Karyotype Variation and Cytophotometric Estimation of *In Situ* DNA Content in Some Minor and Associate Mangroves of India

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Summary Detailed karyotype analysis and cytophotometric estimation of *in situ* 4C nuclear DNA amount as well as interphase nuclear volume (INV) were carried out in *Aegialitis rotundifolia*, *Suaeda nudiflora* and *Thespesia populnea* found in the mangrove forests of Orissa, India. Somatic chromosome number $2n=26$, 34 and 54 were noted in *T. populnea*, *A. rotundifolia*, *S. nudiflora* respectively. Karyotype analysis revealed numerical variations and minute structural alterations of somatic chromosomes in different species. Critical analysis of nuclear DNA also showed significant variations in the 4C DNA content. *S. nudiflora*, a mangrove associate, showed variations at the ploidy level in root meristematic cells suggesting existence of cytotypes in the natural population. Significant correlation was found between chromosome length, volume and 4C DNA content.

Key words *Aegialitis*, Karyotype, Nuclear DNA, *Suaeda*, *Thespesia*.

Mangroves, an assortment of tropical and subtropical trees and shrubs adapted to the marine swampy habitats, represent an unique and dynamic ecosystem of coastal region. 'True', or 'exclusive' or 'major' mangroves occur only in such habitats, or rarely elsewhere. In addition, a loosely defined group of species are often described as 'minor mangroves' and 'mangrove associates', or 'non-exclusive' mangrove species. Out of 20 species belonging to 11 minor mangrove genera and 60 species in 46 genera of mangrove associates in world, 9 species in 6 genera of minor mangroves and about 20 species under 14 genera of mangrove associates are reported from India (Tomlinson 1986, Newbold 1993). In India, mangroves occupy about 6740 km² and about 75 species of mangroves and mangrove associates belonging to 23 families are reported. The diversity of mangroves and the mangrove associates is reported to be high in Bhitarkanika and Mahanadi delta of Orissa (Newbold 1993). The genetic differences among individuals are important measures of the diversity in a population. Assessment of the genetic differences between populations of a species gives a measure of the extent to which such populations are genetically related with each other, while genetic comparison of different species can give information about the extent of divergence and, potentially, when such divergence took place. The geographical isolation of the same species with more frequent self-pollination may cause higher mutation rates, and one intriguing possibility is that an increased mutation rate evolved may up set the low genetic variation resulting from inbreeding (Klekowski *et al.* 1994). In India, mangroves are threatened largely due to human interference resulting in the changes in the tidal pattern and salinity gradient, encroachment of land for cultivation and shrimp culture, grazing of cattle, exploitation for fuel, timber, fodder, and various other purposes. The determination of genetic variability in the minor mangroves and their associates at inter-specific as well as ecotype level may be of great interest to establish the phylogenetic relationships among the species in different mangrove habitats.

Aegialitis rotundifolia Roxb. a minor mangrove of the family Plumbaginaceae is found in the

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high saline soils where as *Suaeda nudiflora* (Willd.) Moq. of Chinopodiaceae and *Thespesia populnea* (L.) Sol. ex Correa of Malvaceae survives in soils with less salinity. Once widely distributed in the tropical and subtropical coastal swamp forests of India, Myanmar and Sri Lanka (Kostermans 1959), *Aegialitis* is now confined to a few pockets on the seashore and along the estuaries in Bhitarkanika mangrove forests of Orissa and becoming endangered (Tomlinson 1986) due to biotic as well as abiotic interferences (Das *et al.* 1999).

