

CYTOLOGICAL STUDIES IN SOME SELECTED MEMBERS OF GENTIANACEAE FROM KASHMIR HIMALAYAS

Younas Rasheed Tantray¹, Reyaz Ahmad Malik¹ and Syed Mudassir Jeelani²

¹Department of Botany, Punjabi University Patiala, Punjab (India)

²Indian Institute of Integrative Medicine, Jammu (India)

ABSTRACT

The family Gentianaceae is known by 1600 species worldwide and its members are commonly found in temperate and sub-tropical areas. In Kashmir Himalayas the family is represented by 55 species. The present work includes the detailed male meiotic studies in 10 accessions belonging to 5 species and 3 genera of family Gentianaceae from Kashmir Himalayas. All the presently investigated wild populations of the species exist at 2x level. The present study reports the existence of varied cytotype for *swertia ciliata* ($n = 13$). The studied accession of *Gentiana carinata* ($n=10$) shows various meiotic abnormalities including cytomixis, chromosome stickiness, unoriented bivalents and abnormal sporads leading to reduction in pollen fertility. Besides, the two species of genus *swertia* (s. *cordata* and s. *petiolata*) depicted the meiotic irregularities in the form of chromosome stickiness and late disjunction of bivalents.

Key words: *Male meiosis, Kashmir Himalayas, cytomixis, chromosome stickiness.*

1. INTRODUCTION

The family Gentianaceae represented by 85 genera covering around 1600 species [1] and is cosmopolitan in distribution but more commonly found in temperate and sub-tropical or at high altitudes in the tropics. In India the family is known by 16 genera and 145 species and is found chiefly in Himalaya, Southern and Western India. From Kashmir 6 genera and 55 species are known which are mainly restricted in alpine parts. The members are annual, biennial, or perennial herbs, shrubs or small trees, usually rhizomatous with opposite, entire and exstipulate leaves, flowers are regular in symmetry, petals 4-5 united, stamens epipetalous and ovary consists of 2 united carpels. Economically, some species are cultivated as ornamentals and making dyes. Many species are used in medicines and as flavourings. A number of biologically active chemical compounds such as alkaloids, seco-iridoids and xanthones, make Gentians of high medicinal value [2]. Besides being cultivated as ornamentals, many species of Gentianaceae are known to contain bitter compounds and various phytochemicals [3]. As far as cytology is concerned 36 species are cytologically known so far from India. The Indian species are

known to have $2n = 16, 18, 20, 22, 24$ and 26 with $2n = 26$ i.e. $x = 13$ being the most common but $x = 8, 9$ and 10 are also represented. As a part of cytomorphological explorations of flowering plants from the Kashmir Himalayas, we have worked out 10 accessions (5 species and 3 genera). The aim of the present study was to workout the detailed meiotic course including microsporogenesis and pollen fertility in all the accessions.

II. MATERIALS AND METHODS

2.1 Collection of materials

For cytomorphological investigations, wild accessions were collected on population basis from the high altitudinal regions of Kashmir Himalayas falling in the altitudinal range of 2500-3300m. The accessions were identified by consulting floras and were compared with the specimens lying in the Herbaria (PUN), Punjabi University, Patiala and Northern Regional Centre, Botanical Survey of India, Dehra Dun (BSD). The duly identified specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala. For meiotic and pollen grain studies, floral buds of appropriate sizes were fixed in a freshly prepared Carnoy's fixative (mixture of alcohol, chloroform and acetic acid in a volume ratio 6:3:1) and preserved in 70% ethanol in a refrigerator.

2.2 Male meiosis and pollen grain analysis

Meiocytes were prepared by squashing the young and developing anthers in 1% acetocarmine. These were observed at various stages of prophase-I, metaphase-I, anaphase-I/II, telophases-I/II and sporads. Pollen fertility was assessed through stainability tests by squashing mature anthers from various blossoms in glycerol-acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were scored as apparently fertile, while shrivelled and flaccid pollen grains with unstained or poorly stained cytoplasm as sterile.

III. RESULTS AND DISCUSSION

A total of 5 species (10 accessions) falling under 3 genera were analyzed for chromosome counts, detailed meiotic course including microsporogenesis and pollen fertility. The information on site of collection, Vouchers, gametic chromosome number, ploidy level, pollen fertility percentage and previous chromosome reports are provided in Table 1. The cytological findings noticed in the accessions of investigated species are briefly discussed here.

