

CYTOMORPHOLOGICAL INVESTIGATIONS IN *OXYRIA DIGYNA* HILL. FROM THE KASHMIR HIMALAYA, INDIA

In the present paper, detailed cytomorphological investigations in Oxyria digyna Hill. from Kashmir Himalaya–India have been reported for the first time. All the of 14 investigated populations of O. digyna are diploid based on $x = 7$. Out of these in two populations 0–2B chromosomes have been recorded for the first time while 6 populations differed significantly in their meiotic characteristics. Meiotic abnormalities during male meiosis observed include inter PMC chromatin transfer (cytomixis). Non-synchronous disjunction of some bivalents, laggards and bridges at anaphases and telophases. Consequent to these meiotic anomalies, microsporogenesis in meiocytes is abnormal resulting in to dyads, triads and polyads with or without micronuclei. The overall effect is seen in reduced pollen fertility. Unreduced pollen grains were observed in some populations, which differed significantly in their size compared to the normal (reduced) pollen grains. It is observed that a smaller frequency of pollen grains differed morphologically in Aharbal and Yosmarg populations. The remaining eight populations showed regular meiotic course, normal microsporogenesis and high percentage of pollen fertility (95.09–99.09 %).

Key words: *Oxyria digyna*, cytomorphology, B-chromosomes, meiotic abnormalities.

Introduction. The mountain Sorrel (*Oxyria digyna* Hill.), a monotypic species of family Polygonaceae is a succulent herb having rounded, cordate or reniform mostly radical leaves and greenish or reddish flowers borne in panicles. In India, it is found in the higher reaches of Himalayas from Kashmir to Sikkim and flowers from May–July. The herb has antiscorbutic and refrigent properties [1, 2]. Its leaf extract is used to improve digestion by tribals in Ladakh [3, 4]. It is a highly cold tolerant species and can survive under continuous snow cover for two to three years in succession [5]. The different populations of the species are quite distinct morphologically and show considerable variation in leaf size, stem / petiole colour and inflorescence size, etc. Cytological data have aided or even made possible taxonomic decisions for example in tracing the origin of hybrids. Though the available literature indicates that the species has been cytologically investigated across the world but

no chromosomal information is available about Indian germplasm of *O. digyna*. To fill in the lacunae, therefore, the present study on meiotic analysis of 14 populations of this species from Kashmir Himalaya was undertaken to understand the cytological status of this species.

Materials and methods. The plant material was collected from the wild populations of *O. digyna* from Kashmir and cold deserts of Ladakh (J & K), India (Fig. 1) during the months of May–July over a period of three years (2008–2011). The voucher specimens of all the cytologically studied populations have been deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). For meiotic analysis the young and unopened floral buds were fixed in freshly prepared carnoy's fixative (6 alcohol : 3 chloroform : 1 acetic acid v/v/v) for 24 hrs and then preserved in 70 % alcohol at 4 °C in the refrigerator. The cytological preparations were made using the squash technique in 2 % acetocarmine. A number of temporary slides were examined to determine the exact chromosome number at different stages and meiotic abnormalities, if any. Pollen fertility was estimated following Marks [6] method using pollen stainability and well filled nature as a measure of fertility. Shrunken and unstained pollens were considered to be apparently infertile. Average size (equatorial and polar axis) of stained pollen grains has been estimated by taking several readings in case of each populations of *O. digyna*. Photomicrographs of chromosome counts and pollen grains were taken from freshly prepared slides using Nikon 80i Eclipse microscope. For previous chromosome reports various indexes to plant chromosome numbers such as Darlington and Wylie [7], Fedorov [8], Moore [9], Goldblatt [10], Goldblatt and Johnson [11], Kumar and Subramaniam [12] and Khatoun and Ali [13] were consulted.