Facets of chromosomal research are gaining importance in the analysis of genomes in different taxa. The chromosomal studies concerning the Indian mangroves were reported for the members of the Sterculiaceae, Rhizophoraceae, Acanthaceae and Avicenniaceae (Raghavan and Arora 1958, Sidhu 1968, Das *et al.* 1994, 1995, 1996). The somatic chromosomes in most of the mangrove species are very tiny; karyotype analysis and karyogram preparation may be useful for precise structural details of the genomes. No detailed karyotype reports are available in *A. rotundifolia*, *S. nudiflora* and *T. populnea* except on chromosome number report $2n=24$, 26 and 52 in *T. populnea* time to time (Toungman 1927, Ford 1938, Hazra and Sharma 1971, Dasgupta and Bhatt 1976, Das *et al.* 1999). Comparisons of nuclear DNA amounts provided useful data in many cytotaxonomic and evolutionary studies (Price 1976, Ohri and Khoshoo 1986, Raina 1990). Data on nuclear DNA content were used to establish interrelationships between the genomic constituents in some mangrove taxa at interspecific level (Das *et al.* 1999). Studies on the estimation of nuclear DNA content were employed to establish interrelationships between the genomic constituents in different taxa at interspecific level (Nagato *et al.* 1981, Das and Mallick 1993a). The present investigation deals with the determination of chromosome number, karyotype analysis with detailed idiogram, interphase nuclear volume and estimation of 4C DNA content in *A. rotundifolia*, *S. nudiflora* and *T. populnea*.

Materials and methods

Seeds of *S. nudiflora* and *T. populnea* as well as propagules of *A. rotundifolia* were collected from the mangrove forests of Bhitarkanika, Orissa, India. The voucher specimens were identified and kept in the herbaria of the Regional Plant Resource Centre. Propagules were grown in the experimental nursery of the Mangrove Research Centre of the Regional Plant Resource Centre, Bhubaneswar.

Young healthy root tips were pretreated in saturated para-dichlorobenzene and aesculine mixture for 3.5 h at 14°C followed by overnight fixation in 1 : 3 propionic ethanol. Chromosome staining was made in 2% propionic orcein after cold hydrolysis in 5 N HCl for 7 min. Root tips were squashed in 45% propionic acid. Well scattered 10 metaphase plates were selected for karyotype analysis of each species. Total chromosome length was measured by adding the length of all chromosomes in the karyotype, and total chromosome volume of a karyotype was calculated applying the formula $\pi r^2 h$, where r and h represented radius and length of the chromosome respectively. Form percentage (F%) of individual chromosome was calculated following the method of Levan *et al.* (1964). Total form percentage (TF%) of a 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 2–2.5 mm, length were fixed in 1 : 3 acetic ethanol for 24 h at 25°C, hydrolysed in 1 N HCl at 4°C for 15 min. After a thorough washing, the root tips were put into Schiff's reagent for 1 h 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, 10 fixed root tips from each species were hydrolysed in 1 N HCl for 12 min at 60°C, washed in distilled water and stained in Schiff's reagent

for 2 h 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 microdensitometer following the method of Sharma and Sharma (1980) with monochromatic light at 550 nm. *In situ* DNA values 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 (67.1 pg) as standard. To find out the significant differences in the 4C DNA content among different species, if any, analytical variation (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.

Results

Chromosome characteristics

Detailed analysis of somatic chromosome of 3 species of mangroves belonging to the family Chenopodiaceae, Malvaceae and Plumbaginaceae showed variations in the chromosome number, size and morphology. The chromosome number was $2n=26$ in *T. populnea*, $2n=34$ in *A. rotundifolia* and $2n=54$ in *S. nudiflora*. In all the studied species, chromosome length varied from 2.06 μm to 6.70 μm . The chromosome size varied within the karyogram of each species from 2.06 μm to 6.70 μm in *A. rotundifolia*; 3.00 μm to 5.15 μm in *S. nudiflora* and 2.56 μm to 4.63 μm in *T. populnea*. In all cases the karyotypes were symmetrical. On the basis of their size and position of the primary and secondary constrictions, a number of common types of chromosome were observed in the 3 species though they differed from one another in the minute details of the karyotype (Tables 1–4). A general description of the representative types is given below (Fig. 1).

Type A had large sized chromosomes with one primary and one secondary constriction. The relative position of the 2 constrictions were median to nearly median and nearly sub-terminal respectively.

Type B chromosomes were medium to long in size 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 chromosomes were medium to long in size with nearly median to median primary constriction.

Type D had medium to long chromosomes with nearly sub-median to sub-median primary constriction.