Gentiana carinata Griseb. Presently analyzed species showed the gametic chromosome count of $n = 10$ as confirmed from the unequivocal presence of 10 bivalents at M-I (fig.1a). The present chromosome count of $n = 10$ is already reported from the study area by [4] and [5, 6] and therefore confirms the earlier reports. The meiotic course is quite abnormal from early prophase throughout the microsporogenesis. The chromosome stickiness is observed in 9% PMCs at metaphase-I (fig.1b). The phenomenon of cytomixis involving chromatin transfer among neighbouring PMCs was also observed at early prophase-I leading to empty PMCs (fig.1c). Besides in 20% of the PMCs an unoriented bivalents were noticed at metaphase-I (fig.1d). All these anomalies

lead to abnormal microsporogenesis with monad, triad and polyad (Fig. 1e) formation, besides the normal tetrads, leading to low pollen fertility of 73 per cent.

***Jaeschkea gentianoides* Kurz.** The analyzed accessions collected from two different localities (Gurez 3000 m, Thajwas 3300 m) exist at $2x$ level with a gametic chromosome count of $n = 11$ which was ascertained from the presence of 11 bivalents at metaphase-1 (fig.1f). The present chromosome count of $n=11$ confirms the earlier report from Kashmir by [7]. Although there is no obvious meiotic irregularity but the pollen fertility is low (72%).

***Swertia ciliata* D. (Don ex. G. Don) B.L Burtt.** The chromosome count of $n = 13$ (fig. lg) recorded in the presently analyzed accession of *Swertia ciliata* from Aharbal (2500 m) adds a new dysploid cytotype to the already reported chromosome number of $n = 12$ by [8] from other parts of India. The individuals depicted highly irregular meiotic course (Table 2) in the form of laggards at anaphase-1 (fig.1h) and unoriented bivalents at metaphase-1 (fig.1i). In microsporogenesis triads (fig. 1j) were also observed besides normal tetrads leading to low pollen fertility of 77.4 percent (Fig. 1k).

***Swertia cordata* Wall.** Three wild accessions collected from Gurez (3000 m), Sunmarg (3000 m) and Baltal (3100 m) uniformly showed the same diploid gametic chromosome count of $n = 13$ which was ascertained from the 13 large sized bivalents at metaphase-I (fig. 1l). The chromosome number of $2n = 26$ confirms the previous cytological report [4] from India. The meiosis shows irregularities (Table 2) in the form of chromatin stickiness at metaphase-I (Fig. 1m) and metaphase-II (Fig. 1n). However, no adverse effect on further microsporogenesis is reported, leading to high pollen fertility (94.6-99.5%)

***Swertia petiolata* Royle.** All the analyzed accessions exist at $2x$ level with a gametic chromosome count of $n = 13$ which was confirmed by the presence of 13 bivalents at M-1 (fig. 1o). The present chromosome count is in agreement with the earlier report by [9, 10] and [4] from Kashmir. The meiotic course is normal and results in high pollen fertility of 86-89.5 percent.

A number of previous researchers have reported various meiotic abnormalities in various angiospermic species growing in Kashmir Himalayas including cytomixis, abnormal spindle activity, syncyte formation [11, 12, 13] which results in the formation of aneuploid and polyploid species therefore playing a significant role in chromosomal evolution and hence speciation.

IV. ACKNOWLEDGEMENTS

The authors are grateful to the University Grant commission, New Delhi for providing financial assistance under Junior Research Fellowship to Younas Rasheed (Award letter no. 2121430298 12/8/2015). The authors are also thankful to Head, Department of Botany, Punjabi University, Patiala for providing necessary laboratory, herbarium and library facilities.