Results. Present investigation on as many as 14 populations of *O. digyna* from Kashmir and Ladakh Himalaya revealed that the plants show noticeable interpopulation morphological variability. The plants clearly exhibit prostrate and erect habit. Popu-

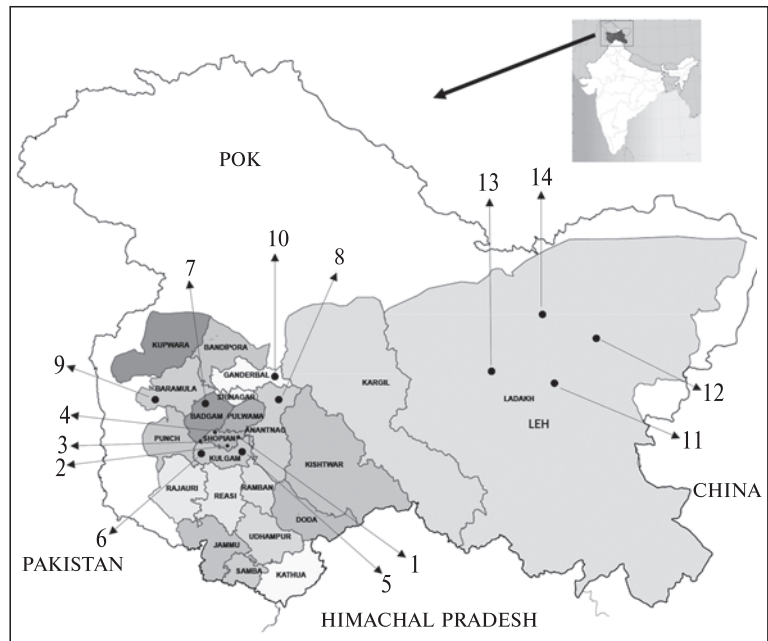


Fig. 1. A map showing different collection sites from where *Oxyria digyna* was collected

lations of *O. digyna* collected from Hirpora (2450 m), Kongwatan (2600 m), Zogila (3528 m) and Khalsar (3300 m) are prostrate where those from remaining areas are erect. The data on detailed observation on various morphological parameters are provided in Table 1. The plants showing prostrate habit grow 10–15 cm in height and form a mat. The plants have small 1.8–2.5 × 2.5–3.0 cm (L × B) leaves with inflorescence

growing up to 5.5 cm long (Table 1). The erect plants show considerable variation in size and can conveniently be placed under three types viz. small (up to 15 cm), medium (25–50 cm) and large (50–90 cm). The plants showing erect habit have 1.5–3.4 cm long and 2.0–5.1 cm broad leaves with inflorescence growing up to 8.5 cm. The colour and texture of leaves and stems also showed interpopulation variations. Populations of *O. digy-*

Table 1. Showing data on different morphological parameters

Accession number	Plant height, cm	Habit	Leaf size, cm			Inflorescence			Stamen number	Fruit size, mm	
			Length	Breadth	L/B	Number	Length, cm	Internode size, cm		Length	Breadth
55223	14	Prostrate	2	2.5	0.80	10	5.5	1.7	6	2	3
55233	32	Erect	2.5	3	0.83	7	6.9	2.5	5	1	3
55231	45	Erect	3.4	5.1	0.66	4	8	4	6	3	4
55235	25	Erect	2.5	3	0.83	3	4.5	1.5	6	2	3
55226	35	Erect	3	3.7	0.81	7	3.2	3.2	7	1	2
55228	15	Prostrate	1.8	2.7	0.66	5	4	2.7	6	1	2
55227	40	Erect	1.5	2	0.75	4	6.5	3.5	7	3	4
56389	15	Erect	2	2.5	0.80	5	3.5	2.7	6	2	3
55230	26	Erect	3.2	4	0.80	8	4.6	2.7	6	1	2
55229	10	Prostrate	2.5	3	0.83	3	3	1.3	6	1	2
55232	10	Prostrate	1.8	2.7	0.66	4	4	2.7	6	1	2
55234	15	Erect	2	2.5	0.80	1	5	1.5	6	1	2
55225	70	Erect	2.5	3	0.83	8	8	3.3	5	4	5
55224	90	Erect	2.5	3	0.83	10	8.5	3.5	5	3	5

