Male Meiotic Studies in Six Species of *Pedicularis* L. from Churdhar and Adjoining Hills of Sirmaur District (H. P.), India

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Summary The genus *Pedicularis* L. comprises many medicinal species in Sirmaur district of Himachal Pradesh, the Northwest Himalayan range. In the present study, male meiotic studies have been carried out in six species of the genus and revealed $n=8$ chromosome number in all taxa. The chromosome number ($n=8$) in *P. heterodonta* and *P. punctata* are new to the world, while *P. oederi* reveals the first chromosome report for Indian populations. Besides, a B chromosome and structural heterozygosity were reported for the first time in *P. hoffmeisteri* and *P. pectinata*, respectively. Further, meiotic anomalies, like chromatin bridges, interbivalent connections, laggards, un-even gamete formation and reduce pollen viability, were also observed in the studied populations.

Key words Chromosome number, Meiotic abnormality, *Pedicularis*, Sirmaur district.

The genus *Pedicularis* L. known popularly as lousewort, belongs to the family Orobanchaceae and comprises about 600 species in alpine and sub-alpine or even in the tundra regions of the northern hemisphere. In India, the maximum diversity of the genus is found in Himalayan range with 81 species, ranging Ladakh to Arunachal Pradesh in east covering Sikkim Himalayas (Yatoo *et al.* 2016). Terminal inflorescences, forked calyx in the anterior end and typical bi-labiate corolla (larger ventral lobes than dorsal) having a hooded upper lip, are few peculiar characteristics of the genus.

In the modern era, due to emerging drug resistance, herbal medicines, due to their multiple beneficial health applications and as an alternative, are gaining increased attention among researchers at global stage (Ganjhu *et al.* 2015). The species of *Pedicularis* are rich in alkaloids, flavonoids, iridoids, lignans, polyphenols, and other phytochemicals (Khodaie *et al.* 2012, Zhao *et al.* 2013, Mura and Iwashina 2015). Several phytochemical extracts of the species are reported to treat various diseases and disorders that include general clinical conditions, various ailments or even in maintaining vitality (Chu *et al.* 2009, Yatoo *et al.* 2016, 2017). The quantitative and qualitative measures of these compounds, generally believed to vary in cyto/morpho/ecotypes. Further, cytogenetics is considered among the key determinants in studying evolutionary concepts in plants owing to meiotic anomalies (Singh *et al.* 2016). Thus,

the present meiotic study will be informative in making chromosomal databases and evolutionary concepts, and even in developing effective medicines and nutraceutical products.

Materials and methods

Eight populations of six species *Pedicularis* (54 individuals) have been collected from various localities of Sirmaur district of Himachal Pradesh in India (Table 1) and studied cytologically. The voucher specimens were submitted in the Herbarium of Botany Department, Punjabi University, Patiala (PUN). For meiotic studies, the young floral buds were fixed in a freshly prepared Carnoy's fixative (ethanol, chloroform, glacial acetic acid in 6:3:1) for 24h, which were transferred to 70% alcohol and stored in a refrigerator at 4°C. Meiotic slides were prepared by a squashing the fixed anthers in 1% acetocarmine. The chromosome number was determined by analyzing 300–350 pollen mother cells (PMCs) at different countable stages of meiosis [metaphase I, II (M I, II) and anaphase I, II (A I, II)] under a microscope and then photomicrographs of detailed meiosis and microsporangogenesis were taken with a digital imaging system of Leica QWin DM3400. Pollen viability was analyzed on mature pollens after heating in glycerol-acetocarmine (1:1) for 24h. More than 250 pollen grains were studied for pollen viability and size variability. The fully-filled pollen grains with stained nuclei were considered as fertile, while shriveled and unstained as sterile.

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Table 1. Table showing details regarding the name of species, a locality with an altitude, chromosome number, ploidy level, size of pollen grains, and remarks on the meiotic course of previous worked out species.