REFERENCES

- [1] DJ Mabberley 2008, *Mabberley's Plant Book:A Portable Dictionary of Plants Classification and Uses* (Third Edition), Cambridge University Press, Cambridge, UK.
- [2] JS Negi, P. Singh and B. Rawat, Chemical constituents and Biological Importance of *Swertia*: A Review, Current Research in Chemistry, 3, 2011, 1–15.
- [3] A Singh, Phytochemicals of Gentianaceae, A review of pharmacological properties. International Journal of Pharmaceutical sciences and Nanotechnology, 1, 2008, 33-36.
- [4] KN Vasudevan, Contribution to the cytotaxonomy & cytogeography of the flora of the Western Himalayas (with an attempt to compare it with the flora of Alps). Part II. Bericht der Schweizerischen Botanischen Gesellschaft, 85, 1975, 210– 252.
- [5] V Jee, U. Dhar and P. Kachroo, Chromosomal conspectus of some alpine subalpine taxa of Kashmir Himalaya, Chromosome Information Service, 39, 1985, 33–35.
- [6] V Jee, U. Dhar and P. Kachroo, Cytogeography of some endemic taxa of Kashmir Himalaya, Proceedings of the Indian National Science Academy, B 55, 1989, 177–184.
- [7] RN Gohil, M. Ashraf and R. Raina, Cytotaxonomical conspectus of the flora of Kashmir II. Chromosome numbers of 51 dicotyledonous species, Herba Hungarica, 20, 1981, 43–49.
- [8] PN Mehra and LS. Gill, In: IOPB chromosome number reports XVI, *Taxon*, 17, 1968, 199–204.
- [9] TN Khoshoo and SR. Tandon, Cytological, morphological and pollination studies on some Himalayan species of *Swertia*, Caryologia, 16, 1963, 445–477.
- [10] AK Koul and PN Gohil, Cytotaxonomical conspectus of the flora of Kashmir, I. Chromosome numbers of some common plants, Phyton, 15, 1973, 57–66.
- [11] MIS Saggoo and U. Farooq, Cytology of *Rheum*, a vulnerable medicinal plant of Kashmir Himalaya, Chromosome Botany, 6, 2011, 41-44.
- [12] RA Malik and RC. Gupta, Meiotic studies in some selected members of Gamopetalae from Kashmir Himalaya, Plant Systematics and Evolution, 299, 2013, 1549-1560.
- [13] RA Malik, RC. Gupta, S. Kumari and AH. Malik, Cytomictic anomalous male meiosis and $2n$ pollen grain formation in *Mertensia echinoides* Benth. (Boraginaceae) from Kashmir Himalaya. Scientific World J. 2014, Article ID 134192, 7 pages doi: 10.1115/2014/134192.

