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Additions to the cytology of *Saxifraga* (Saxifragaceae) from the Western Himalayas, India

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ABSTRACT: We present a cytological study of species of the genus *Saxifraga* (*Saxifragaceae*) by analysing 8 accessions covering 5 species from the Western Himalayas. New intraspecific cytotypes in *S. filicaulis* Wall. ex Ser. (n = 8) and *S. pallida* Wall. ex Ser. (n=8) are reported. *Saxifraga brunonis* Wall. ex Ser. (n = 8) is reported for the first time from India. The occurrence of B-chromosomes in *S. parnassifolia* D. Don ($2n$=16+0–1B) has been found for the first time. Chromosome number of *S. pallida* Wall. ex Ser. (n=8) is different from the previous report. In the course of meiosis *S. brunonis* and *S. parnassifolia* show abnormality, such as cytomixis, chromatin stickiness, laggards and chromatin bridges. Abnormal microsporogenesis results in heterogeneous-sized pollen grains and reduced pollen fertility.

Keywords: cytomixis, meiotic abnormalities, *Saxifraga*, Western Himalayas.

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INTRODUCTION

The genus *Saxifraga* L. (*Saxifragaceae*) comprises more than 440 species which are spread over Europe, Northern Africa, Asia and the American subcontinents (Webb & Gornall 1989) of which 50 species are found in India (Aswal & Mehrotra 1994). From the cytological point of view, the genus *Saxifraga* is rather inadequately studied. This is due not only to practical complications relating to fixation of the material and to chromosome separation (Favarger 1965) but also to the occurrence of several chromosome numbers within a single species (Love & Love 1961, Fedorov 1969, Moore 1982). The chromosome numbers of *Saxifraga* have been most frequently recorded from the species distributed in the temperate regions of Europe and North America and also from the Arctic regions (see Fedorov 1969, Goldblatt 1984). Among species of the Western Himalayas, chromosome numbers of only eight species have been reported (Kumar *et al.* 2011, Kumar & Singhal 2011). Chromosome numbers reported for species of *Saxifraga* range from 2n= 10 to 220 and include multiples of nearly every number from 5 to 30, and aneuploidy is apparently common in the genus (Fedorov 1969, Spongberg 1972). Thus, as part of an ongoing cytological investigation of flowering plants in India, a chromosomal study on some species of *Saxifraga* has been undertaken.

MATERIALS AND METHODS

For meiotic studies, flowering buds were collected from different localities of selected areas of the Western Himalayas (Table 1). Smears of appropriate-sized flower buds were made after fixing with the Carnoy's fixative, using the standard acetocarmine technique. Pollen fertility was estimated by mounting mature pollen grains in a 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 a Nikon 80i eclipse Digital Imaging System. Voucher

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specimens are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

RESULTS

A meiotic study on a population basis was carried out on 8 accessions covering 5 species belonging to the genus *Saxifraga* from different localities with an altitudinal range of 2,600 - 3,600 m of the Western Himalayas, India. The data regarding locality, altitude, accession number, ploidy level, present and previous chromosome numbers of these species are presented in Table 1. Brief observations for each species are presented and discussed below.

***Saxifraga brunonis* Ser.**

Saxifraga brunonis grows in moist rock crevices at high altitudes between 3,000-3,600 m.

At present, two accessions have been studied from Churdhar (3,600 m) and Tisri (3,000 m) showing chromosomal counts of 2n=16, as confirmed from the presence of 8 bivalents at diakinesis (Fig. 1a). The present chromosome count of 2n=16 is the first cytological report from India, and is in conformity with a previous report from Nepal (Wakabayashi & Ohba 1988). The pollen fertility was reduced to 70-72%.

***Saxifraga cernua* L.**

Saxifraga cernua was collected from moist hill crevices between 3,000-3,600 m altitudes.

Meiotic studies of the species depicted 2n=16 as counted from the presence of 8 bivalents at metaphase-I (Fig. 1b) in two populations. The present chromosome count of 2n=16 is in conformity with a previous report from the Kashmir Himalayas (Jeelani *et al.* 2012). The course of meiosis was found to be normal, resulting in regular tetrad formation. The pollen fertility was quite high at 96-97%.

***Saxifraga filicaulis* Wall. ex Ser.**

Saxifraga filicaulis is found on moist places between 3,000-3,600 m altitudes.

Meiotic studies of the species depicted 2n=16 as seen in the PMCs at diakinesis (Fig. 1c) in two populations. This chromosomal count of 2n=16 is a new diploid report as the species was earlier known to have 2n=24 and 32 (Wakabayashi 1997). The course of meiosis was found to be perfectly normal with high pollen fertility (93-96%).