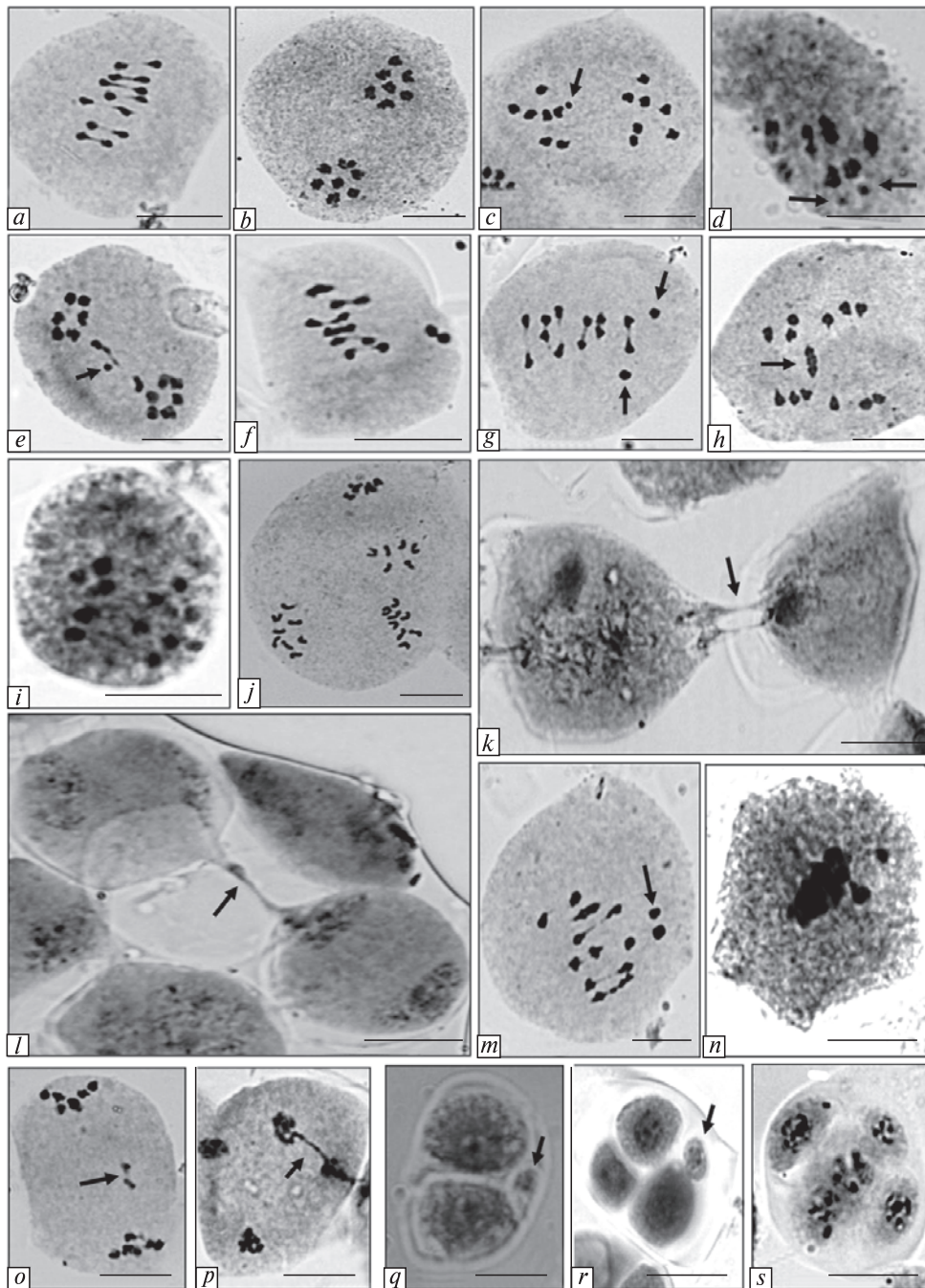


Fig. 2. *a* – a PMC at metaphase I showing 7_{II}; *b* – a PMC with 7:7 distribution at anaphase I; *c* – a PMC showing 1B chromosome (arrowed); *d* – a PMC showing 2B chromosomes (arrowed); *e* – a PMC showing laggard and B chromosome; *f* – a PMC with unoriented bivalent at metaphase I; *g* – a PMC showing early disjunction of one bivalent at metaphase I; *h* – a PMC showing late disjunction of bivalent at anaphase-I; *i-j* – PMC's showing unequal distribution of chromosomes at anaphase-I and anaphase-II; *k-l* – PMC's showing chromatin transfer (arrowed); *m* – a PMC showing extra chromatin material (arrowed); *n* – a PMC showing stickiness of chromosomes; *o* – a PMC showing laggards at anaphase-I; *p* – a PMC showing chromatin bridge at anaphase II; *q-r* – diad and triad with micronuclei (arrowed); *s* – a polyad. Scale bar = 10 μ m

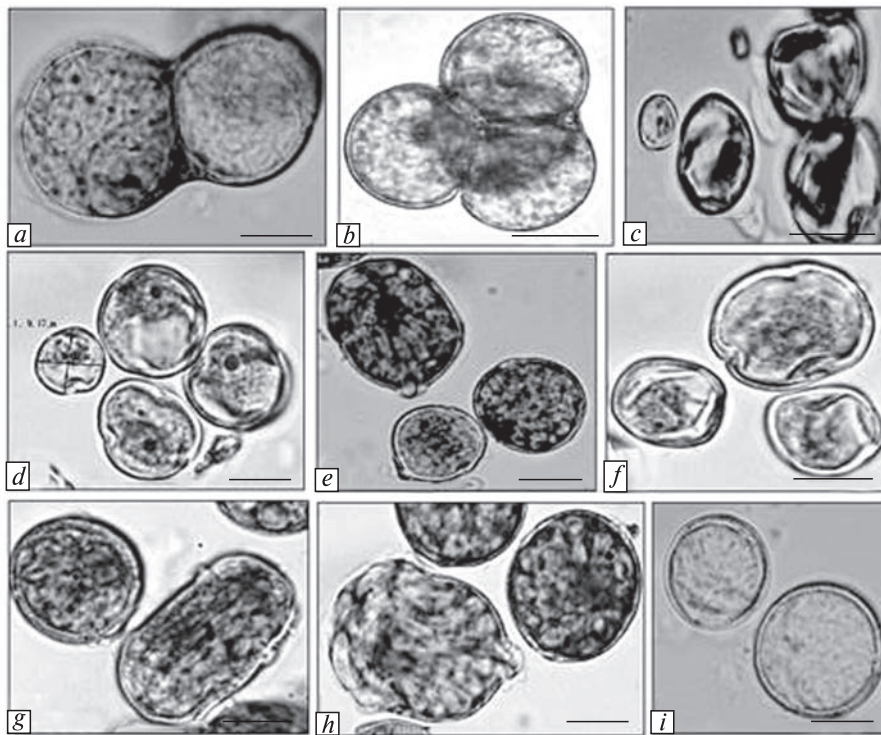


Fig 3. *a-b* – pollen grains showing fusion and remaining as a unit, *c-h* – apparently fertile heterogeneously sized pollen grains. (Observe the different sizes and shapes of the pollen grains), *i* – sterile pollen grain. Scale bar = 10 μ m

na collected from Zaznar (3200 m), Lidderwat (3500 m), and Turtuk (3100 m) possessed perfect greenish stems with green and shiny leaves. In some populations *viz* Keller (2200 m), Yosmarg (2300 m) the stem and margins of leaf were perfect dark brown. Interestingly, all the prostrate plants possess 6 anthers per flower while in the erect plants the number of anthers per flower varied between 5–7 (majority with 6 anthers per flower). We also present detailed cytological observations made on various populations of *O. digyna* inhabiting different localities of Kashmir Himalaya ranging in altitude from 2200–3528. Information on locality, altitude, geographic coordinates plant accession numbers, meiotic chromosome number, percentage of pollen fertility and average size of pollens of the presently studied populations are provided in Table 2. Meiotic investigations in the plants of all the 14 populations revealed that all share the same chromosome number of $n = 7$. This can be confirmed by the presence of 7 bivalents at metaphase-I (Fig. 2, *a*), equal distribution of chromosomes at anaphase-I (Fig. 2, *b*). In two

