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Cyto-morphology of Some Gamopetalous Species from Jammu Area (Jammu & Kashmir), India

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Meiotic course and pollen fertility was investigated in 11 gamopetalous species from Jammu Division (Jammu & Kashmir), India. The varied hexaploid (6x) chromosome count for *Anaphalis adnata* (n = 21), *Veronica cana* (n = 21) and diploid (2x) for *Synedrella nodiflora* (n = 12) are reported for the first time. Further 0-2B chromosomes are reported for the first time in *Artemisia roxburghiana*. While, tetraploid (4x) chromosomal count in *Veronica laxa* (n = 16) is the first report from India.

Keyword: Cytomorphology, *Gamopetalae*, Jammu, meiosis.

Jammu and Kashmir, the northern most state of India is situated among the Himalayan mountains and divided into three divisions: Jammu, Kashmir valley and Ladhakh. Jammu division is located between 32°43′48″ N 74°52′12″ E, varies from from 330–4200 m in altitude and covers an area of 26,293 km^2. The total forest area in Jammu region is 9,481.98 hectares and ranges from thorn scrub to temperate and alpine types (Champion and Seth 1968). From the Jammu region, 660 species in 431 genera of angiosperms (215 species in 155 genera belong to subclass *Gamopetalae*) have been recorded by Sharma and Kachroo (1981). This region is the source of some medicinal and aromatic plants. Some workers (Koshoo and Sobti 1958, Kapoor et al. 1963, Koul and Gohil 1973, Sharma and Kachroo 1981, Jee et al. 1989, Masood and Shafi 2005, Malik et al. 2011a, Jeelani et al. 2011) have cytologically explored the flora of Jammu & Kashmir. But little work has been done on the cytology of Jammu region, such as *Plantago* spp. (Sharma et al. 1984), *Swertia* spp. (Khoshoo and Tondon 1958, Chakraborty et al. 2009). With the phytogeographical wealth of this region in mind, the present cytological study is an attempt to add new or varied cytotypes.

Materials and Methods

Appropriate sized flower buds were collected and fixed in Carnoy's fixative (Alcohol: Chloroform: Acetic acid in 6: 3: 1 ratio). After 24 hrs, the buds were transferred to rectified alcohol till further use. Meiotic studies were made by squashing anthers in standard 1% acetocarmine solutions. Photographs of pollen mother cells (PMCs) were made in freshly prepared slides using a Nikon 80i Eclipse Microscope. Pollen fertility was estimated by their stainability in 1% glyceroacetocarmine. Filled and stained pollen grains were considered fertile whereas shrunken and unstained pollen grains were taken as sterile. Voucher specimens were submitted to Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

Table 1. Locality with altitude, accession number, chromosome number, ploidy level, habit, pollen fertility and pollen size of the species studies

	Locality with altitude (m)	Accession no. (PUN)	Chromosome number (n)	Ploidy level	Habit*	Pollen fertility (%)	Pollen size (μm)
Asteraceae							
Ageratum conyzoides P1	Basohli, 678	56473	9	2x	AH	80	22.1 × 28.3
P2	Bani, 1280	56469	18	4x	AH	95	21.4 × 25.6
Anaphalis adnata	Dullangal, 2000	56487	21^{oo}	6x	PH	85	15.5 × 23.3
Artemisia capillaris	Sarthal, 2187	56464	9	2x	PH	89	11.6 × 19.4
Artemisia roxburghiana	Bani, 1280	56465	9+0-2B[1]	2x	PH	85	9.0 × 14.2
Blumea laciniata	Bani, 1280	56483	9	2x	AH	89	11.4 × 15.9
Erigeron multicaulis	Sarthal, 2187	56461	9	2x	AH	85	16.3 × 19.5
Senecio kunthianus	Duggan, 2200	56472	20	4x	PH	74	16.1 × 20.3
Synedrella nodiflora	Basohli, 678	56491	12^{oo}	2x	AH	79	15.7 × 18.5
Scrophulariaceae							
Lindenbergia indica	Basohli, 678	56497	25	2x	AH	80	13.5 × 16.1
Veronica cana	Banjal, 1406	56495	21^{oo}	6x	PH	95	13.8 × 14.5
Veronica laxa	Sarthal, 2187	56515	16^{o}	4x	PH	92	15.5 × 17.6

*Habit. AH. Annual herb. PH. Perennial herb. PS. Perennial shrub. BS. Biennial shrub. T. Tree.
^{oo} First ever cytotype reports on the world basis.
[1] First B-chromosome report.
^{o} First cytotye report from India.

