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Short Communication

Chromosomal diversity in three species of genus *Rosa* L. (Rosaceae) from district Kangra of Himachal Pradesh

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Abstract

The chromosome number, detailed male meiosis, microsporogenesis and pollen fertility were examined in three species of the genus *Rosa* namely, *Rosa brunonii* (2n=14), *R. indica* (2n=14) and *R. macrophylla* (2n=14, 28). Of these, 2n=14 for *R. brunonii* and *R. indica* and 2n=28 for *R. macrophylla* makes a new records at India level. The course of meiosis varies from normal to abnormal in different populations of the species. The anomalous taxa are marked with meiotic abnormalities in the form of cytomixis, chromosomal stickiness, unoriented bivalents, formation of laggards and bridges resulting in abnormal microsporogenesis and production of heterogenous sized fertile pollen grains. All the taxa with normal meiotic course show high pollen fertility.

Key words: Chromosome number, meiotic abnormalities, *Rosa*, district Kangra

The genus *Rosa* comprises of 200 species, mainly distributed in the northern hemisphere [1]. From India, 10 species [2], including 3 from district Kangra, Himachal Pradesh [3], are known. The genus includes spiny shrubs having stems usually prickly; leaflets 7-9, sharply toothed, oval; carpels free from calyx tube. Roses are best known as ornamental plants grown for the flowers in the gardens and sometimes even indoors. These have also been used for commercial perfumery and commercial cut flower crops. They also have minor medicinal uses [4]. Cytological studies including chromosomal counts, karyotypes and meiotic configuration frequencies by means of traditional squashing and pressing [5], C-banding [6] as well as FISH [5] have been made in the genus. The genus is known to exhibit well marked inter- and intraspecific chromosomal diversity, both at diploid and polyploid levels. With an aim to mark out the intraspecific genetic variants of *Rosa*, to be recommended for pharmaceutical testing, the meiotic studies have been carried out on population basis from different localities of district Kangra.

For meiotic studies, flower buds were collected from different localities of district Kangra (Table 1). Smears of appropriate sized flower buds were made after fixing these in Carnoy's fixative, using standard acetocarmine technique. Pollen fertility was estimated by mounting mature pollen grains in glycero-acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shrivelled and unstained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Nikon 80i Eclipse Digital Imaging System. Voucher specimens are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala.

Detailed cytological studies were carried out on 7 populations belonging to 3 species of the genus *Rosa*. The data regarding location, altitude, accession number, present and previous chromosome number reports, ploidy level, meiotic course of presently worked out species has been presented in Table 1. The results for each species with new/varied chromosome counts and abnormal meiotic course are discussed below.

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Cytological studies

R. brunonii Lindl. (=*R. clavigera* H. Lév.): Both the accessions reveal the chromosome number 2n=2x=14 in the PMCs at meiotic metaphose I (M-I) (Fig. 1), being reported for the first time from India and conforming to the previous reports from outside India (Table 1).

R. indica L. (=*R. cymosa* Tratt.): Both the accessions reveal 2n=2x=14 chromosome number at M-I (Fig. 2), making the first cytological report for the species from India, however, it is noted to be in conformity with the earlier reports from outside India (Table 1).

R. macrophylla Lindl: Two accessions depict the PMCs with 2n=2x=14 chromosome number at M-I (Fig. 3) and third one showed 2n=28 at Diakinesis (Fig. 4). The count of 2n=14 is in accordance with the previous reports from India and outside India (Table 1), whereas, 2n=28 is reported for the first time from India and is in line with such reports, only from outside India (Table 1).

