International Journal of Advance Research in Science and Engineering
Volume No.06, Issue No. 11, November 2017
www.ijarse.com
IJARSE
ISSN: 2319-8354

CYTOLOGICAL STUDIES IN SOME SELECTED MEMBERS OF GENTIANACEAE FROM KASHMIR HIMALAYAS

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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 irregularties in the form of chromosome stickiness and late disjunction of bivalents.

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

I. 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 by16 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 belonging t

o 5 species and 3 genera having chromosome count of 2*n* = 20, 22 and 26. The aim ofthe present study was to w ork out the detailed meiotic course including microsporogenesis and pollen fertility in all the accessions.

II. MATERIALS AND METHODS

2.1 Collection of materials

For cytomorphogical 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.

2.2Male 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-1, metaphase-1, anaphase-1/II, telophases-1/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-1(fig.1b). The phenomenon of cytomixis involving chromatin transfer among neighbouring PMCs was also observed at early prophase-1 leading to empty PMCs (fig.1c). Besides in 20% of the PMCs an unoriented bivalents were noticed at metaphase-1(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 2*x* 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]. Alhough 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) uniformally 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 2*n* = 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 2*x* 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.

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International Journal of Advance Research in Science and Engineering
Volume No.06, Issue No. 11, November 2017
www.ijarse.com
IJARSE
ISSN: 2319-8354

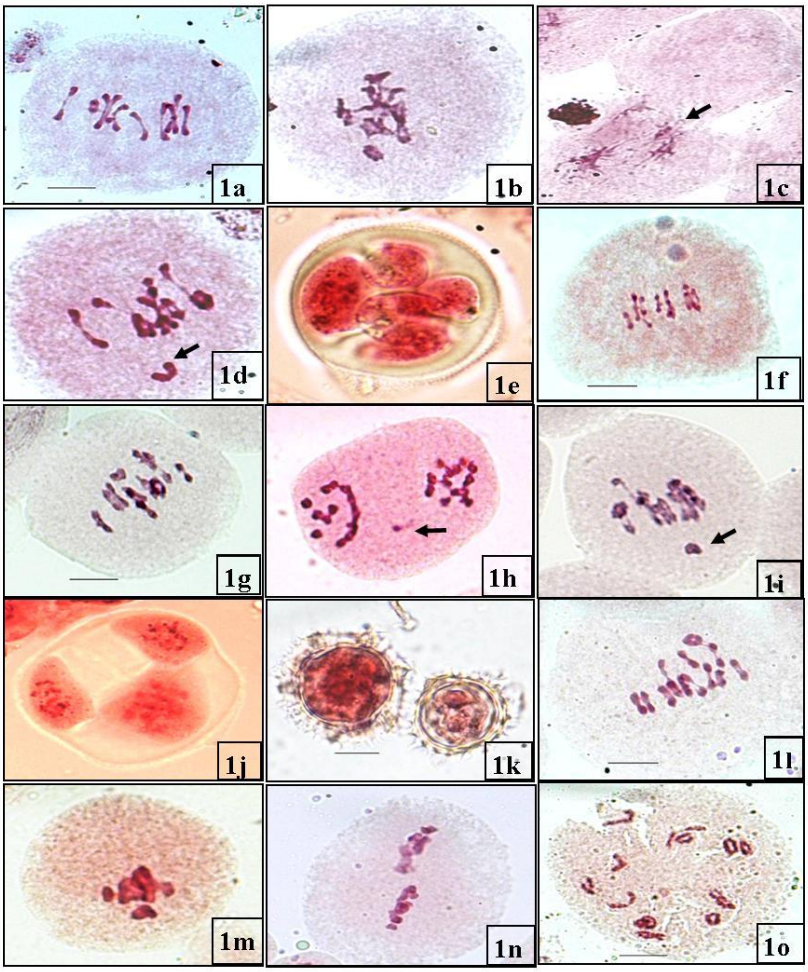

Figure1 Male meiosis in Gentianaceae. *Gentiana carinata*: (a) a PMC showing 10 bivalents at M-1; (b) a PMC showing chromatin stickiness at M-1; (c) cytomixis showing enucleated and syncytic PMC (arrowed); (d) a PMC showing unoriented bivalent at M-1(arrowed); (e) Polyad. *Jaeschkea gentianoides*: (f) 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. *Swetia petiolata*: (o) a PMC at early M-1 showing 13 bivalents.

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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. *Gentiana carinata* Grisel (= *Ericala carinata* D.Don G.Don)	Thajwas Hill 3300	56941	10	2x	73	**2*n* = 20:** Vasudevan 1975a; Jee *et al.* 19 1989 **2*n* = 40:** Mehra & Gill 1968a
2. *Jaeschkea gentianoides* H (= *J. oligosperma* (Grisel	Gurez 3000	57006	11	2x	72	**2*n* = 18:** Koul & Gohil 1973 **2*n* = 22:** Gohil *et al.* 1981, 1981a
	Thajwas 3300	57007	11	2x	69	
3. *Swertia ciliata* (D.Don e: Don) B.L.Burtt.	Aharbal 2500	54357	13	2x	77	**2*n* = 24:** Mehra & Gill 1968 **2*n* = 26:** Malik *et al.* 2011b
4. *S. cordata* Wall	Gurez 3000	57050	13	2x	98	**2*n* = 26:** Vasudevan 1975
	Sonmarg 3000	57051	13	2x	99	
	Baltal 3100	57052	13	2x	95	
5. *S. petiolata* Royle (= *S. lahulensis* A. Kerner *S. obtusipetala* Gruning.)	Gulmarg 2800	55187	13	2x	89	**2*n* = 26:** Khoshoo & Tandon 1963; Kou Gohil 1973;
	Tangmarg 2600	56969	13	2x	85	Vasudevan 1975a
	Sonmarg 3100	56970	13	2x	86	

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

Meiotic abnormality	*S. ciliata*	*S. cordata*			*S. petiolata*		
	P-1	P-1	P-2	P-3	P-1	P-2	P-3
Chromatin stickiness	--/--	42.8 (21/45)	52.9 (27.51)	42.1 (16/38)	--/--	--/--	--/--
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