Result and Discussion

Eleven wild species growing wild, belonging to nine genera from different localities of Jammu region were cytomorphologically investigated. The information on name of the species, locality with altitude, accession number, chromosome number, ploidy level, habit, pollen size and fertility are presented in Table 1.

Ageratum conyzoides (*Asteraceae*)

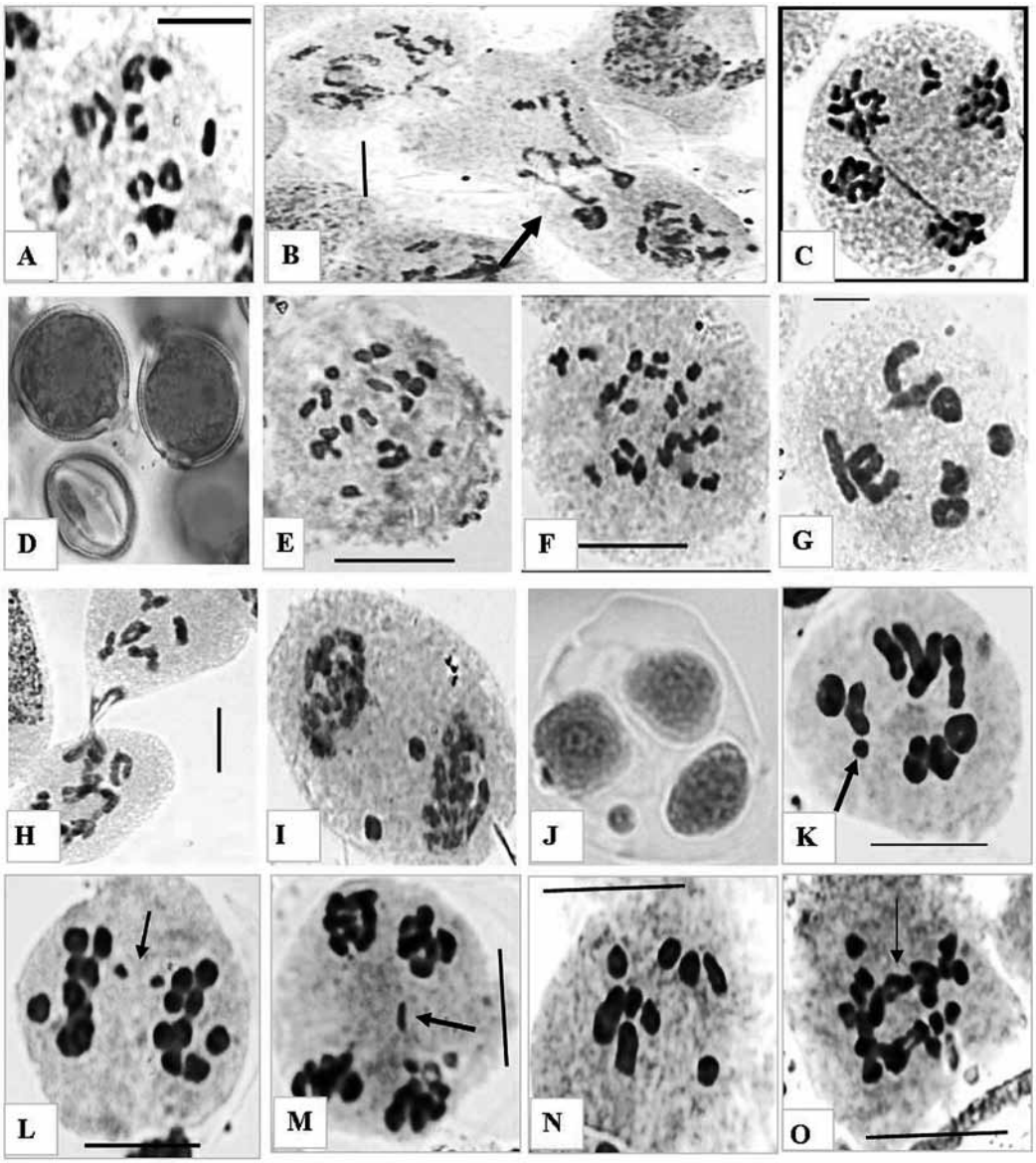
The species is widely distributed in tropical and subtropical regions in grasslands, forests, agriculture, plantation and horticulture fields between 1400–1500 m. The species shows a great morphological variation. Two populations, P1 and P2 (Table 1) are found to be diploid (n = 10; Fig. 1A) and tetraploid (n = 20; Fig. 1D), respectively. Besides a little morphological disparity, the diploid cytotype showed irregular meiosis in the form of chromatin transfer (Fig. 1B) and chromatin bridges (Fig. 1C) in 20% and 9.25% of PMCs respectively, which leads to reduced pollen fertility (Fig. 1D). Population (P2) is normal with high pollen fertility. Both the cytotypes are quite common and reported persistently from different parts of the world (Okunade 2002). B-chromosomes are also observed in diploid cytotype by Gill and Gupta (1971). Both the cytotypes have earlier been reported to have normal meiosis and quite indistinct morphology (Gupta and Gill 1984).

