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Cytopalynology of Woody Taxa of Family Rubiaceae from North and Central India

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Chromosomes of four species i.e. *Gardenia latifolia* (n=11), *Ixora barbata* (n=11), *Pavetta tomentosa* (n=11) and *Xeromphis uliginosa* (n=11) are counted for the first time. Diploid race for *Ixora rosea* is reported against the earlier record of triploid only. Except for one hexaploid *Hymenodictyon excelsum* (n=33) and three tetraploid species (*Adina cordifolia* n=22, *Anthocephalus cadamba* n=22, and *Mitragyna parvifolia* n=22), the rest were diploids. Cytomixis was noticed in *Serissa foetida*. The base number x=11 seems well established in the woody members of the family.

The results of palynology reveal that at family level there was no correlation between pollen grain size and ploidy level.

Key Words: Cytology, Palynology, Rubiaceae, Woody taxa

Introduction

Rubiaceae is one of the largest families of flowering plants with about 500 genera and 6,000 species (Airy Shaw 1973). Its members are largely pantropical in distribution and also found in subtropical regions, some even extending up to the temperate as well as arctic regions (Lawrance 1951). These economically important plants attracted attention of the cytologists all over the world. So far the cytology of about 19% of 91 genera and 550 species from India has been worked out.

Material and Methods

Material was mainly collected from forests around Dehradun, Mussoorie, Nainital in Uttar Pradesh (North India) and Hoshangabad, Pachmarhi in Madhya Pradesh (Central India). In addition, some cultivated taxa from Patiala, Chandigarh and Dehradun were also collected. Meiotic studies were done on young floral buds after fixation in Carnoy's fluid, subsequently squashed using standard aceto-carmine technique. Desirable slides were mounted in euparal after usual dehydration in ethanol. Pollen fertility was determined according to Marks (1954). Pollen characteristics were noted following standard acetolysis technique. Size measurements were taken on unacetolysed pollen grains mounted in 1 : 1 glycero-safranin mixture. In the pollen description, terminology of Erdtman (1952) and Nair (1964) has been used. Voucher specimens were deposited in PUN.

Observations

Cytology

Twenty-four woody species including six commercial timbers were investigated. Information about recorded chromosome numbers and collection localities is summarised in table 1. Except for *Serissa foetida* where cytomixis occurs, meiosis in other species is normal leading to the formation of well-filled viable pollen grains.

Chromosome numbers of nine species have been recorded for the first time from India. There was no earlier information for four species viz., *Gardenia latifolia* (n = 11). *Ixora barbata* (n = 11), *Pavetta tomentosa* (n = 11) and *xeromphis uliginosa* (n = 11). The investigated species are all diploid except three tetraploid species, viz., *Adina cordifolia* (n = 22), *Anthocephalus cadamba* (n = 22) and *Mitragyna parvifolia* (n = 22), and a hexaploid species—*Hymenodictyon excelsum* (n = 33).

Out of the total PMCs observed in *Ixora rosea* (n = 11) at anaphase I, 19.7% showed an irregular distribution of 12 : 10 chromosomes, which results into 46.0% pollen sterility. This irregular distribution is possibly due to certain spindle abnormalities.

Cytomixis has been noticed in *Serissa foetida* (n = 11). 23.9% of the total PMCs observed show cytoplasmic channels connecting each other, and even at prophase they show the actual transfer of chromatin material. At a time 2-8 PMCs are involved in cytomixis. Accordingly, in different PMCs at diakinesis and metaphase I, variations in chromosome number from n = 9 - 12 were observed. In normal PMCs, 11 bivalents were counted (figure 8).

Palynology

Pollen morphology of 18 species from 12 genera was observed (figures 9a–z, aa–jj).