***Saxifraga pallida* Wall. ex Ser.**

Saxifraga pallida is also found on open rocky slopes at 3,000 m altitude. Flowering and fruiting is seen during the months of July-August.

Table 1. Locality/altitude, accession number, ploidy level/ meiotic course, present and previous chromosome number reports of the examined species of genus *Saxifraga* L.

Taxa	Locality/ Altitude (m)	Accession (PUN) number	Ploidy level (x)/ Meiotic course	Chromosome numbers	
				Present n number and respective figures	Previous*
***Saxifraga brunonis* Wall ex. Ser.**	Churdhar 3,600	56796	2x/A	8	***2n* =16** Wakabayashi and Ohba 1988
	Jamnala 2,600	56797	2x/A	8	
***S. cernua* L.**	Churdhar, 3,600	57355	2x/N	8	***2n*=16** Jeelani *et al.* 2011
	Tisri 3,000	57356	2x/N	8	
***S. filicaulis* Wall. ex Ser.**	Tisri 3,000	57887	2x/N	8	***2n*=24, 32** Wakabayashi 1997
	Churdhar, 3,600	57888	2x/N	8	***2n*=66** Vargas & Nieto Felinor 1995
***S. pallida* Wall. ex Ser.**	Tisri 3,000	57337	2x/N	8	***2n* = 22,44** Wakabayashi 1992
***S. parnassifolia* D. Don (=*Saxifraga diversifolia* var. *parnassifolia* (D.Don) Ser**	Haripurdhar 2.600	56717	2x/A	8+0-1B	***2n* =16** Kumar and Singhal 2011
	Sangrah 2.200	56171	2x/A	8	