populations *viz* Lidderwat (3100 m) and Yosmarg (2300 m) 0–2B chromosomes were detected (Fig. 2, *c, d*). The meiotic course was normal in majority of the populations. As many as six populations of *O. digyna* worked out from Hirpora (2450 m), Turtuk Ladakh (3100 m), Aharbal (2200 m), Yosmarg (2300 m), Changmar Leh Ladakh (3000 m) and Lidderwat (3100 m) showed meiotic anomalies to a range of 8.94 to 23.13 % PMC's during further course of male meiosis. Few PMC's with un-oriented bivalents at metaphase-I (Fig. 2, *f*) and non-synchronous disjunction of 1–2 bivalents was seen in few populations (Fig. 2, *g, h*). Unequal distribution of chromosomes was observed at anaphase-I and anaphase-II stages (Fig. 2, *i, j*) in plants collected from Yousmarg and Lidderwat. As many as five populations exhibited the phenomenon of cytomixis (Table 2). Narrow cytoplasmic channels forming 1–2 chromatin strands involving 2–5 PMC's were observed and the transfer of chromatin material was seen to be both uni and bidirectional (Fig. 2, *k, l*). The percentage of PMC's involved in chromatin material transfer

ranged between 7.14–10.01 %. The transfer of chromatin material resulted in the formation of hyperploid cells (Fig. 2, *m*). During the present study all the six abnormal populations showed chromosomal stickiness with high percentage (4.34 %) in the Hirpora population (2450 m) and lowest (1.49 %) in the Changmar (3000 m) Leh Ladakh population (Fig. 2, *n*). Similarly presence of laggards

and bridges at A-I and A-II (Fig. 2, *o, p*) were the most common aberrations among all the abnormal populations. Consequent to these abnormalities, microsporogenesis was abnormal characterised by the presence of monads, diads, triads and polyads with or without micronuclei. (Fig. 2, *q-s*).

Pollen fertility was reduced in all the abnormal populations (69–80 %). The above mentioned mei-

Table 2. Information on locality, altitude, geographic coordinates, plant accession numbers, meiotic chromosome number, meiotic abnormalities, percentage of pollen fertility and average size of pollens

Locality (Altitude, coordinates)	Accession number (PUN)	<i>n</i>	PMCs with meiotic abnormalities, %						Pollen fertility, %	Average pollen size, μm
			CYM	STC	UOB	BRG	LAG	MPT		
Shopian: Hirpora, 2450 m, 33° 47' N 74° 45' E	55223	7	7.60	4.34	2.17	5.43	6.52	–	73.91	22.31 × 21.68, 17.20 × 16.71
Shopian: Dhobijan, 3000 m, 33° 37' N 74° 32' E	55233	7	–	–	–	–	–	–	96.95	16.74 × 15.34
Shopian: Zaznar, 3200 m, 33° 38' N 74° 46' E	55231	7	–	–	–	–	–	–	95.04	16.61 × 15.68
Shopian: Keller, 2200 m, 33° 43' N 74° 83' E	55235	7	–	–	–	–	–	–	98.07	16.70 × 15.87
Kulgam : Aharbal, 2200 m, 33° 38' N 74° 47' E	55226	7	9.09	3.89	2.59	7.79	6.49	–	70.12	28.79 × 27.91, 22.59 × 19.91, 16.44 × 15.53
Kulgam : Kongwatan, 2600 m, 33° 36' N 74° 46' E	55228	7	–	–	–	–	–	–	98.09	16.87 × 15.86
Badgam: Yosmarg, 2300 m, 33° 47' N 74° 39' E	55227	7+0-2B	10.01	4.28	–	7.14	5.71	2.85	70.01	30.11 × 29.71, 24.93 × 23.67, 17.55 × 16.54
Anantnag: Lidderwat, 3500 m, 4° 04' N, 75° 14' E	56389	7+0-1B	7.14	2.85	–	–	4.28	5.71	73.33	27.80 × 26.97, 16.06 × 15.74, 5.29 × 4.28
Baramulla: Tangmarg, 2500 m, 34° 32' N, 25° 17' E	55230	7	–	–	–	–	–	–	97.10	16.60 × 15.57
Ganderbal: Zogila, 3528 m, 34° 16' N, 75° 28' E	55229	7	–	–	–	–	–	–	96.09	17.79 × 16.68
Leh: Khalsar, 3300 m, 34° 31' N, 77° 41' E	55232	7	–	–	–	–	–	–	98.53	17.29 × 16.25
Leh: Changmar, 3000 m, 34° 49' N, 77° 05' E	55234	7	–	–	1.49	–	4.47	2.98	80.59	21.06 × 20.05, 16.72 × 15.32
Leh: Bogdan, 2900 m, 34° 48' N, 77° 02' E	55225	7	–	–	–	–	–	–	97.06	16.54 × 15.59
Leh: Turtuk, 3100 m, 34° 50' N, 76° 49' E	55224	7	9.75	3.65	2.43	6.09	7.31	3.65	69.51	25.23 × 23.57, 17.57 × 16.86, 11.56 × 10.23