Oblate-spheroidal, prolate-spheroidal and subprolate pollen grains were observed. The oblate-spheroidal grains happen to be the most common—found in 14 species. The smallest grains were seen in *Wendlandia exserta* (6–8 μm × 6–8 μm) and the largest in *Spermadictyon sauveolens* (42 - 44 μm × 42 - 44 μm). Sizewise the polleng rains may be categorised from very small to medium size (Erdtman 1952). Accordingly, the pollen grains of the tetraploid *Mitragyna parvifolia* fall under small size. Detailed information on pollen characters and fertility is given in table 1.

Pollen studies of the two distinct forms of *Xeromphis spinosa* reveal that plants with long spines from Dehradun forest have large pollen (24–28μm × 24–28 μm) as compared with plants without spines from Pachmarhi forest which have smaller grains (12–16 μm × 12–16 μm), but the relevant types are cytologically similar with n=11. Pollen grains usually remain united in tetrads in case of *Gardenia latifolia* and *G. thunbergia*.

Discussion

Ixora rosea in the present investigation was found to possess n=11 (diploid) in contrast to an earlier report of 2n=33 (triploid) by Sharma and Chatterjee (1960). Three cultivated ornamental shrubs, viz., *Ixora bandhuca* (67.0%), *I. barbata* (61.5%) and *Mussaenda frondosa* (75.5%) show low pollen fertility, the reason for which is not known.

The occurrence of cytomixis in plants is normally attributed to meiotic abnormalities (cf. Stebbins 1958). *Serissa foetida* is a normal diploid with n=11, where this phenomenon seems to be under some genetic control as postulated by Brown and Bertke (1974). However, incomplete cytokinesis during premeiotic mitosis or defective cell wall formation etc. (Sarvella 1958) cannot be ruled out.

All the investigated genera except *Hamelia*

Table 1 *Data on chromosome numbers and morphology of pollen grains in certain woody species of Rubiaceae from North and Central India*

S.No.	Taxon	Localities	Chromosome number/s*	%age pollen fertility	Pollen grain characteristics**			
					Size in μm	Shape	Aperture	Exine
1	2	3	4	5	6	7	8	9
1.	*Adina cordifolia* Hook.f.ex Brandis	Dehradun : Mohand, 400 m	n=22 (fig. 1)	—	—	—	—	—
		Nainital : Ranibag, 800 m	n=22	—	—	—	—	—
2.	*Anthocephalus cadamba* Miq.	Chandigarh : 300 m	n=22	—	—	—	—	—
3.	*Coffea bengalensis* Roxb.++	Dehradun : Bindal Nala, 600 m	n=11	88.4	22–24×26–28	Oblate-spheroidal	3-zonocolporate; ectocolpium 15.0×2.5μm; endocolpium circular, dia. 3.3 μm	1.6 μm thick, sexine as thick as nexine, surface reticulate, lumina smooth, polygonal to circular, 0.8 μm in dia.
4.	*Gardenia florida* Linn.++	Dehradun : F.R.I. 600 m	n=11	—	—	—	—	—
5.	*G. latifolia* Ait.+	Dehradun : F.R.I., 600 m	n=11 (fig. 2)	100	21–23×21–23 (tetrad 36–44×36–44)	Oblate-spheroidal	3-zonoporate; pore circular, rimmed, avg. dia. 3.8 μm	2.5 μm thick, sexine thicker than nexine, surface faintly areolate
6.	*G. spathulifolia* Stapf. ex Hutch.++	Dehradun : F.R.I., 600 m	n=11	—	—	—	—	—
7.	*G. thunbergia* Linn.++	Dehradun : F.R I., 600 m	n=11	100	24–26×24–26 (tetrad 44–48×44–48)	Oblate-spheroidal	3-zonoporate; pore circular, rimmed, avg. dia. 4.6 μm	2.6 μm thick, sexine thicker than nexine, surface faintly areolate
8.	*Hamelia patens* Jacq.	Dehradun : 600 m	n=12	88.0	21–24×21–24	Oblate-spheroidal	3-zonocolporate; ectoc. 15.0×2.0 μm; endoc. circular, avg. dia. 4.2 μm	2.3 μm thick, sexine thicker than nexine; surface reticulate, lumina polygonal to circular up to 1.7 μm in dia.
9.	*Hymenodictyon excelsum* Wall.	Nainital : Lalkuan, 350 m	n=33	—	—	—	—	—