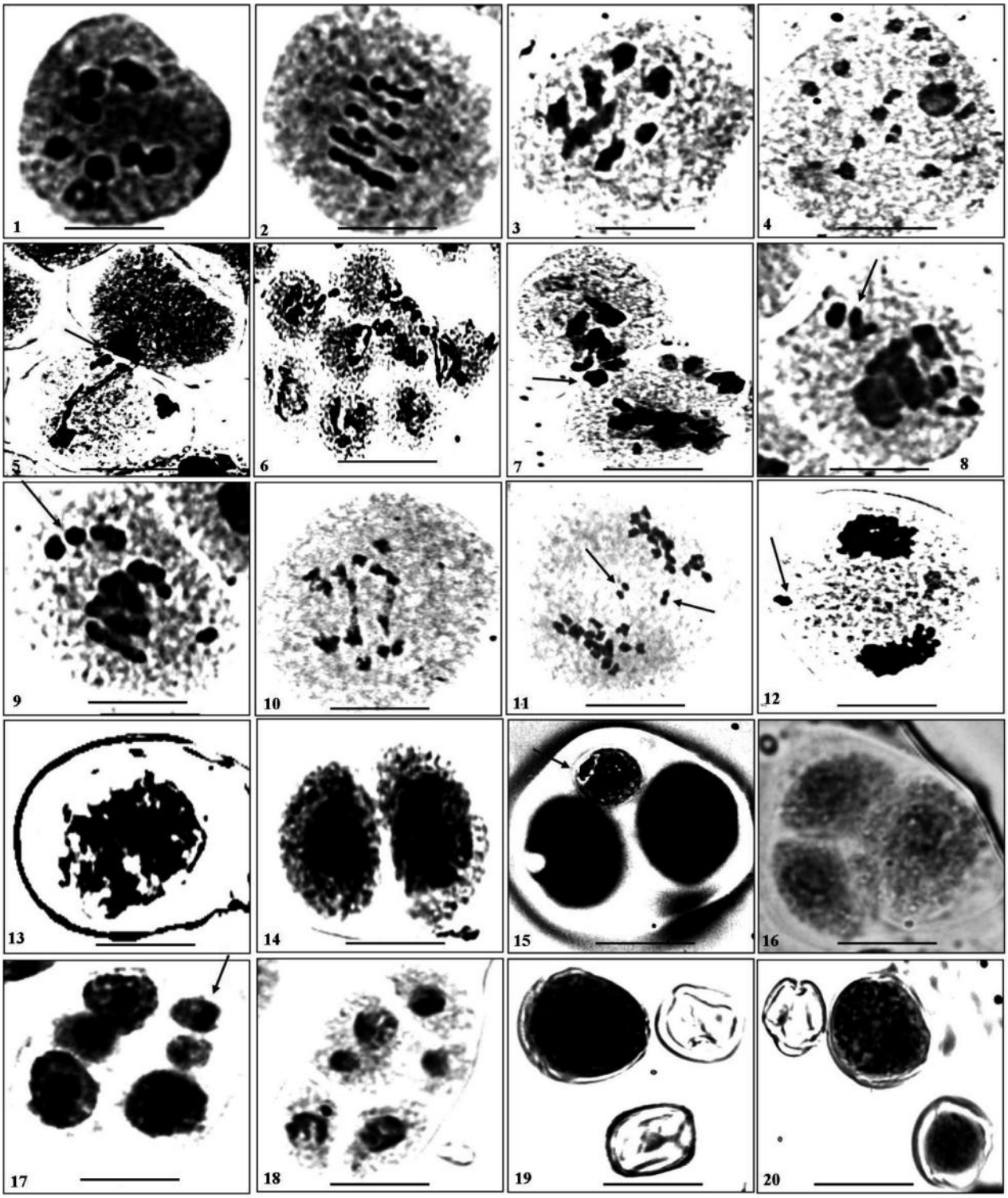
A perusal of cytological cumulative literature brings to light that 200 species/379 cytotypes of the genus *Rosa,* including 5 species from India, have been cytologically studied earlier. The chromosome numbers in the genus vary considerably from 2n=14 to 70 with 2n=2x=14 (71.50%) being the most common followed by 2n=4x=28 (52%), 2n=5x=35 (33%), 2n=6x=42 (21%), 2n=3x=21(6%), 2n=8x=56 (2.5%), [7-9]. From India, 5 species are cytologically worked out with two chromosome number counts i.e., 2n=14 and 28. Genus *Rosa* exhibits a typical polyploidy series with a basic chromosome number of x=7. The predominated euploid cytotypes in *Rosa* ranges from 2n=2x=14 to 2n=8x=56 [10]. The hexaploid cytotype (2n=6x=42) for *R. sweginzowii* from China [11], octaploid cytotype (2n=8x=56) for *Rosa acicularis* from Europe and Asia [12] and decaploid cytotype (2n=10x=70) for *R. praelucens* from China [13] was known earlier.

The meiotic abnormalities have been recorded in one-one population of all the three species of *Rosa.* In such populations, abnormalities in the form of cytomixis, chromatin stickiness, unoriented bivalents, bridges, laggards have been observed at different stages of meiosis (Figs. 5-12 and Table 2). It is obvious that chromatin transfer from early prophase to pollen formation stage has been observed for all the populations with the highest percentage recorded in population of *R. brunonii* (Figs. 5-7 and Table 2). All these meiotically abnormal populations show the presence of chromosomal laggards and bridges at anaphase and telophase quite frequently. Chromatin stickiness involving few or often complete clumping of bivalents is seen from prophase-I to metaphase-I in most of these populations (Fig. 8). Cytomixis and chromatin stickiness are considered to be the result of genetic factors [22, 23] and environmental factors [24] as well as genomic-environmental interaction [25] and seems to be equally applicable to the presently investigated populations. The unoriented bivalents at

Table 1. Data showing location, altitude, accession number, present and previous chromosome number reports, ploidy level and meiotic course of presently worked out species of genus *Rosa* L.