Note. CYM – cytomixis, STC – stickiness, UOB – un-oriented bivalent, BRG – bridge, LAG – laggard, MPT – multipolarity.

otic irregularities lead to the formation of heterogeneous sized apparently fertile pollen grains which can be categorised into large, small and normal sized pollens (Table 2). Some pollen grains, though in small frequency could not complete their cytokinesis after T-II stage of meiosis and therefore remained together as a unit (Fig. 3, *a, b*). The occurrence of large sized pollen grains was observed in Aharbal (4.71 %), Yosmarg (5.91 %) and Liderwat (5.93 %) populations along with normal sized pollen grains. These large pollen grains might be un-reduced or hyperploid in nature. The diameter of such pollen grains ranged from 27.80×26.97 to $30.11 \times 29.71 \mu\text{m}$, while the diameter of the normal sized pollen grains was $15.53 \times 16.41 \mu\text{m}$. Besides, the normal pollen grains smaller pollen grains ($5.29 \times 4.28 \mu\text{m}$) were also present. It was interesting to see that in Aharbal and Yosmarg populations a small percentage of pollen grains also differed morphologically. The different shapes of pollen grains observed were round, oval and elliptical (Fig. 3, *c-h*).

Discussion. *Oxyria* is a monotypic genus and exhibits morphological and chromosomal differences. The present diploid chromosome number of $n = 7$ for the species is the first report from India. It is also note worthy that the B chromosomes (0–2B) have been reported for the first time in the genus. Earlier studies revealed $2n = 14, 28, 42$ (diploid, tetraploid and hexaploid cytotypes) [13–19].

B chromosomes can be present or absent in different individuals within a population and do not pair or recombine with any of the A chromosomes during meiosis [20]. It is believed that B chromosomes may have arisen by the fragmentation of A chromosomes or by a greater degree of heterochromatinization [21]. Generally it is accepted that B chromosomes do not have any effect on the growth and development of plants, but it is now shown that pollen abortion in *Ornithogalum candatum* is related to B chromosomes [22]. It seems that there is no correlation between B chromosomes and pollen sterility.

Meiotic abnormalities. Present study has revealed the following meiotic abnormalities in this species.

Cytomixis in PMCs. Migration of chromatin material among adjacent meiocytes occurs through cytoplasmic connections originating from the plasmodesmata formed within the anther tissues [23]. The role of cytomixis in plant evolution is considered an additional mechanism for the origin

of aneuploidy and polyploidy as it results in the formation of unreduced pollen grains as reported in several plant species [23–25]. In some cases, cytomixis may lead to the migration of whole chromatin among neighbouring meiocytes and lead to the formation of un-reduced gametes. In the present case, hyperploid nature was seen in some PMC's and the product of such PMC's in these individuals yield variable sized fertile and sterile pollen grains.