Continued

10.	*Ixora bandhuca* Roxb.	Patiala: Punjabi Univ. campus, 250 m	n=11	67.0	28–30×24–26	Subprolate	3-zonocolporate; ectoc. with thick margins, 21.7×3.3 μm; endoc. slightly lolongate, 5.0×4.6 μm	1.8 μm thick, sexine thicker than nexine, surface reticulate, lumina smooth, almost circular, dia. 0.8 μm
11.	*I. barbata* Roxb.+	Dehradun: F.R.I., 600 m	n=11 (fig. 3)	61.5	26–30×24–26	Prolate-spheroidal	3-zonocolporate; ectoc. 17.5×2.8 μm; endoc. circular, avg. dia. 3.8 μm	2.3 μm thick, sexine thicker than nexine, surface areolate
12.	*I. coccinea* Linn.	Chandigarh: 300 m	n=11	—	—	—	—	—
13.	*I. parviflora* Vahl	Hoshangabad: Singhanama, 500 m	n=11	88.5	24–28×24–28	Oblate-spheroidal	3-zonocolporate; ectoc. 21.4×2.9 μm; endoc. circular, avg. dia. 4.2 μm	2.8 μm thick, sexine thicker than nexine, surface reticulate, lumina smooth, almost circular, diam. 0 4 μm
14.	*I. rosea* Wall. ex. Roxb.+	Dehradun: F.R.I., 600 m	n=11 (fig. 4)	54.0	22–26×18–20	Subprolate	3-zonocolporate; ectoc. 20.8×3.3 μm; endoc. slightly lolongate, 5.4×4.5 μm	2.4 μm thick, sexine thicker than nexine, surface reticulate, lumina smooth, polygonal to circular, dia. 0.8 μm
15.	*Mitragynal parvifolia* (Roxb.) Korth.	Hoshangabad: Singhanama, 500 m	n=22 (fig. 5)	90.8	14–17×14–17	Oblate-spheroidal	3-zonocolporate; ectoc. 12.5×2.5 μm; endoc. slightly lolongate, 3.8×3.3 μm	1.3 μm thick, sexine and nexine of same thickness, surface reticulate, lumina almost circular, 0 8 μm in dia.
		Nainital: Lalkuan, 350 m	n=22	—	—	—	—	—
16.	*Mussaenda frondosa* Linn.	Dehradun: F.R.I., 600 m	n=11	75.5	16–18×16–18	Oblate-spheroidal	3-zonocolpate; colpium 9.2×1.7 μm	1.3 μm thick, sexine and nexine of same thickness surface reticulate, lumina almost circular, dia. 0.8 μm
17.	*M. luteola* Del.	Hoshangabad: Pipariya, 400 m	n=11	100	12–19×12–19	Oblate-spheroidal	3-zonocolpate: colpium 11.7×2.0 μm	1.3 μm thick, sexine and nexine of same thickness, surface reticular, lumina circular, dia. 0.8 μm
18.	*Pavetta tomentosa* Roxb.ex Sm.+	Dehradun: Daat, 600 m	n=11 (fig. 6)	95.5	24–27×19–22	Subprolate	3-zonocolporate; ectoc. 20.7×3.3 μm; endoc. lolongate, 4.8×3.8 μm	1.7 μm thick, sexine thicker than nexine, surface reticulate, lumina polygonal to circular, dia. 0.8 μm