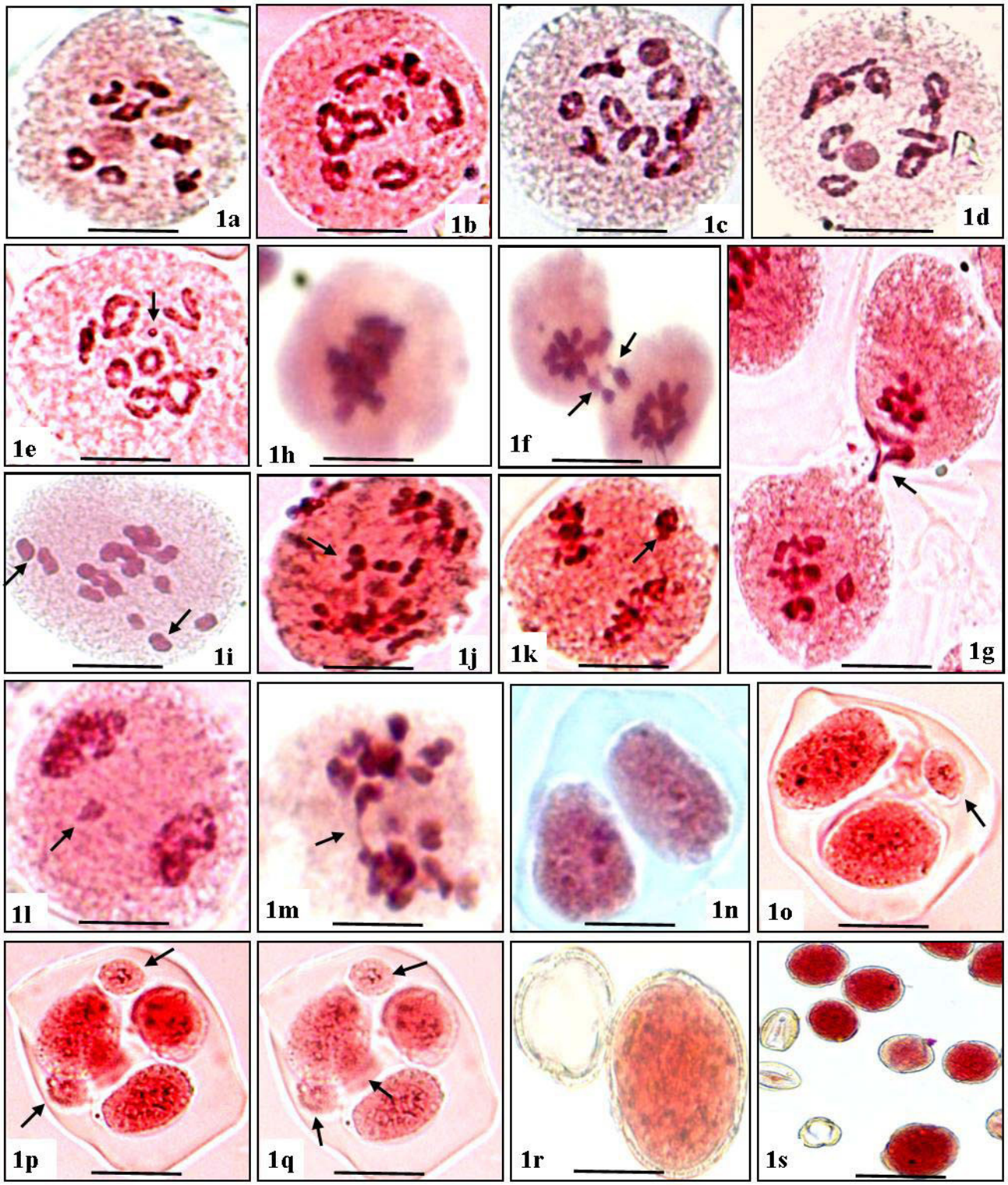

Figure (1a-1s):
a.- *Saxifraga brunonis* (n=8), PMC at diakinesis. **b.**- *S. cernua* (n=8), PMC at M-I. **c.** - *S. filicaulis* (n=8), PMC at diakinesis. **d.** - *S. pallida* (n=8), PMC at diakinesis. **e.**- *S. parnassifolia* (n=8+0-1B), PMC at M-I. **f-g**- Transfer of chromatin (arrowed) in pollen mother cells (PMCs) at different stages of meiosis. **h.** - PMC showing chromatin stickiness at M-I. **i.** - PMC showing unoriented bivalents (arrowed) at M-I.. **j-l**.- PMC showing laggards (arrowed) at different stages of meiosis. **m.** - PMC showing bridges (arrowed) at A-I **n.** - Diad **o.** - Diad with micronuclei. (arrowed). **p.**- Triad with micronuclei. (arrowed). **Q.** - Tetrad with micronuclei (arrowed). **r.** -Heterogenous sized fertile pollen grains. **s.**- Fertile and sterile pollen grains. **Scale 10 µm.**

Table 2. Cytological overview of *Saxifraga* investigated at present on the basis of complete information including previous as well as present chromosome number reports.

S. no. Name of genus (Habit)	Number of species Intra-specific euploids (Respective base numbers in parenthesis)				Various euploid levels Intraspecific aneuplods	Number of cytotypes (Chromosomal races) = Known 2n chromosome numbers (figures in parenthesis give number of species/ cytotypes)	Number of species with more than one cytotype		Base numbers (common one/s underlined and doubtful with ?) [Proposed by Darlington & Waylie, 1955]
	Taxon-omically known +World ++India	Cytologically known Total (%)	Diploids	Polyploids (%)			Intra-specific euploids (Respective base numbers in parenthesis)	Intraspecific aneuplods	
I	II	III	IV	V	VI	VII	VIII	IX	X
Saxifraga L. (Herbs perennial, rarely annual or biennial).	+440	250 (55.55)	40	209 (83.60)	2x,4x, 6x,8x, 10x,14x,20x,	**43**= 10(1),12(3), 13(1),14(1), 16(26), 18(3), 20(15), 22(12), 23(1), 24(8), 26(38), 27(2), 28(34), 30(9), 32(21), 36(3), 38(6), 39(2), 40(10), 42(1), 44(7), 46(1), 48(6), 50(1), 52(12), 56(12), 58(1), 60(5), 62(1), 63(1), 64(7), 66(2), 70(3), 76(1), 78(1), 80(6), 84(1), 90(1), 92(1), 112(3), 120(1), 124(1)	2(5), 1(7), 8(8), 2(11), 3(13)	50	x=5,6,7,8,9,13 [**8-14**]
	++50	8 (16.00)	6	2 (25.00)	2x,4x	**4**= 16(8), 20(1), 32(2), 34(1)	1(8)	1	x=5,8

A chromosome number of 2n=16 was counted in the PMCs at diakinesis (Fig. 1d), making this the first cytological report for this species from India. However, the species has previously been known to have 2n=22 and 44 from Nepal (Wakabayashi 1992, 1997). Normal meiosis resulted in pollen fertility being as high as 93.5%.

***Saxifraga parnassifolia* D. Don (=*Saxifraga diversifolia* Wall. ex Ser. var. *parnassifolia* (D. Don) Ser.**

Saxifraga parnassifolia The species is found on grassy rocks between an altitude range of 2,600-3,000 m.