Chromosome stickiness. Chromosome stickiness is characterised by chromosome clustering during different phases of cell cycle and it ranged from a mild phenomenon involving a few chromosomes to an extensive one involving the entire chromosome complement. Genetic and environmental factors as well as genomic-environmental interactions have been considered to be the reason for chromosome stickiness in different plant species [26, 27].

Spindle abnormalities. Spindles play a crucial role in chromosome alignment during metaphase [25]. Any disturbance in the spindle apparatus may result in the random dis-orientation of chromosomes in the PMC's. Multipolar spindles also produce un-balanced and sterile gametes. Environmental influence and genotypic interactions have been seen responsible for abnormal spindle formation [26].

Non-synchronous disjunction. Generally speaking, different chromosomes of a complement disjunct almost simultaneously, but in some taxa, non-synchronization in the disjunction of bivalents have been reported. These anomalies in the chromosome separation might be due to different rates of terminalisation of various chromosomes of a complement [28], changed homology of chromosomes [29], or absence of coordination between chromosome and spindle [30]. Sometimes due to late disjunction, bivalents lag behind forming micronuclei which ultimately lead to abnormal microsporogenesis. As a consequence of late disjunction of bivalents bridges are often noticed at anaphases and telophases due to interlocking of chiasmata. The delayed separation of some bivalents in the presently investigated species caused irregular distribution of chromosomes at A-I ($8 + 6$) and A-II ($8 + 8 + 6 + 6$). This phenomenon can be considered of immense cytological significance as it can lead to the formation of gametes with $n+1$ or $n-1$ number of chromosomes causing numerical variation in chromosome number.

Un-reduced (2n) pollen grains. The trend to form 2n gametes in plants is highly variable and it varies among individuals within a single taxonomic group or even among flowers of an individual plant [31]. The production of unreduced gametes is heritable, governed by many genes and increases with increasing environmental stress, eg. Frost, wounding, herbivory, water deficit and lack of nutrients [32]. Different cytological mechanisms are responsible for the production of 2n gametes. It has been shown that 2n gametes in *Brachiaria brizantha* are produced from total absence either of the first or the second cytokinesis [33]. Further, influence of seasonal and environmental factors such as high and low temperature on 2n gamete formation have been shown [31]. Besides, cytomixis might have produced un-reduced pollen grains in this species. Different methods (morphological, flow cytometry, cytological) have been used to detect 2n gametes. The most direct method of screening for 2n pollen involves the examination of the range of the size of pollens produced by an individual, as with increase in DNA content the cell volume increases which in turn influence the pollen diameter.

Although the cytological status of these heterogenous sized pollen grains could not be ascertained but certainly their role in the production of aneuploid and polyploid plants could not be ruled out, because of the apparent fertile nature of these pollen grains.

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ЦИТОМОРФОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ *OXYRIA DIGYNA* HILL. ИЗ КАШМИРА (ГИМАЛАИ, ИНДИЯ)

Приводятся детальные цитоморфологические исследования *Oxyria digyna* Hill из Кашмира (Гималаи, Индия). Все 14 изученных популяций являются диплоидными, где $x = 7$. Из них в двух популяциях впервые описаны 0–2В хромосомы, тогда как шесть популяций сильно различались по своим мейотическим характеристикам. Аномалии мейоза при микроспорогенезе включали цитомиксис, несинхронное рас-

хождение некоторых бивалентов, задержки и мосты в анафазах и телофазах. Возникающий в связи с этим аномальный микроспорогенез приводит к формированию диад, триад и полиад как с микроядрами, так и без них. Общим эффектом является снижение фертильности пыльцы. В некоторых популяциях наблюдали нередуцированные пыльцевые зерна, которые по величине значительно отличались от нормальных. В популяциях Aharbal и Yosmarg некоторые пыльцевые зерна отличались морфологически. Оставшиеся восемь популяций проявляли нормальный ход мейоза, нормальный микроспорогенез и высокий процент фертильности пыльцы (95,09–99,09 %).

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