Table 1 *(Contd.)*

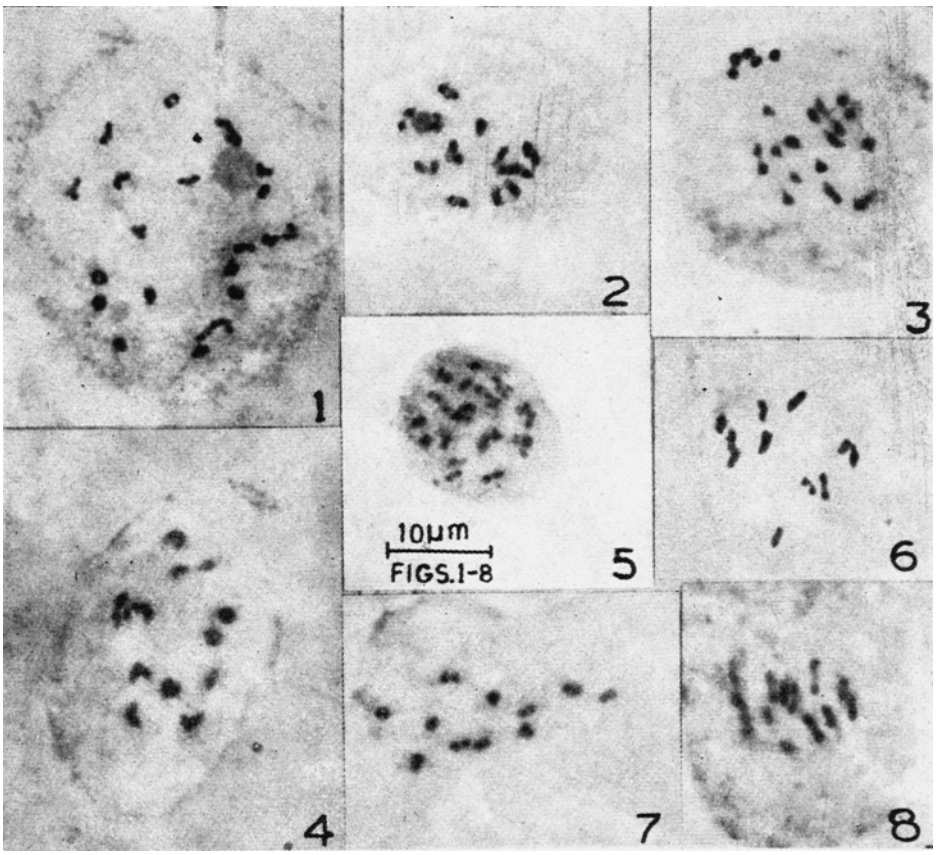
1	2	3	4	5	6	7	8	9
19.	*Randia tetrasperma* Benth. & Hook.f.	Mussoorie: Camel's back road, 1,850 m	n=11	100	28–32×28–32	Oblate-spheroidal	3-zonoporate; pore circular, rimmed, dia. 5.0 μm	2.6 μm thick, sexine thicker than nexine, surface faintly areolate
20.	*Serissa foetida* Lamk.++	Dehradun: F.R.I., 600 m	n=11	57.2	36–40×36–40	Oblate-spheroidal	3-zonocolpate; colpium 27.7×4.8 μm	2.6 μm thick, sexine thicker than nexine surface reticulate, lumina polygonal to circular, dia. up to 1.7 μm
21.	*Spermadictyon suaveolens* Roxb.	Mussoorie: Kempty fall, 1,350 m	n=11	100	42–44×42–44	Oblate-spheroidal	3-zonocolpate; colpium 20.8×3.3 μm	3.0 μm thick, sexine considerably thicker than nexine, surface reticulate, lumina polygonal to circular, dia. 1.7 μm
22.	*Wendlandia exserta* DC.	Pachmarhi: Manda deo caves, 1,000 m	n=11	100	6–8×6–8	Oblate-spheroidal	3-zonocolporate; ectoc. 6.2×1.7 μm, endoc. circular, avg. dia. 2.5 μm	1.2 μm thick, sexine and nexine of same thickness, surface reticulate, lumina almost circular, dia. 0.4 μm
		Nainital: Dogaon, 1,000m	n=11	—	—	—	—	—
23.	*Xeromphis spinosa* (Thunb.) Keay (*Randia dumetorum* (Retz. Poir.)							
	a. Form with long spines:	Dehradun: Mohand, 400 m	n=11	100	24–28×24–28	Oblate-spheroidal	3-zonoporate; pore circular, rimmed, dia. 4.2 μm	1.3 μm thick, sexine thicker than nexine, surface faintly areolate
	b. Form without spines:	Pachmarhi: Air strip, 1,050 m	n=11	100	12–16×12–16	Oblate-spheroidal	3-zonoporate; pore circular, rimmed, dia. 3.3 μm	1.2 μm thick, sexine thicker than nexine, surface faintly areolate
24.	*X. uliginosa* (Retz.) Maheshwari+ (*R. uliginosa* DC.)	Dehradun: Lachiwala, 600 m	n=11 (fig. 7)	100	24–28×26–32	Oblate-spheroidal	3-zonoporate; pore circular, rimmed, dia. 4.6 μm	2.0 μm thick, sexine thicker than nexine, surface faintly areolate
		Hoshangabad: Matkuli, 450 m	n=11	100	24–28×26–32	Oblate-spheroidal	3-zonoporate; pore circular, rimmed, dia. 4.6 μm	2.0 μm thick, sexine thicker than nexine, surface faintly areolate