A detailed analysis of the somatic complements and different chromosomal characteristics of

Table 1. Detailed karyotype analysis in *Aegialitis rotundifolia* ($2n=34$), a minor mangrove

Chromosome type	Number of chromosome	Whole length of chromosomes (μm)	Length of short arm (μm)	Form percentage F%
B	2	9.26	4.12	44.49
			2.06	22.24
C	6	21.63	9.24	42.71
C	4	12.36	6.16	49.83
C	2	5.14	2.06	40.07
C	2	4.12	2.06	50.00
D	2	13.39	3.08	23.00
D	2	11.32	3.08	27.20
D	4	20.60	6.16	29.90
D	6	24.72	6.18	25.00
D	4	12.36	4.12	33.33

Table 2. Detailed karyotype analysis in *Suaeda nudiflora* ($2n=54$), a mangrove associate

Chromosome type	Number of chromosome	Whole length of chromosomes (μm)	Length of short arm (μm)	Form percentage F%
A	2	10.30	5.14	49.90
			1.82	17.66
A	2	9.26	4.12	44.49
			1.02	11.01
A	2	8.24	3.08	37.37
			1.02	12.37
B	2	10.30	4.12	40.00
			1.02	19.02
C	2	11.30	5.14	45.48
C	4	20.60	10.28	49.90
C	4	20.60	8.24	40.00
C	4	18.50	8.24	44.45
C	8	32.96	12.32	37.37
C	6	21.60	8.58	39.72
D	2	10.30	3.08	29.90
D	4	18.52	6.28	33.90
D	8	24.00	8.24	34.33
D	4	16.48	4.12	25.00

Table 3. Detailed karyotype analysis in *Thespesia populnea* ($2n=26$), a mangrove associate

Chromosome type	Number of chromosome	Whole length of chromosomes (μm)	Length of short arm (μm)	Form percentage F%
B	2	9.26	4.12	44.49
			3.08	33.26
B	2	8.24	4.12	50.00
			2.56	31.06
C	2	10.30	4.08	39.61
C	2	9.26	4.08	44.06
C	2	8.24	4.08	49.51
C	2	8.24	3.08	37.37
C	2	7.20	3.08	42.77
C	2	5.18	2.06	39.76
D	4	16.48	4.12	25.00
D	4	14.40	4.12	28.61
D	2	6.18	1.02	16.50

the 3 studied species (Tables 1–3, Figs. 2–4) showed species-specific variation in the genomic behavior. Noticeable variation in the somatic chromosome number was observed in *S. nudiflora* ($2n=54$) having diploid chromosomes $2n=40$ (Fig. 5) and $2n=34$ (Fig. 6) in the root tip cells and was not statistically significant. Total genomic chromosome length varied from $51.49 \mu\text{m}$ in *T. populnea* to $116.48 \mu\text{m}$ in *S. nudiflora*. The maximum genomic chromosome volume was $114.11 \mu\text{m}^3$ in *S. nudiflora* followed by $67.45 \mu\text{m}^3$ in *A. rotundifolia* and $42.81 \mu\text{m}^3$ in *T. populnea* (Table 4). Significant interspecific variations of chromosome length, volume, INV and nuclear DNA content was observed (Table 5). Analysis of TF% showed symmetric karyotypes having sub-median chromosomes in the karyotype. Significant correlation (0.996) was observed between the length and volume of chromosome (Table 6).

Table 4. Somatic chromosome number, chromosome length, chromosome volume, TF%, INV and 4C DNA content in a minor-mangrove and 2 mangrove associates

Species	2n	Karyotype formula	No of SCC	Genomic chromosome length ($\mu\text{m} \pm \text{SE}$)	Genomic chromosome volume ($\mu\text{m}^3 \pm \text{SE}$)	4C DNA content (pg $\pm \text{SE}$)	TF%	INV ($\mu\text{m}^3 \pm \text{SE}$)	GCL/ chromosome	GCV/ chromosome	4C DNA/ chromosome
PLUMBAGINACEAE											
<i>Aegialitis rotundifolia</i>	34	2B+14C+18D	2	67.45 $\pm$ 1.32	54.15 $\pm$ 1.46	35.72 $\pm$ 1.24	34.92	517.53	1.98	1.59	1.05
CHENOPODIACEAE											
<i>Suaeda nudiflora</i>	54	6A+2B+28C+18D	8	116.48 $\pm$ 2.18	114.15 $\pm$ 3.78	42.45 $\pm$ 1.56	36.44	1846.52	2.15	2.11	0.79
MALVACEAE											
<i>Thespesia populnea</i>	26	2A+2B+12C+10D	4	51.49 $\pm$ 1.45	42.81 $\pm$ 1.56	28.56 $\pm$ 0.98	35.09	632.41	1.98	1.64	1.09

