	91.46±0.99	6 31±0.08	42.64±0.37	132.77±2.45
Asparagaceae							
Asparagus racemosus **var.** *javanica*	20	2A+4B+10C+4D	42.44±1.22	141.42±1.27	12.63±0 15	38.65±0.28	367 50±3.40
Poaceae							
Porteresia coarctata	48	4A+28C+16D	91 64±2.10	74 86±0.65	16 63±0 46	38.97±0 41	507 82±4 60

defixum and *A. racemosus* var. *javanica.* A general description of the chromosome types is given below (Fig. 6).

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

Type B comprised medium to long sized chromosome with sub-median primary constriction and terminal secondary constriction 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.

Total genomic chromosome length varied from 21.22µm in *A. racemosus* var. *javanica* to 46.93µm in *C. defixum.* The maximum genomic chromosome volume was 167.695µm^3 in *C. defixum*, followed by 70.71µm^3 in *A. racemosus* var. *javanica,* 60.65µm^3 in *C. ciliata*, 45.73µm^3 in *C. cephalotes* and 37.43µm^3 in *P. coarctata.* The total form percentage (TF%) in these species varied from 30.01% in *C. ciliata* to 42.64% in *C. cephalotes*. Analysis of TF% showed sub-median chromosomes of *C. ciliata* of *Araceae* and *C. defixum* of *Amaryllidaceae*; median chromosomes were more in other three species.

INV and 4C nuclear DNA content

Interphase nuclear volume (INV) differed significantly in the 5 studied species. The INV was comparatively less in *C. cephalotes* (132.77µm^3) in contrast to more in *C. defixum* (4391.15µm3). The frequency distribution of the interphase nucleus showed a prominent peak around the mean all the studied species except *C. defixum* which showed a minor peak beside the major peak. The nuclear DNA

Table 2. Cytological parameters of 5 different species of monocot mangroves.

Species/Family	2n	GCL	GCV	ACL	ACV	ADNA	Genome size
		(µm±SE)	(μm^3±SE)	(µm±SE)	(μm^3±SE)	(pg. ±SE)	(Mbp)
Amaryllidaceae							
Crinum defixum	22	46.93	167.69	4.266	15.245	2.143	11551
Araceae							
Cryptocoryne ciliata	22	41.48	60.65	3.770	5.514	0.470	2535
Cyperaceae							
Cyperus cephalotes	20	22.75	45.73	2.275	4.573	0.315	1545
Asparagaceae							
Asparagus racemosus **var. *javanica***	20	21.22	70.71	2.122	7.071	0.631	3094
Poaceae							
Porteresia coarctata	48	45.82	37.43	1.909	1.559	0.346	4074

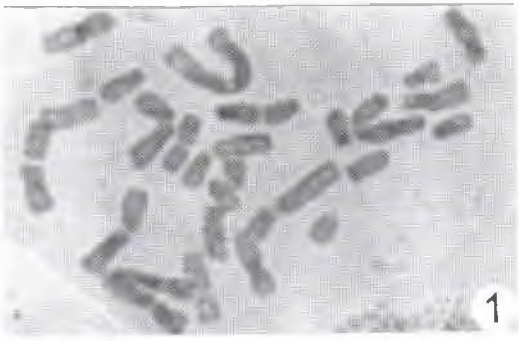
GCL=Genomic chromosome length, GCV=genomic chromosome volume, ACL=average chromosome length, ACV=Average chromosome volume, ADNA= Average DNA per chromosome

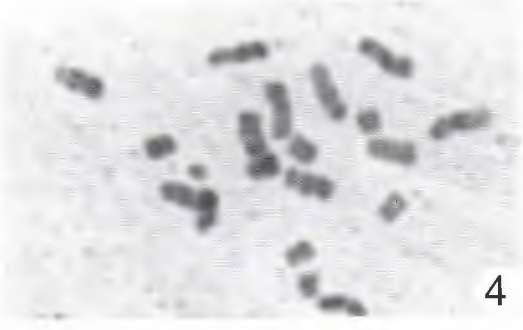
amount differed significantly among the species from 6.31pg in *C. cephalotes* to 47.15pg in *C. defixum.* The calculated genome size also varied from 11,551 Mbp in *C. defixum* to 1545 Mbp in *C. cephalotes* (Table 2). The average nuclear DNA content also significantly correlated with average chromosome volume and length.