* For previous reports reference is made to Darlington & Wylie (1955), 'Index to Plant chromosome numbers' (1956 onwards), 'IOPB chromosome numbers reports' (1965 onwards), Löve & Löve (1961, 1974, 1975) and Fedorov (1969) and selected references from Biological Abstracts (1970 onwards).

** For unworked species proper material was not available.

\+ New or different chromosome number reports.

++ First chromosome number report from India.



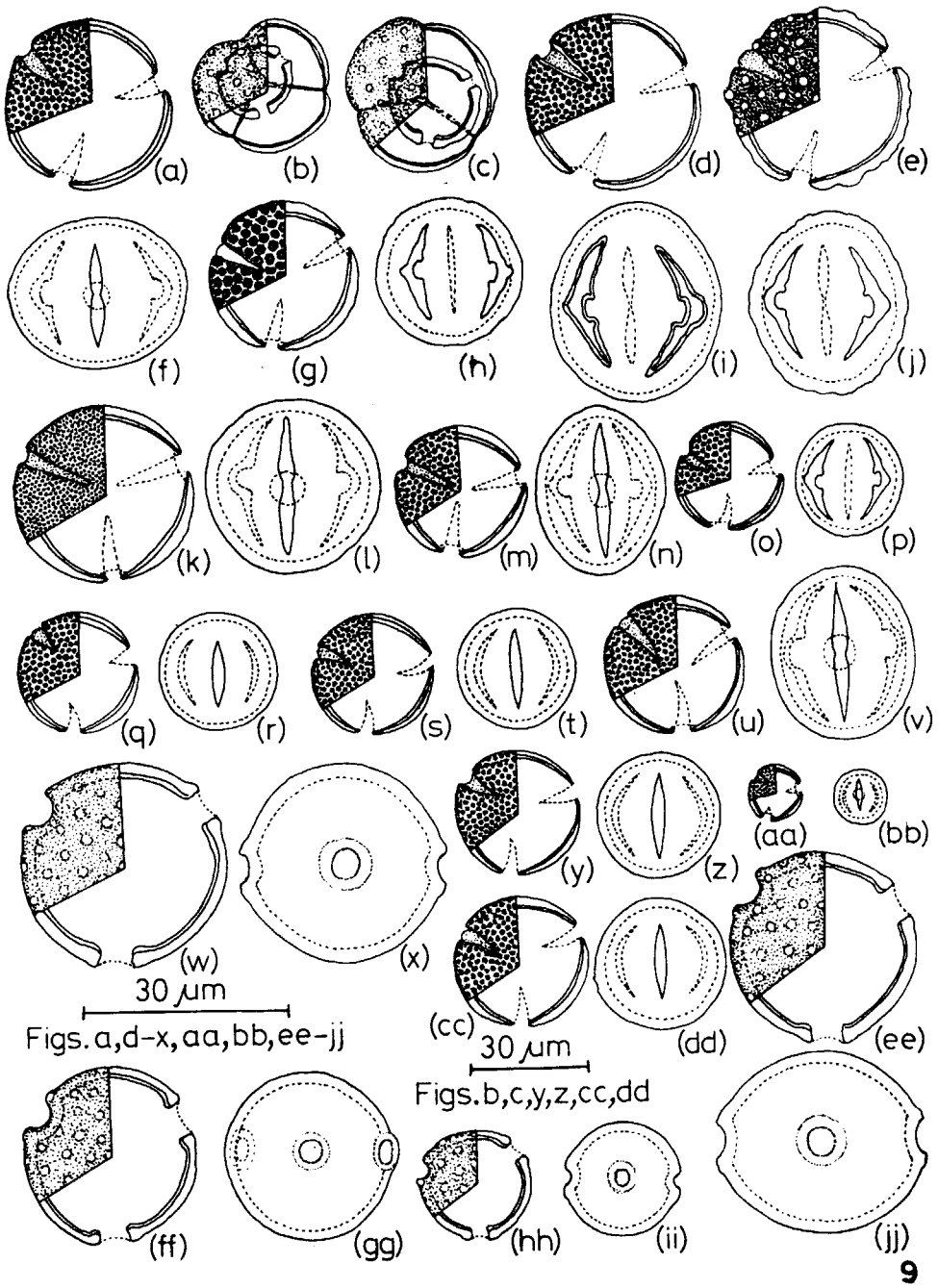
Figures 1–8 Meiotic chromosomes of Rubiaceae. **1**, *Adina cordifolia*, Diakinesis, n=22; **2**, *Gardenia latifolia*, Diakinesis, n=11; **3**, *Ixora barbata*, Mixed A–I, 2n=22; **4**, *I. rosea*, M–I, n=11; **5**, *Mitragyna parvifolia*, M–I, n=22; **6**, *Pavetta tomentosa*, M–I, n=11; **7**, *Xeromphis uliginosa*, M–I, n=11; **8**, *Serissa foetida*, M–I, n=11

(x=12) are based on x=11—a number common in woody members of the family. This gives support to the contention of Lewis (1980) that Rubiaceae fundamentally has x=11 in woody tropical tribes. May be x=11 is a derived number as was suggested by Sharma and Chatterjee (1960) and Mathew and Philip (1979) who considered this number to have been evolved through amphidiploidy from basic number x=5 and/or x=6.