Two accessions were studied from Sangrah (2,200 m) and Haripurdhar (2,600 m) exhibiting chromosome numbers of 2n=16 + (0-1B) counted in the PMCs at metaphase-I (Fig. 1e). The presence of a B chromosome in the species is reported for the first time. Otherwise, the species has previously also been shown to have 2n=16 from the Manimahesh hills of Himachal Pradesh (Kumar & Singhal 2011) and from Rolwaling Himal, Nepal (Wakabayashi & Ohba 1988), and 2n=20 from the northwest Himalayas in India (Mehra & Dhawan 1971) and Nepal (Malla *et al.* 1984). Pollen fertility was found to be low (75-80%).

Meiotic abnormalities. All the populations belonging to two species, namely, *S. brunonis* and *S. parnassifolia* depicted abnormal meiosis. The phenomenon of cytomixis involving transfer of chromatin material was observed involving groups of proximate PMCs at various meiotic stages from diakinesis to telophase-II (Figs. 1f, g; Table 2). Further, transfer of chromatin material was either partial or complete with the formation of hypo and hyperploid

Table 3. Data on cytomixis and meiotic abnormalities in different species of *Saxifraga.*

Accession Number	Cytomixis		Meiotic course showing PMCs with			
	% of PMCs involved Meiosis–I/ Meiosis–II	Number of PMCs involved	Chromosomal Stickiness at M–I (%)	Unoriented bivalents at M–I (%)	Bridges at Meiosis –I/ Meiosis–II (%)	Laggards at Meiosis –I/ Meiosis–II (%)
S. brunonis						
56796	4.00 (3/75)/ 2.94(2/68)	2–4	-----	-----	-----/----	5.79 (4/69)/ -----
56797	3.48(3/86)/ -----	2-4	-----	-----	-----	4.76(3/63)
S. parnassifolia						
56717	7.50(6/80)/ ----	2-3	-----	4.49(4/89)	------	11.25(9/80)/ ----
56171	-----	-----	3.22(3/93)	-----	1.04(1/96)/ -----	3.26(3/92)/ 1.09(1/91)

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

Table 4. Data on abnormal microsporogenesis in different species of *Saxifraga.*

Accession Number	Microsporogenesis (Values in %)							
	Monads		Dyad		Triads		Tetrads	
	WMN	WM	WMN	WM	WMN	WM	WMN	WM
S. brunonis								
56796	-------	-------	3.40 (3/88)	1.13 (1/88)	2.27 (2/88)	-------	88.63 (78/88)	4.54 (4/88)
56797	-------	-------	-------	2.15 (2/93)	-------	4.30 (4/93)	88.17 (82/93)	5.37 (5/93)
S. parnassifolia								
56717	-------	-------	-------	5.71(4/70)	-------	-------	87.14(61/70)	7.14(5/70)
56171	-------	-------	-------	4.49(4/89)	-------	-------	92.13(82/89)	3.37(3/89)

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

PMCs (Fig. 1f). Chromatin stickiness involved the whole chromosome complement and was seen mostly as a single clumped mass at prophase-I to metaphase-I (Fig. 1h). Unoriented bivalents at metaphase-I (Fig. 1i) were also noted (Table 3 [2]). Late or non-disjuncting bivalent bridges as well as chromosomal laggards were more common in all the populations (Figs. 1j-m). All these abnormalities lead to the formation of abnormal microsporogenesis with the presence of dyads, triads or polyads (Figs. 1n-q; Table 2 [3]). Micronuclei at the tetrad stage were also observed in most of these species. Ultimately heterogeneous-sized pollen grains would be formed accompanied by reduced pollen fertility (Figs. 1r, s).

DISCUSSION

From chromosome numbers determined so far (Table 4), including the presently-added cytotypes, of 440 taxonomically known species, 250 (56.8%) species have been investigated in the form of 43 cytotypes with a wide range of chromosome numbers: 2n =10, 12, 13, 14, 16, 18, 20, 22, 23, 24, 26, 27, 28, 30, 32, 36, 38, 39, 40, 42, 44, 46, 48, 50, 52, 56, 58, 60, 62, 63, 64, 66, 70, 76, 78, 80, 84, 90, 92, 112, 120, 124, 206-220. The genus is polybasic with x=5, 6, 7, 8, 9, 10, and 13, somewhat in line with numbers previously presented by Darlington & Wylie (1955) of x=8-14, 17. The most common number is x=8. As many