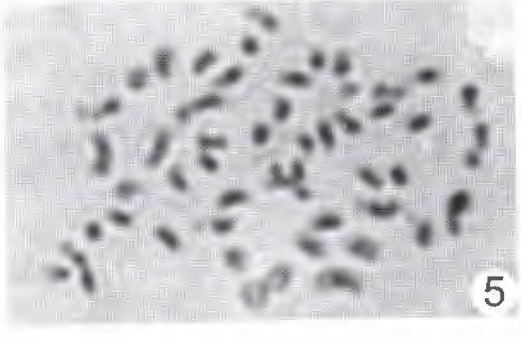
Discussion

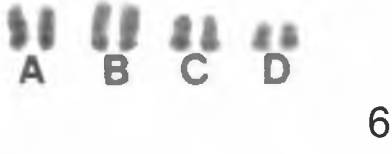
Karyotype, chromosome length, volume and TF%

A critical analysis of karyotype in 5 monocot mangrove associates revealed numerical and structural alterations of chromosomes. The maximum average size of the chromosome was observed in *C. defixum* (4.2µm) and the minimum found in *P. coarctata* (1.9µm). Although, there were a several reports on somatic chromosome numbers in *C. defixum* like 2n=19,20,20+B,21,22,24,30,50,60, (Subramanian 1979) 2n=22 (Tandon and Mathur 1966, Raina 1978) we confirm the earlier report of 2n=22 chromosomes. Again, in *P. coarctata* we confirm the chromosome count 2n=48 which matches with earlier report of Krishnaswamy and Chandrasekharan (1957). Although the somatic chromosome number 2n=22 in *C. ciliata* is in accordance with the previous report still it differed from 2n=33 as reported by Jacobsen (1977). In *A. racemosus* var. *javanica* the somatic chromosome varied as 2n=20 2n=30 and 2n=40, that was reported earlier we found only the diploid form 2n=20 again for that same taxon. *A. racemosus* var. *javanica* and *C. defixum* have type A, B C and D chromosome while *C. cephalotes* have type C and D chromosome. Type A, C, D chromosomes were found in *P. coarctata* whereas type B, C, D, were noted in *C. ciliata* . Only type C and D chromosomes without any secondary constrictions were observed in *C. cephalotes*









Figs. 1-6. Somatic metaphase plates of different species of mangroves (×3216). -1.*Crinum defixum* (2n=22). -2. *Cryptocoryne ciliata* (2n=22). -3. *Porteresia coarctata* (2n=48). -4.*Cyperus cephalotes* (2n=20). -5. *Asparagus racemosus* var. *javanica* (2n=20). -6. Type chromosomes present in different species of monocot mangroves.

out of 5 studied monocot species. The dose variation of C and D chromosomes among the species was more prominent in *P. coarctata* and *C. cephalotes* where most of the chromosomes are median constricted. Interestingly, *C. defixum* and *A. racemosus* var. *javanica* had mostly the same type of chromosomes in its karyotype besides two additional type D chromosomes in *C. defixum*. The TF% of around 35% to 42% in the karyotype which got reflected in their respective karyotypes suggest involvement of more nearly median chromosomes in the karyotype architecture except 30% TF% in *C. ciliata*. All above facts suggest a homogenous chromosome morphology in all studied species although they belong to different families. 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 (1.90μm) and volume (1.55μm^3) was observed in *P. coarctata*. 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 chromosome volume per chromosome that was 15.25μm^3 in *C. defixum* (2n=22), 7.07μm^3 in *A. racemosus* var. *javanica* (2n=20), 5.51μm^3 in *C. ciliata* (2n=22), 4.57μm^3 in *C. cephalotes* (2n=20) and 1.55μm^3 in *P. coarctata* (2n=48). Interphase nuclear volume showed significant correlation with 4C DNA content (r=0.934) and INV (r=0.966) where as no such correlation was obtained with chromosome length (r=0.966). These characters were independent and differential interaction of genomic characteristics lead to the variation in DNA content (Yamaguchi and Tsunado 1969). Critical investigation on 4C DNA amount showed significant variations among the species (Table 2). The maximum 4C DNA amount (47.15pg) in *C. defixum* and the minimum (6.31pg) were noted in *C. cephalotes*. Average DNA amount per chromosome also varied markedly (Table 2). The chromosome volume, however, did not show any significant correlation with chromosome length (r=0.381). The genome size (1C DNA in Mbp) also varied significantly from 11551 Mbp in *C. defixum* to 1545Mbp in *C. cephalotes*. The genome size among the studied monocot mangrove associates showed moderately low i. e 1545Mbp to 4074Mbp where as maximum length was obtained in *C. defixum*. The variation of DNA amount as well as genome length in all 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 minor and associate mangroves, were reported for the first time, such type of variations were noticed in several other species (Price 1976).