A total of only 53 woody species from 18 genera have been cytologically investigated in India. A perusal of data given in table 2

Table 2. *Cytological data for woody members of Rubiaceae from India*

Habit	Total number of taxa	Dip-loid	Poly-ploid	Poly-ploidy %	Highest ploidy
Trees	27	16	11	40.74	8x
Shrubs	26	22	4	19.38	6x
Total	53	38	15	28.30	—



Figures 9a–z, and aa–jj. Palynograms in family Rubiaceae: **a, f,** *Coffea bengalensis*; **b,** *Gardenia latifolia*; **c,** *G. thunbergia*; **g, h,** *Hamelia patens*; **d, i,** *Ixora bandhuca*; **e, j,** *I. barbata*; **k, l,** *I. parviflora*; **m, n,** *I. rosea*; **o, p,** *Mitragyna parvifolia*; **q, r,** *Mussaenda frondosa*; **s, t,** *M. luteola*; **u, v,** *Pavetta tomentosa*; **w, x,** *Randia tetrasperma*; **y, z,** *Serissa foetida*; **cc, dd,** *Spermadictyon suaveolens*; **aa, bb,** *Wendlandia exserta*; **ff, gg,** *Xeromphis spinosa* (with long spines); **hh, ii,** *X. spinosa* (without spines); **ee, jj,** *X. uliginosa*