2n=diploid chromosome number, SCC=number of secondary constricted chromosome, GCL=genomic chromosome length, GCV=genomic chromosome volume, INV=interphase chromosome volume.

Table 5. ANOVA of genomic chromosome length, volume, TF%, INV and 4C DNA content in a minor mangrove and 2 mangrove associate species

Source	DF	SS	MS	F
Genomic chromosome length				
Between species	2	35.23	17.61	31.44**
Within species	27	15.12	0.56	—
Total	29			
Genomic chromosome volume				
Between species	2	28.55	14.27	19.54**
Within species	27	19.67	0.73	—
Total	29			
Total form percentage (TF%)				
Between species	2	245.34	112.67	33.94**
Within species	27	89.56	3.32	—
Total	29			
Interphase nuclear volume (INV)				
Between species	2	546.86	273.43	430.59**
Within species	197	125.23	0.63	—
Total	199			
4C DNA content				
Between species	2	55.68	27.84	18.81**
Within species	27	40.22	1.48	—
Total	29			

** Significant at $p \geq 0.001$, DF=degree of freedom, SS=sum of squares, MS=mean squares, F=variance ratio.

Table 6. Correlation coefficient values of different chromosomal and nuclear parameters in a minor mangrove and 2 mangrove associate species

Cytological parameters	r values	Cytological parameters	r values
Genomic chromosome length vs. genomic chromosome volume	0.996	Average chromosome length vs. average chromosome volume	0.996
Genomic chromosome length vs. 4C DNA content	0.954	Average chromosome length vs. average 4C DNA content	-0.992
Genomic chromosome volume vs. 4C DNA content	0.923	Average chromosome volume vs. average 4C DNA content	-0.997
Genomic chromosome length vs. INV	0.926	Average chromosome length vs. average INV	0.387
Genomic chromosome volume vs. INV	0.956	Average chromosome volume vs. average INV	0.446
4C DNA content vs. INV	0.772	Average 4C DNA content vs. average INV	-0.271

INV and 4C nuclear DNA amount

Interphase nuclear volume (INV) differed significantly in the 3 studied species (Table 4). The INV was comparatively less in *A. rotundifolia* ($517.53 \mu\text{m}^3$) than *T. populnea* ($632.41 \mu\text{m}^3$), *S. nudiflora* had the highest ($1646.52 \mu\text{m}^3$). The frequency distribution of the interphase nucleus showed a prominent peak around the mean in *S. nudiflora* whereas 2 other species showed a minor peak close to their mean values (Fig. 7). The nuclear DNA amount differed significantly (Tables 4, 5) among the species from 28.56 pg in *T. populnea* to 42.45 pg in *S. nudiflora*. Moderate correlation was observed between the INV and nuclear DNA amount ($r=0.772$). 4C DNA content showed high correlation (Table 6) with chromosome length ($r=0.954$) and chromosome volume ($r=0.923$).

Figs. 1–6. 1) Type chromosomes present in different species of minor and associate mangroves. 2–4) Somatic metaphase plates of different species of minor and associate mangroves. 2) *Aegialitis rotundifolia* showing $2n=34$ chromosomes ($\times 3216$). 3) *Suaeda nudiflora* showing $2n=54$ chromosomes ($\times 1940$). 4) *Thespesia populnea* showing $2n=26$ ($\times 3216$). 5, 6) Metaphase plates of *S. nudiflora* showing $2n=40$ and $2n=34$ chromosomes respectively ($\times 3216$).

Fig. 7. Graph showing frequency distribution of interphase nuclear volume (INV) in different species of minor and associate mangroves.