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References

Bennet M. D., Bhandal P. & Leitch, I. J. 2000: Nuclear DNA amounts in angiosperms and their modern uses-807 new estimates. - Annals of Botany. 86: 859-905.

Bennet M. D., Leitch I. J. & Hanson, L. 1998: DNA amounts in two samples of angiospermids. -Anals of Bot. 82 (Supplement A): 121-134.

Das A. B. & Mallick R, 1993a: Nuclear DNA and chromosome changes within the tribe Ammineae. -Cytobios 74: 197-207.

Das A. B. & Mallick R. 1993b: Karyotype diversity and interspecific 4C DNA variation in Bupleurum. -Biologia Plantarum, 35(3): 355-363.

Das A. B., Basak U. C. & 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., Mukherjee A. K. & Das P. 2001: Molecular phylogeny of Heritiera Aiton (Streculiaceae), a tree mangrove: variations in RAPD markers and nuclear DNA content. -Botanical Journal of the Linnean Society . -136(2):221-229.

Grant V. 1971: Plant Speciation. Columbia University Press, New York.

Jacobsen N. 1977: Chromosome number & taxonomy in Cryptocornye (Araceae). -Bot. Not. 130:71-87

Jagtap G., Vishwas S. C. & Arvind G., Untawale, 1993: Mangrove Ecosystem of India: A need for protection (Synopsis). -AMBIO 22: 252-254.

Jena S., Sahoo P., Mohanty S., Das A. B. & Das P. 2002: Karyotype variation and cytophotometric estimation of in situ DNA content in some minor and associate mangroves of India. -Cytologia 67(1):15-24.

Levan A., Fredya K. & Sandberg A. 1964: Nomenclature for centromeric position on chromosome. -Heridity 52: 201-220.

Krishnaswamy N. & Chandrasekharan P. 1957: Note on naturally occurring tetraploid species of Oryza. -Sci. cult.23:308-310.

Price H. J. 1976: Evolution of DNA contents in plants. -Bot. Rev. 42:27-52.

Raina S. N. 1978: Genetic mechanisms underlying evolution in Crinum. -Cytologia 43:575-580.

Rath S. P. & Patnaik S.N. 1978: Cytological studies in Cyperaceae with special reference to its taxonomy II. -Cytologia 43: 643-653.

Sarkar A. K. & Chatterjee U.1979: Chromosome studies in Cryptocornye (Araceae). – Caryologia 32: 1-4.

Sharma A. K. & Bhattacharya B. 1957: Cytology of 6 species of Asparagus & Lilium. -Phyton 8: 1-12.

Sharma A. K. & Sharma A. 1980: Chromosome techniques: theory and practice. 3rd edition. -Butterworths, London.

Sheidai M. & Inamdar A. C. 1997: Cytomorphology of Asparagus taxa using miltivariate statistical analysis. –Nucleus 40: 7-12.

Sokal P. R. & Rohlf F. J. 1973: Introduction to biostatistics. -Freeman, San Francisco.

Subramanian D. 1979: Cytopolymorphism in Crinum defixum Ker. -Sci. Cult. 45: 110-112

Tandon S. L. & Mathur M. 1966: Cytology of Crinum defixum (Chromosome number, 2n=22, diploid). -Curr. Sci. 35: 156-157.

Thombre M. V. 1959: Chromosome numbers in some common flowering plants. -Curr. Sci. 28: 206-207.

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.

Yamaguchi Y. & Tsunado S. 1969: Nuclear volume, nuclear DNA content and radiosensitivity in Brassica and allied genera. -Jap. J. Breed. 19: 35.