as 209 (83.6%) species have been found to be polyploid, reaching 11x. In total, 14 species show intraspecific euploidy (1 species based on x=7, 8 species based on x=8, 2 species based on x=10 and 3 species based on x=13). A significant proportion of species (50) have been shown to exhibit intraspecific aneuploidy. On the basis of data for India, however, of 50 reported species, 8 (16.0%) species have been distributed in the form of 4 cytotypes (2n=16, 20, 32, 34) based on x= 8, 10, with 2 (25.0%) species being polyploid, reaching 4x, and including one species each with intraspecific euploidy and aneuploidy. These earlier reports of chromosomes in *Saxifraga* were mostly from outside India, except for a few reports from the Western Himalayas (Mehra & Dhawan 1971, Kumar *et al.* 2011, Kumar & Singhal 2011). Previously, cytomorphological studies of the genus had been reported only from the Western Himalaya, India (Kumar *et al.* 2011; Kumar & Singhal 2011). From India, chromosomal counts are known for the species *S. diversifolia* with 2n=2x=16, 20, *S. diversifolia* var. *diversifolia* with 2n=2x=16, *S. filicaulis* with 2n=2x=16, *S. flagellaris* with 2n=2x=16, *S. flagellaris* ssp. *komarovii* (A. Los.) Hulten with 2n=4x=32, *S. hirculus* L. var. *hirculus* with 2n=4x=32, *S. ligulata* Wall. 2n=2x=34, *S. stracheyi* with 2n=2x=34 from the Western Himalaya (Mehra & Dhawan 1971, Kumar *et al.* 2011, Kumar & Singhal 2011) and *S. stolonifera* with 2n=4x=32 from the Eastern Himalaya (Sharma & Sarkar 1967-1968) based on x=10 and x=8 respectively. For the other two species, *S. brunonis* has been cytologically studied for the first time from India and *S. pallida* revealed intraspecific aneuploidy. After pooling the present and previous data and on the basis of information thus generated for a total of eight species of *Saxifraga* from India, these findings indicate that the genus is probably still very complex cytologically. Only after detailed cytological studies will it be possible to determine actual chromosome numbers for species of *Saxifraga*, as well as base chromosome numbers for the genus.

The meiotic abnormalities in the form of cytomixis, chromatin stickiness, unoriented bivalents, inter-bivalent connections, bridges, laggards or multipolar spindle have been observed at different stages of meiosis. These meiotic abnormalities indicate the existence of intraspecific genetic diversities. Such genetic differences have been seen previously in different plant species (Baptista-Giacomelli *et al.* 2000, Sheidai *et al.* 2003). Cytomixis and chromatin stickiness are considered to be the result of genetic factors (Bellucci *et al.* 2003, Ghaffari 2006, Fadaei *et al.* 2010) and environmental factors (Nirmala & Rao 1996) as well as genomic-environmental interaction (Baptista-Giacomelli *et al.* 2000), and seem to be equally applicable to the presently investigated populations. Cytomixis or occurrence of multipolar cells and meiotic irregularities in anaphase segregation of chromosomes may be possible mechanisms for the formation of large sized pollen grains and low pollen fertility in these meiotically abnormal populations, as has been reported earlier in several angiosperms (Gupta *et al.* 2009, Kumar *et al.* 2008). Hypo- and hyperploid PMCs formed due to cytomixis (Falistocco *et al.* 1995, Fadaei *et al.* 2010) accompanied by other meiotic abnormalities may lead to anomalous microsporogenesis resulting in the formation of variable-sized pollen grains possibly with an aneuploid condition (Sheidai *et al.* 2003, Sheidai & Fadael 2005) . It is quite clear that aneuploidy and polyploidy remain to be the chief mode of evolution in *Saxifraga*.

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REZIME

Prilozi za citologiju roda *Saxifraga* L. sa zapadnih Himalaja, Indija

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U radu se navode novi citološki podaci za pet vrsta iz roda *Saxifraga* sa Zapadnih Himalaja. Navode se novi intraspecijski citotipovi za *S. filicaulis* Wall. ex Ser. (n = 8) i *S. pallida* Wall. ex Ser. (n=8). Po prvi put se navodi citotip *Saxifraga brunonis* Wall. ex Ser. (n = 8) iz Indije. Takodje se po prvi put navodi prisustvo B hromozoma kod *S. parnassifolia* D. Don (2n=16+0–1B). Broj hromozoma zabeležen kod *S. pallida* Wall. ex Ser. (n=8) razlikuje se od prethodnih navoda za ovu vrstu. Tokom mejoze kod *S. brunonis* i *S. parnassifolia* uočavaju se nepravilnosti, kao što je citomiksis, lepljivi hromatin ili hromatinski mostovi. Abnormalije pri mikrosporogenezi rezultuju u formiranju polena različite veličine i smanjuju njegov fertilitet.

Ključne reči: citomiksis, mejotske ambnormalije, *Saxifraga*, Zapadni Himalaji.