Discussion

Karyotype, genome length, volume and TF%

The karyotype analysis in all the studied species showed variations in the number of chromosomes, the size as well as the position of secondary constrictions on the chromosome arms (Tables 1–4). The somatic chromosome numbers $2n=54$ in *S. nudiflora* was reported for the first time against the earlier report $2n=34$ (Sidhu 1961). The variations in the chromosome number in root tips of *S. nudiflora* $2n=40$ (Fig. 4) and $2n=34$ (Fig. 5) and $2n=54$ (Fig. 3) suggested the occurrence of cytotypes in Bhitarkanika mangrove forests which might have occurred due to cross pollination. The size and the type of chromosomes in different species also differed. All the 4 types of chromosomes *i.e.* Type A, B, C and D were observed in *S. nudiflora* and *T. populnea* (Figs 2a–4a), where as Type A chromosome was absent in *A. rotundifolia*. The maximum numbers of Type A chromosomes (6 numbers) was obtained in *S. nudiflora* and the minimum of 2 in *T. populnea*. The maximum 28 median sized Type C chromosomes were present in *S. nudiflora* where Type D (sub-median chromosomes) were 18 only. Type C and D chromosomes in the other 2 species were more or less equal in number showing symmetric karyotype as evident from the corresponding karyograms (Figs. 2a–4a) suggesting stability of chromosomes in the genome without any outbreeding during the course of evolution. The TF% of around 34% to 36% in the karyotype which got reflected in their respective karyotypes suggest involvement of more sub-median chromosomes in the karyotype architecture. All these facts suggest a homogenous chromosome morphology in all the studied species although they 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 (Stebbins 1971, Das 1991, Das *et al.* 1999).

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 chromosome length ($1.98 \mu\text{m}$) and volume ($1.59 \mu\text{m}^3$) was observed in *A. rotundifolia*. The total chromosome volume and length in all the 3 species also showed positive correlation ($r=0.996$). 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 $1.59 \mu\text{m}^3$ in *A. rotundifolia* ($2n=34$), $1.64 \mu\text{m}^3$ in *T. populnea* ($2n=26$) and $2.11 \mu\text{m}^3$ in *S. nudiflora* ($2n=54$). Interphase nuclear volume showed significant correlation with chromosome length (0.926), chromosome volume (0.956) and 4C DNA content (0.772). But INV per chromosome did not show any positive correlation with all these parameters (Table 6). These characters were independent and differential interaction of genomic characteristics lead to the varia-

Figs. 2a–4a. Karyograms of different species of minor and associate mangroves. 2a) *A. rotundifolia*. 3a) *S. nudiflora*. 4a) *T. populnea*.

tion in DNA content (Yamaguchi and Tsunado 1969). Critical investigation on 4C DNA amount showed significant variations among the species (Table 5). The maximum 4C DNA amount (42.45 pg) in *S. nudiflora* and the minimum (35.72 pg) were noted in *A. rotundifolia*. Average DNA amount per chromosome also varied markedly (Table 4). The chromosome volume, however, showed high correlation with 4C DNA amount ($r=0.923$). The variation of DNA amount 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). 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 *et al.* 1980, Das and Mallick 1993a, b) for stable structural gene function during macro-evolution for maintaining genetic heritability (Sharma 1983).