Anaphalis adnata (*Asteraceae*)

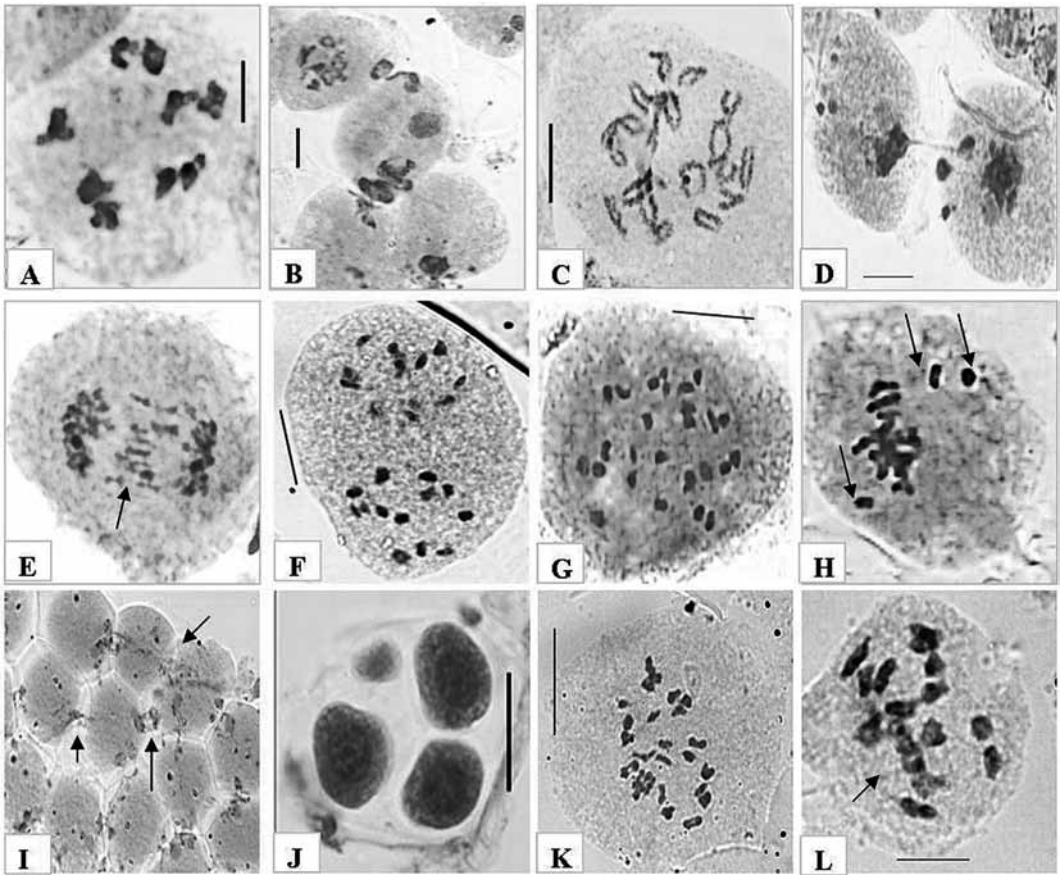
The species is widely distributed in the Western and Eastern Himalayas, Gangetic plain and Deccan plateau in shady slopes and crevices of rock up to 3200 m (Hajra et al. 1995). Cytological investigations reveal that, for the species, this is first ever hexaploid (n = 21; Fig. F) cytotype. Previously, tetraploid (n = 14) cytotype was reported frequently from north and south India (Mehra et al. 1965, Gupta et al. 1989).