Figures 9 a, b, c, d, e, g, k, m, o, q, s, u, w, y, aa, cc, ee, ff, hh: Polar view

Figures 9 f, h, i, j, l, n, p, r, t, v, x, z, bb, dd, gg, ii, jj: Equatorial view

reveals that trees in relation to shrubs exhibit higher incidence of polyploidy (40.74%). Further, it is interesting to note that out of nine timber taxa of the family in India, five are polyploids at 4×(4) and 6×(1) levels. Intraspecific polyploidy is exhibited only by *Xeromphis spinosa*, diploid is from Nainital (Mehra & Bawa 1969; 2n=22) and Pachmarhi and Dehradun (present studies, n=11) whereas the tetraploid is recorded from Assam (Mehra & Bawa 1969; n=22).

Pollen grain size was quite variable in the family and no correlation between pollen grain size and polyploidy could be drawn, as many of the diploid species had pollen grains larger than the tetraploid taxon *(Mitragyna parvifolia)*.

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References

Airy Shaw H K 1973 *Dictionary of Flowering Plants and Ferns* 8th ed. (London : Cambridge Univ. Press)

Brandis D 1906 *Indian trees* 5th ed. (Dehradun)

Brown W V and Bertke E M 1974 *Textbook of Cytology* 2nd ed. (Missouri : Mosby. Saint Louis)

Dalington C D and Wylie A P 1955 *Chromosome Atlas of Flowering Plants* (London : George Allen and Unwin Ltd.)

Erdtman G 1952 *Pollen morpholoogy and Plant Taxonomy* (Stockholm : Almavist and wiksell)

Fedorov An A (ed.) 1969 *Chromosome Numbers of Flowering Plants* (Leningrad : Academy of Sci. of U.S.S.R. Komaror Bot. Inst).

Lawrence G H M 1951 *Taxonomy of Vascular Plants* (New York : The Macmillan Company)

Lewis W H 1980 Polyploidy in angiosperms : Dicotyledons, in *Polypioidy : Biological Relevance* pp. 241–268 ed. W H Lewis (New York : Plenum Publ. Corpn.)

Love A and Love D 1961 *Chromosome Numbers of Central and North-West European Plant Species Opera Botanica* (Stockholm : Almqvist and Wiksell)

_____ and _____ 1974 *Cytotaxonomical Atlas of the Slovenian Flora* (Lehre : J Cramer)

_____ and _____ 1975 *Cytotaxonomical Atlas of Arctic Flora* (Vaotus : J Cramer)

Marks G E 1954 An acetocarmine glycerol jelly for use in pollen fertiliy counts; *St. Technology* **29** 277

Mathew P M and Philip K O 1979 Studies on South Indian Rubiaceae I Cytology of genus *Ophiorrhiza*; *Nucleus* **22** 47–50

Mehra P N and Bawa K S 1969 Chromosomal evolution in tropical hardwoods; *Evolution* **23** 466–481

Nair P K K 1964 Pollen morphology; in *Advances in Palynology* pp. 203–224 ed. P K K Nair (Lucknow; Nationol Bot. Gardens,

Sarvella P 1958 Cytomixis and loss of chromosomes in meiotic and somatic cells of Gossypium; *Cytologia* **23** 41–24

Sharma A K and Chatterjee T 1960 Chromosome studies in *Ixora*; *Genetics* **31** 421–447

Stebbins G L 1958 The inviability, Weakness and sterility of interspecific hybrids; *Adv. Genet.* **9** 147–215