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Cytopalynology of some members of Rutaceae

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Abstract. Cytopalynology of 13 species of the family Rutaceae from North and Central India do not reveal any correlation between polyploidy and pollen size. Partial pollen malformation in certain species does not seem to be due to detectable cytological reasons. Multivalent formation in some trees of *Citrus jambhiri* ($2n=18$) is due to reciprocal translocations. The newly counted species, *C. jambhiri* ($2n=18$) and the monotypic genus *Limonia* (*L. crenulata*, $n=9$) and all other species except for *Zanthoxylum armatum* ($n=33$) are based on $x=9$. Though the family is polybasic, $x=9$ appear to be its original base number. The chromosomal heterogeneity in the family ($2n=14-162$) coupled with dysploid series of base numbers ($x=7-19$) confirm that polyploidy and aneuploidy have played a considerable role in speciation.

Keywords. Cytopalynology; Rutaceae; dysploid; aneuploid.

1. Introduction

Rutaceae, a widely distributed family of about 900 species of trees and shrubs (Airy Shaw 1973) are represented in India by 20 genera and 50 species (*cf.* Mehra 1976), of which nine are commercial timbers (Pearson and Brown 1932). It is equally important in horticulture for its citrus fruits. Keeping in view the large size of the family, the palynological observations are meagre. The only studies dealing with pollen characteristics are those of Erdtman (1952), Nair and Mehra (1962), Bamzai and Randhawa (1965) and Nair (1965). The family is also not adequately known cytologically, and the cumulative worldwide data* indicate that the chromosome counts of only 25% of the species are available. Some work has been carried out on the Himalayan taxa by Mehra and Khosla (1973) but information from the other regions of the country is lacking. In view of the considerable heterogeneity in chromosome numbers ($2n=14-162$) involving euploidy as well as aneuploidy, the present studies were undertaken on North and Central Indian taxa to correlate cytopalynological characteristics.

2. Material and methods

For meiotic studies flower buds of appropriate size were fixed in Carnoy's fluid, saturated with iron acetate. PMCs were squashed subsequently, in 1% acetocarmine

*Based on Darlington and Wylie (1955), Index to Plant Chromosome Numbers' (1956 onwards), 'IOPB Chromosome number reports' (1965 onwards), Löve and Löve (1961, 1974, 1975), Fedorov (1969) and selected references from the Biological Abstracts.

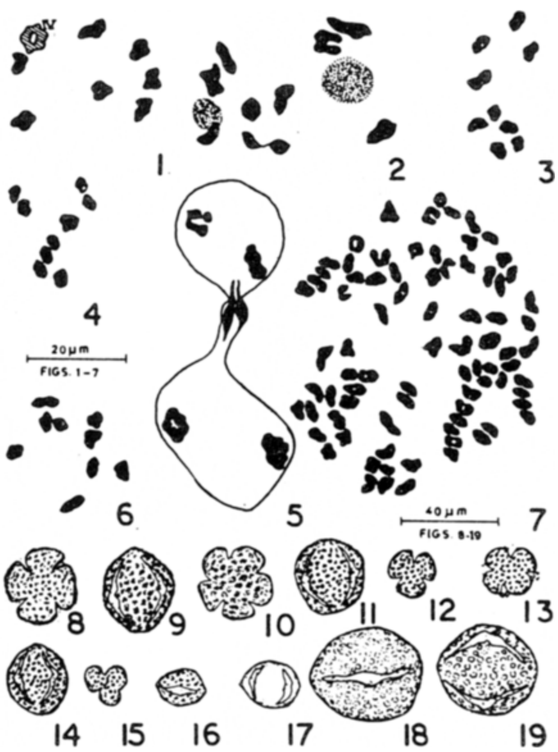
and desirable preparations were made permanent in euparal.

Pollen fertility was judged on the basis of their stainability with glycero-acetocarmine mixture (1:1) and well-filled nature. Pollen grain characteristics which are worked out from mature pollen grains, stained in 1% safranin and mounted in glycerol, are based on Erdtman (1952) and Nair (1964).

3. Observations

Cytopalynological investigations on 13 species belonging to 8 genera have been carried out on wild and cultivated taxa growing in Northern and Central India. Four species of *Citrus* studied presently, viz., *C. jambhiri*, *C. maxima*, *C. medica* and *C. sinensis* have the same chromosome number ($n=9$) and are diploid. Multiple associations in 22.3% PMC are recorded in some trees of *C. jambhiri* (figure 1).

Abnormal nucleolar activity exists in *Citrus medica* (figure 2), *Feronia limonia* ($n=9$), and in the normal and structurally heterozygous plants of *C. jambhiri* ($n=9$,



Figures 1-19. **1-7.** Meiosis in pollen mother cells. **1.** *Citrus jambhiri*, M-I with $2n=18=1_{IV}+7_{II}$ **2.** *C. medica*, diakinesis showing 9_{II} and two nucleoli **3.** *Clausena wampi*, M-I with 9_{II} **4.** *Fortunella japonica*, M-1 with 9_{II} **5.** *Glycosmis pentaphylla*, two PMC showing chromatin transfer **6.** *Limonia crenulata*, M-I with 9_{II} **7.** *Geijera parviflora*, M-I with 81_{II}. **8-19.** Pollen grains **8.** *Citrus jambhiri* **9.** *C. maxima* **10.** *C. medica* **11.** *C. sinensis* **12.** *Clausena wampi* **13.** *Feronia limonia* **14.** *Fortunella japonica* **15.** *Geijera parviflora* **16.** *Glycosmis pentaphylla* **17.** *Limonia crenulata* **18.** *Murraya koenigii* **19.** *M. paniculata.*

$2n=18$). Multiple nucleoli (1-5/PMC) formation though not very frequent, is recorded at diakinesis in *C. medica* (figure 2) and *C. jambhiri*. Nucleolus which is single in *F. limonia* persists even at late M-I or early A-I. Cytomixis is noticed in some of the PMC in *Glycosmis pentaphylla* with $2n=18$ (figure 5) but it does not lead to any numerical variation in chromosome number and reduction in pollen fertility.