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References

- Das, A. B. 1991. Chromosomal variability in relation with 4C DNA content in the subtribe Carinae. *Cytologia* **56**: 627–632.
- and Mallick, R. 1993a. Nuclear DNA and chromosome changes within the tribe Ammineae. *Cytobios* **74**: 197–207.
- and — 1993b. Karyotype diversity and interspecific 4C DNA variation in *Bupleurum*. *Biologia Plantarum* **35**: 355–363.
- , Basak, U. C. and Das, P. 1994. Karyotype diversity in three species of *Heritiera*, a common mangrove tree on the Orissa coast. *Cytobios* **80**: 71–78.
- , — and — 1995. Chromosome number and karyotype diversity in the Rhizophoraceae found in mangrove forests of Orissa. *Cytobios* **81**: 27–35.
- , — and — 1996. Karyotype analysis and 4C nuclear DNA estimation in three species of *Acanthus*, a mangrove associate from coastal Orissa. *Cytobios* **87**: 151–159.
- , — and — 1999. Genetic erosion of wetland biodiversity in Bhitarkanika forest of Orissa, India. *Biologia* **54**: 415–422.
- Dasgupta, A. and Bhatt, R. P. 1976. IOPB Chromosome Number Reports LIII. *Taxon* **25**: 483–500.
- Ford, C. E. 1938. A contribution to cytogenetical survey of the Malvaceae. *Genetica* **20**: 5–6.
- Hazra, R. and Sharma, A. 1971. Further studies on cytotaxonomy of Malvaceae. *Genet. Iber.* **23**: 145–166.
- Klekowski, E. J., Lowenfeld, R. and Hepler, P. K. 1994. Mangrove genetics II. Out crossing and lower spontaneous mutation rates in Puerto Rican *Rhizophora*. *Intern. J. Plant Sci.* **155**: 373–381.
- Kostermans, A. J. G. J. 1959. Monograph of the genus *Heritiera* Aiton (Sterculiaceae). *Reinwardtia* **4**: 465–483.
- Levan, A., Fredya, K. and Sandberg, A. 1964. Nomenclature for centromeric position on chromosome. *Heredity* **52**: 201–220.
- Newbold, C. 1993. Wetland Research and Survey Priorities in the British Isles and Their Relevance to India. In: Biodiversity Conservation Forests, Wetland and Desert, British High Commission, British Council Division, Tata Energy Research Institute, New Delhi. pp. 54–67.
- Nagato, Y., Yamamoto, K. and Yamashita, H. 1981. Variation of DNA content in Asian rice. *Jap. J. Genet.* **56**: 483–493.
- Ohri, D. and Khoshoo, T. N. 1986. Plant DNA contents and systematics. In: Dutta, S. K. (ed.). *DNA Systematics*. Vol. II. Plants, CRC Press, Florida. pp. 2–19.
- Price, H. J. 1976. Evolution of DNA content in plants. *Bot. Rev.* **42**: 27–52.
- , Bachman, K., Chambers, K. L. and Riggs, J. 1980. Detection of intraspecific variation in nuclear DNA content in *Microseris douglasii*. *Bot. Gaz.* **141**: 195–198.
- Raghavan, R. S. and Arora, C. M. 1958. Chromosome numbers in Indian medicinal plants. II. *Proc. Ind. Acad. Sci.* **47**: 352–358.
- Raina, S. N. 1990. Genome Organisation and Evolution in the Genus *Vicia*. In: Kawano, S. (ed.). *Biological Approaches and Evolutionary Trends in Plants*. Academic Press, London. pp. 183–201.
- Sharma, A. K. 1983. Additional Genetic Materials in Chromosomes. In: Brandham, P. E. and Bennett, M. D. (eds.). *Kew*

- Chromosome Conf. II. Allen and Unwin Ltd., London. pp. 35–42.
- and Sharma, A. 1980. Chromosome Techniques: Theory and Practice. 3rd edition. Butterworths, London.
- Sidhu, S. S. 1961. Chromosomal studies of some mangroves. Proc. 48th Indian Sci. Congr. Part 3: 302–303.
- 1968. Further studies on the cytology of mangrove species of India. Caryologia **21**: 353–357.
- Sokal, P. R. and Rohlf, F. J. 1973. Introduction to Biostatistics. Freeman, San Francisco.
- Stebbins, G. L. 1971. Chromosomal Evolution in Higher Plants. Edward Arnold (Publishers) Ltd, London.
- Tomlinson, P. B. 1986. The Botany of Mangroves. Cambridge University Press, Cambridge, London. pp. 312–352.
- Toungman, W. 1927. Studies in the cytology of the Hibisceae. Annal. Bot. **41**: 755–778.
- Van't Hof, J. 1965. Relationships between mitotic cycle duration, S period duration and the average rate of DNA synthesis in the root meristem cells of several plants. Expl. Cell Res. **39**: 48.
- Yamaguchi, Y. and Tsunado, S. 1969. Nuclear volume, nuclear DNA content and radiosensitivity in *Brassica* and allied genera. Jap. J. Breed. **19**: 35.
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