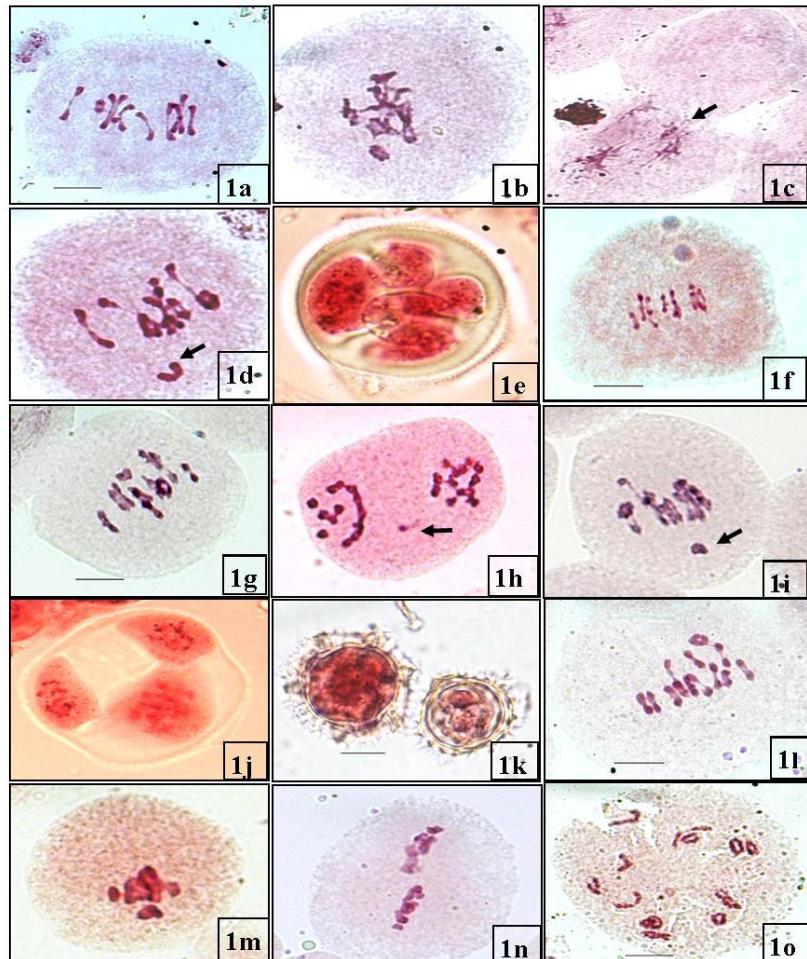


Figure 1 Male meiosis in Gentianaceae. *Gentiana carinata*: (a) a PMC showing 10 bivalents at M-1; (b) a PMC showing chromatin stickiness at M-1; (c) cytomixis showing enucleated and syncytic PMC (arrowed); (d) a PMC showing unoriented bivalent at M-1 (arrowed); (e) Polyad. *Jaeschkea gentianoides*: (f) a PMC showing 11 bivalents at M-1. (g) *Swertia ciliata*: a PMC showing 13 bivalents at M-1; (h) PMC at A-1 with a laggard (arrowed); (i) a PMC showing off-plate bivalent at M-1 (arrowed); (j) Triad; (k) Apparently fertile and sterile pollen grains. *Swertia cordata*: (l) a PMC showing 13 bivalents at M-1; (m) a PMC showing chromatin stickiness at M-1; (n) a PMC showing chromatin stickiness at M-II. *Swertia petiolata*: (o) a PMC at early M-1 showing 13 bivalents.

Table 1. Information on site of collection along with altitude, vouchers (PUN), gametic chromosome number, ploidy level, pollen fertility (%age) and previous counts on the cytologically investigated species from Kashmir Himalayas.

Taxon	Site of collection with altitude (in meters)	Vouchers PUN	Gametic chromosome number (n)	Ploidy level	Pollen fertility (%age)	Previous reports
1. <i>Gentiana carinata</i> Griseb. (= <i>Ericala carinata</i> D.Don ex G.Don)	Thajwas Hill 3300	56941	10	2x	73	2n = 20: Vasudevan 1975a; Jee et al. 1985; 1989 2n = 40: Mehra & Gill 1968a
2. <i>Jaeschkeia gentianoides</i> Kurz. (= <i>J. oligosperma</i> (Griseb.)	Gurez 3000 Thajwas 3300	57006 57007	11 11	2x 2x	72 69	2n = 18: Koul & Gohil 1973 2n = 22: Gohil et al. 1981, 1981a
3. <i>Swertia ciliata</i> (D.Don ex G.Don) B.L.Burtt.	Aharbal 2500	54357	13	2x	77	2n = 24: Mehra & Gill 1968 2n = 26: Malik et al. 2011b
4. <i>S. cordata</i> Wall	Gurez 3000 Sonmarg 3000 Baltal 3100	57050 57051 57052	13 13 13	2x 2x 2x	98 99 95	2n = 26: Vasudevan 1975
5. <i>S. petiolata</i> Royle (= <i>S. lahulensis</i> A. Kerner S. <i>obtusipetala</i> Gruning.)	Gulmarg 2800 Tangmarg 2600 Sonmarg 3100	55187 56969 56970	13 13 13	2x 2x 2x	89 85 86	2n = 26: Khoshoo & Tandon 1963; Koul & Gohil 1973; Vasudevan 1975a

Table 2: Meiotic abnormalities in the population of 3 species of Swertia

Meiotic abnormality	<i>S. ciliata</i>	<i>S. cordata</i>			<i>S. petiolata</i>		
Chromatin stickiness	<u>P-1</u> --/--	P-1 42.8 (21/45)	P-2 52.9 (27.51)	P-3 42.1 (16/38)	P-1 --/--	P-2 --/--	P-3 --/--
Unoriented bivalents	19.2 (5/26)	--/--	--/--	--/--	--/--	--/--	--/--
Non-synchronous disjunction	09.6 (3/31)	--/--	--/--	--/--	5.8 (2/34)	5.5 (1/8)	--/--
Chromatin bridge	14.2 (4/28)	--/--	--/--	--/--	5.2 (1/19)	6.4 (2/31)	4.7 (1/21)
Laggards	04.0 (2/50)	--/--	--/--	--/--	--/--	--/--	--/--
Pollen fertility %	77.4	99.5	98.0	94.6	89.5	86.3	86.6

Figures in the parentheses denote frequency of respective abnormal PMCs