Artemisia capillaris (*Asteraceae*)

This species is widespread in temperate



Figs. 1. Chromosomes of some gamopetalous species from Jammu & Kashmir, India. A–E. *Ageratum conyzoides,* P-I (n = 10) . A. PMC at metaphase-I. B. PMCs involved in cytomixis. C. Chromatin bridge at anaphase-II. D. Heterogeneous sized pollen grains, P-2 (n = 20). E. PMC at metaphase-I. F. *Anaphalis adnata* (n = 21): PMC at metaphase-I. G–J. *Artemisia capillaris* (n = 9): G. PMC at metaphase-I, H. Cytomixis at metaphase-I, I. Laggards at telophase-I, J. Triad with micronucleus. K–M. *Artemisia roxburghiana* (n = 9). K. PMC at metaphase-I with 1B chromosome. L. 2B chromosomes at anaphase-I. M. 1B chromosome at telophase-II. N–O. *Blumea laciniata* (n = 9). N. PMC at metaphase-I showing 2 large sized bivalents. O. Late disjunction at anaphase-I. Scale = 10 µm.



Figs. 2. Chromosomes of some gamopetalous species from Jammu & Kashmir, India. A–B. *Erigeron multicaulis* (n = 9). A. PMC at metaphase-I. B. PMCs at early stages showing transfer of chromatin material. C–E. *Senecio kunthianus* (n = 20). C. PMC at Diakinesis. D. PMC involved in chromatin transfer. E. Late disjunction at anaphase-I (arrow). F. *Synedrella nodiflora* (n = 12): PMC at anaphase-I. G–J. *Lindenbergia indica* (n = 25). G. PMC at metaphase-I. H. Unoriented bivalents at metaphase-I (arrows). I. Cytomixis. J. Triad with micronucleus. K. *Veronica cana* (n = 21). PMC at metaphase-I. L. *Veronica laxa* (n = 16): PMC at metaphase-I. Scale = 10 μm.

Europe, Asia, northern Africa, Alaska and north America on waste places in nitrogen rich soil (Cardini and Haung 1998). In India, it is distributed in the Indo-Gangetic plains and the Western Himalayas between 1500–3500 m. The diploid (n = 9) chromosomal count has been confirmed from the presence of 9 bivalents at metaphase-I (Fig. 1G). The species also shows some cytomixis at metaphase-I (Fig. 1H) and laggards at telophase-I (Fig. 1I) in some of the PMCs (4.67%). Consequently, the microsporogenesis was abnormal and showed presence of diads and triads (Fig. 1J) with or without micronuclei, which leads to heterogeneous sized pollen grains and low pollen fertility (67%). The present chromosome count has been reported by many workers (Gupta et al. 2012). Besides, the species show diploid cytotypes based on x = 8, 9, 10, 11 and intraspecific polyploids (2x, 4x) on x = 9

(Gupta et al. 2012). Valles and Torrel (1995) has reported B-Chromosomes in the species.

Artemisia roxburghiana (*Asteraceae*)

The species occurs along the roadsides in Nepal, south China, north-east Afghanistan and the Western Himalayas between 1000–3500m. The diploid chromosome count (n = 9 + 1B) is confirmed at metaphase-I (Fig. 1K). The presence of 0-2B chromosomes have been observed in most of the PMCs (83%) at Diakinesis, metaphase-I & II and anaphase-I & II (Figs. 1L, 1M). Although, intraspecific polyploid races n = 9 (Bala and Gupta 2011), and n = 18 (Koul and Bakshi 1984) have been reported earlier. B-chromosomes has been reported for the first time for this diploid cytotype (n = 9+0-2B).

Blumea laciniata (*Asteraceae*)

The species occurs throughout the plains of India in forest clearings, along roadsides and in waste places up to 1200 m. The diploid chromosome count (n = 9) is confirmed at metaphase-I with two larger sized bivalents (Fig. 1N) which showed some irregular segregation at anaphase-I (Fig. 1O). Athough, the chromosome count has already been confirmed by many workers, but other report of n = 10 (Gupta 1969) and n = 11 (Mehra and Remanandan 1975, Daruwalla 1995) seems to be due to miscounting, as the species has two bivalents with almost double the size of other bivalents.