Palynological studies concern 12 species (figures 8-19) and for *Zanthoxylum armatum* mature pollen grains were not available at the time of collection. Till now no information is available about the pollen characteristics of *Clausena wampi* ($2n=18$), *Geijera parviflora* ($2n=162$), *Glycosmis pentaphylla* ($2n=18$), *Limonia crenulata* ($2n=18$) and *Murraya paniculata* ($2n=18$). Pollen grains in the present species are quite variable which are either oblate (*Limonia crenulata*), prolate spheroidal (*Citrus jambhiri, C. maxima, C. medica, C. sinensis, Feronia limonia, Fortunella japonica, Murraya koenigii, M. paniculata*), subprolate (*Clausena wampi, Glycosmis pentaphylla*) or prolate (*Geijera parviflora*) type. Exine is thin ranging from psilate (*Limonia crenulata*) to faintly reticulate (9 species) to granulose (*Murraya koenigii, M. paniculata*). Aperturewise pollen are either 3-zonocolporate (*Clausena wampi, Geijera parviflora, Glycosmis pentaphylla, Murraya koenigii, M. paniculata*), 4-zonocolporate (*Citrus jambhiri, C. maxima, C. sinensis, Feronia limonia, Fortunella japonica*) or 5-zonocolporate (*Citrus medica, Limonia crenulata*). Pollen grains are generally medium sized ranging from 20-22 μm × 16-18 μm (*Glycosmis pentaphylla*) to 44-46 μm × 40-42 μm (*Murraya paniculata*). Reduction in pollen fertility in *Citrus maxima* (91%), *C. sinensis* (42%), *Feronia limonia* (98%), *Fortunella japonica* (93%), *Geijera parviflora* (92%), *Limonia crenulata* (49%) and the Pachmarhi population of *Murraya paniculata* (85%) cannot be attributed to any apparent cytological reasons, for synapsis, chromosomal distribution and cytokinensis are normal. It might be due to cryptic structural heterozygosity or reasons other than cytological. However, pollen sterility (32%) recorded in some trees of *Citrus jambhiri* appears to be the consequence of reciprocal translocations.

4. Discussions

Of the 13 species investigated, the chromosome count of $n=9$ for *Citrus jambhiri* and *Limonia crenulata* (figure 6) is the first record which also includes the new report for the monotypic genus *Limonia*. Additional cytotype of $n=81$ (figure 7) is recorded for *Geijera parviflora*, an exotic species which is earlier recorded with $2n=108$ by Smith-White (1954) from Australia and $2n=144$ by Rao (1967) from the cultivated source (FRI, Dehra Dun). Incidentally all the intraspecific cytotypes are polyploid (12*x*, 16*x*, 18*x*) which are based on $x=9$. *Clausena wampi* ($n=9$) (figure 3) and *Fortunella japonica* ($n=9$, figure 4) are counted for the first time from India but confirm the earlier reports from elsewhere by Krug (1943) and Longley (1925) for the species respectively. Multiple associations in the diploid species, *C. jambhiri* ($2n=18$) have been attributed to the reciprocal translocations by Singhal and Gill (1981).

The family represents a great heterogeneity in chromosome numbers ($2n=14$, 16,18,20,21,22,24,26,28,30,32,34,36,38,40,42,45,48,50,54,56,60,64,66,68,70,72,80, 89-90, 108, 136, 144, 162) indicating that it has passed through an active phase of numerical chromosomal evolution leading to delimitation of various genera and species. Analysis of data on the 237 species (57 genera) counted so far shows that $x=9$ is by far the most common in the family. As many as 147 species have chromosome number $n=9$ or in its multiple. Intraspecific polyploidy based on $x=9$ is exhibited by 30 species, with the only exception of *Zanthoxylum ovalifolium* and *Z. americanum* which are based on $x=17$. The other common base numbers are $x=18$ (31 species) and $x=8$ (25 species). Though the family is polybasic and the base number constitute a regular dysploid series ($x=7$-19), $x=9$ seems to be the original from which the rest might have been derived through polyploidy and/or aneuploidy. The present species *Geijera parviflora* has the highest chromosome number recorded in the family and is at the 18-ploid level. Thus, polyploidy seems to have played a significant role which is also apparent from its existence in 38% of the species. The correlation of pollen grain size with chromosome number in the present species reveals that the smallest (20-22 × 16-18 µm, *Glycosmis pentaphylla*) and largest (40-46 × 40-42 µm, *Murraya paniculata*) pollen grains exist in the diploid species ($2n=18$). Furthermore, *Geijera parviflora* ($2n=162$) which is a very high polyploid ($18x$) also possess small-sized pollen grains indicating that there might not be any correlation between pollen grain size and chromosome number and the level of polyploidy in the different species of the family. All the four species of *Citrus* are diploid with ($2n=18$) and possess almost similar pollen grains but aperturewise *C. medica* differs from the other three by having 5-zonocolporate type pollen grains.

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