Taxa	Locality/altitude (m)/accession	Chromosome numbers		Ploidy level/ meiotic course
		Present (2n)	Previous[#]	
R. brunonii Lindl. (=***R. clavigera*** H.Lév.)	Triund, 3,000/56313	14	2n=14 Hurst [14-16]; Lewis & Basye [17]	2x/N**
	Dharmkot, 2,800/56314	14		2x/A*
R. indica L. (=***R. cymosa*** Tratt.)	Triund, 3,000/56315	14	2n=14 Hurst [15]; Reimann-philipp [18]	2x/N
	Dharmkot, 2,800/56316	14		2x/A
R. macrophylla Lindl. (=***R. alpina*** L. var. ***macrophylla***)	Boh, 1,900/55909	28	2n=14 Täckholm [19]; Hurst [14-16]; Mehra & Dhawan [20]; Sandhu & Mann [21] 2n= 28 Hurst [15];	4x/A
	Dharmkot, 2,800/55131	14		2x/N
	Ranhear, 800/52682	14		2x/N

*A=Abnormal, **N= Normal; #Figure in parentheses indicate the Ref.



Figs. 1- *R. brunonii* (2n=14), PMC at M-I. 2- *R. indica* (2n=14), PMC at M-I. 3- *R. macrophylla* (2n=14), PMC at M-I. 4- *R. macrophylla* (2n=28), PMC at diakinesis. 5, 6 & 7 PMCs involved in cytomixis showing hypo- and hyperploid cells. 8- PMCs showing chromosomal stickiness at M-I. 9- PMC showing unoriented bivalent at M-I. 10- PMC showing bridge at anaphase-I. 11- PMC showing laggards at anaphase-I (*R. macrophylla*). 12- PMC showing laggards at telophase-I. 13- Monad. 14- Diad. 15- Diad with micronuclei. 16- Triad. 17- & 18- Tetrad with micronuclei. 19- Heterogenous sized fertile pollen grains. 20- Fertile and sterile pollen grains. Scale 10 µm.

metaphase-I have also been observed with highest frequency noted in the populations of *R. brunonii* (Fig. 9 and Table 2). These meiotic abnormalities lead to abnormal microsporogenesis and formation of unreduced large and smaller sized fertile pollen grains (Figs. 13-20 and Table 3). The pollen grains comprise 7-9% large sized one in the different populations. The frequency of pollen grain fertility has also been observed to be lower in such populations ranging from 59-61%. On the other hand the pollen fertility in the normal populations has been seen to be high (77-85%). The occurrence of giant pollen grains, possibly the unreduced 2n pollen grains have, earlier been reported in several species [26-28]. The unreduced gametes are known to produce higher polyploidy levels through polyploidization [21]. These meiotic anomalies indicate the existence of intraspecific genetic diversities. Such genetic differences have been earlier seen in different plant species [25].

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Table 2. Data on cytomixis, meiotic course, pollen fertility and pollen grain size in different populations of species of *Rosa* L.

Taxa/accessions	Cytomixis			Meiotic course showing PMCs with			Pollen grain fertility(%)	Average size of fertile pollen grains (µm)	
	PMCs Involved Meiosis-I/II (%)	No. of PMCs involved	Chromosomal stickiness at M-I (%)	Unoriented bivalents at M-I (%)	Bridges at A-I &T-I/ A-II &T-II (%)	Laggards at A-I &T-I/ A-II & T-II (%)		Larger	Smallar
R. brunonii *56314*	22.85 (24/105)/ 12.50 (14/112)	2-5	13.91 (16/115)	11.96 (14/117)	8.69 (10/115) 5.88 (6/102)	4.72 (6/127) 3.63 (4/110)	59.90	21.21×20.67	18.89×18.02
R. indica *56316*	12.30 (16/130)/ 7.81(10/128)	2-4	12.17 (14/115)	5.88 (6/102)	6.03 (7/116) 5.98 (7/117)	11.51 (16/139) 5.83 (7/120)	57.78	20.76×20.11	17.89×17.01
R. macrophylla *55909*	8.00 (10/125)/ 8.18 (9/110)	2-3	9.00 (10/111)	3.38 (4/118)	13.79 (16/116) 6.77 (8/118)	6.55 (8/122) 13.60 (17/125)	61.23	19.43×19.00	16.89×16.00

Figures in parenthesis denote observed number of abnormal PMCs in the numerator and total number of PMCs observed in denominator

Table 3. Data on abnormal microsporogenesis in different populations of species of *Rosa* L.

Taxa/accessions	Microsporogenesis									
	Monads		Diads		Triads		Tetrads		Polyads
	WMN (%)	WM (%)	WMN (%)	WM (%)	WMN (%)	WM (%)	WMN (%)	WM (%)	
R. brunonii 56314	3.50 (4/114)	—	5.26 (6/114)	2.63 (3/114)	7.01 (8/114)	3.50 (4/114)	62.28 (71/114)	14.03 (16/114)	1.75 (2/114)
R. indica 56316	2.83 (3/106)	1.88 (2/106)	3.77 (4/106)	—	4.71 (5/106)	0.94 (1/106)	68.86 (73/106)	15.09 (16/106)	1.88 (2/106)
R. macrophylla 55909	0.98 (1/102)	—	3.92 (4/102)	2.94 (3/102)	1.96 (2/102)	—	75.49 (77/102)	13.72 (14/102)	—

WMN = without micronuclei; WM = with micronuclei.
Figures in parenthesis denote observed number of abnormal PMCs in the numerator and total number of PMCs observed in denominator.

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