Erigeron multicaulis (*Asteraceae*)

The species is well spread in temperate and alpine Himalayas up to 2000–4000 m. Meiotic study revealed the presence of nine bivalents at metaphase-I (Fig. 2A). Further, some of the PMCs (38.07 %) showed laggards at anaphase-I & II and chromatin mass transfer (28%) at prophase-I to anaphase-I (Fig. 2B), leading to the formation of hypo/- and hyperploids cells. The present report is in conformity with the previous reports (Mehra et al. 1965, Gupta et al. 1989) from India. Beside this, another hexaploid cytotype with n = 27 has been reported by Koul (1964). Thus the species exists at diploid and hexaploid levels on x = 9.

Senecio kunthianus (*Asteraceae*)

The species is confined to the Western Himalayas up to 1200–4000 m. The species is diploid (n = 20; Fig. 2C) and further meiotic course is found to be abnormal. Chromatin transfer (Fig. 2D) and late disjunction (Fig. 2E) has been observed in 12% and 16% of PMCs, respectively. The same chromosome number has been reported previously by Gupta et al. (2010) in the population from Lahaul-Spiti district of Himachal Pradesh.

Synedrella nodiflora (*Asteraceae*)

The species is native to tropical America and now considered as pantropical weed and usually grows as a weed of agricultural land and waste places. Meiotically, it is first ever diploid chromosomal count (n = 12; Fig. 2F) for this species. However, earlier the chromosome report of n = 9, 18 (Nirmala and Rao 1984), n = 17, 38 (Jose and Mathew 1995), n = 19 (Gajapathy 1962) and n = 20 (Gupta and Gill 1984) have been reported from India. From outside India n = 19 (Powell and King 1969) and n = 9 (Peng and Hsu 1978) have been reported. Thus the species shows a lot of chromosomal diversity.

Lindenbergia indica (*Scrophulariaceae*)

This polymorphic species is found between 400–2100 m throughout India. The gametophytic chromosome count of n=25 has been confirmed by 25 bivalents at metaphase-I (Fig. 2G). Further, meiotic course showed unoriented bivalents (Fig. 2H), laggards (7%), chromatin transfer (26%, Fig. 2I), consequently micronuclei (Fig. 2J) appear which form heterogenous sized pollen grains. This chromosome count has been confirmed previously by Bala and Gupta (2011) from Kangra District (Himachal Pradesh). Besides, other cytotypes from India are of 2n =

28 (Bhattacharyya 1967) and 2n = 30 (Trivedi and Trivedi 1992), and from outside India 2n = 30 (Khatoon and Ali 1993) from Pakistan.

Veronica canna (*Scrophulariaceae*)

The species is native to temperate Himalayas, distributed at an altitude of 2800–4100 m in moist places. Present investigation found, new varied chromosome count of hexaploid cytotype with n = 21 (Fig. 2K) based on x = 7. Prior to this, the species is known to have three other cytotypes, n = 25 (Mehra and Vasudevan 1972), n = 26 (Vasudevan 1975) and n = 23 (Malik et al. 2011b) from the Western Himalayas.

Veronica laxa (*Scrophulariaceae*)

The species is very rare, and found between 2100–3500 m. Meiotic studies revealed the species to be tetraploid (n = 16) with the presence of 16 bivalents at metaphase-I (Fig. L). It is the new chromosome report for the species from India. Earlier reported chromosome number (n = 23) is quite common in the Western Himalayas (Mehra and Gill 1968, Vasudevan 1975) and outside India (Hong and Zhang 1990).

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R. C. Gupta, A. Mehra, V. Singh：**インド，ジャム・カシミール地方産合弁花類数種の細胞学的研究**

インド，ジャム・カシミール地方に分布する11種の合弁化類について，減数分裂と花粉の稔性を調べた．その結果，*Anaphalis adnata*（キク科）と *Veronica cana*（ゴマノハグサ科）に六倍体が，*Synedrella nodiflora*（キク科）に二倍体が初めて報告された．さらに，キク科ヨモギ属の *Artemisia roxburghiana* にB染色体が初めて見出された．*Veronica laxa* に見られた四倍体はインドでは初めての報告となる．

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