Name of species, plant number, voucher number (PUN)	Locality, altitude in meters, geographical co-ordinates	Chromosome number (n)	Ploidy level (x)	Pollen size ($\mu\text{m} \pm \text{SD}$)	Pollen number viability (%)
<i>P. gracilis</i> Wall. ex Benth. P-1 60053	Churdhar, 3650, 30°52'N, 77°28'E	8	2	30.6±0.4×32.5±1.2	85
<i>P. heterodonta</i> Pancic ex Janka P-1 59664	Churdhar, 3650, 30°52'N, 77°28'E	8	2	s-12.5±0.2×14.2±0.9 l-22.5±1.1×25.3±1.3	65
P-2 60054	Tisri, 3650, 30°51'N, 77°27'E	8	2	s-11.6±0.4×13.9±0.6 l-20.9±1.2×23.8±1.4	61
<i>P. hoffmeisteri</i> Klotzsch P-1 58623	Churdhar, 3650, 30°52'N, 77°28'E	8+0-1B	2	s-12.6±0.3×13.6±0.9 l-22.9±0.9×24.0±1.0	58
<i>P. oederi</i> Vahl P-1 59502	Churdhar, 3650, 30°52'N, 77°28'E	8	2	s-12.6±0.5×13.6±0.5 l-22.9±1.3×24.0±1.3	65
P-2 60050	Jamnala, 3650, 30°50'N, 77°26'E	8	2	s-10.2±0.2×12.1±0.5 l-23.4±1.3×27.5±1.6	65
<i>P. pectinata</i> Wall. ex Benth. P-1 59784	Churdhar, 3650, 30°52'N, 77°28'E	8	2	23.9±1.5×25.6±1.6	79
P-2 60055	Chapdhar, 3650, 30°49'N, 77°25'E	8	2	24.3±1.6×26.4±2.1	76
<i>P. punctata</i> Decne. P-1 58622	Nauradhar, 3650, 30°48'N, 77°25'E	8	2	s-14.6±0.5×15.0±0.4 l-20.6±1.4×23.1±2.3	65

s-small pollen grain, l-large pollen grain, μ =mean, SD=Standard deviation.

Results and discussion

Chromosome numbers

P. gracilis showed $n=8$ in the PMCs (Fig. 1A), and this count confirms the previous reports (Mehra and Gill 1968, Amano 1999).

P. heterodonta revealed $n=8$ at M I (Fig. 1B), which is the first chromosome report.

P. hoffmeisteri showed B chromosome (Fig. 1C). Previously, the species is reported with $2n=16$ by Saggoo and Srivastva (2009) from Lahaul-Spiti district of Himachal Pradesh (2009) and Amano (1999) from Nepal. Due to the presence of B chromosome, the meiotic abnormalities in the species are low compared to the others (Table 2), which resemble many previous studies (Houben 2017).

P. oederi reveals the first chromosome number of $n=8$ (Fig. 1D) from India (Singh *et al.* 2016). Earlier, the species is reported with the same chromosome number ($2n=16$) from the Furkotská dolina valley of Slovakia (Uhríková and Dúbravcová 2000).

P. pectinata showed $n=8$ (Fig. 1E) and the chromosome number supports the previous report by Saggoo and Srivastava (2009). The species reveals structural heterozygosity (Fig. 1E), the quadrivalent/s in PMCs at M I, are thought to arise due to partial homology in non-homologous pairs that forms an association in the form of reciprocal translocations. The similar phenomenon has been discussed in genera, like *Oenothera*, *Datura*,

Fig. 1. A PMC with 8_{II} at diakinesis in *P. gracilis* (A), a PMC showing 8_{II} at M I in *P. heterodonta* (B), a PMC with $8_{II}+1B$ (arrowed) at M I in *P. hoffmeisteri* (C), a PMC at diakinesis showing 8_{II} in *P. oederi* (D), a PMC showing $1_{IV}+6_{II}$ configuration (arrowed) at M I in *P. pectinata* (E), a PMC with 8_{II} at M I in *P. punctata* (F). Scale bars=10 μm .

Table 2. Data on the meiotic course in the studied populations of six species of *Pedicularis* from Churdhar and adjoining hills of Sirmaur (H.P.).

Plant number	PMCs with chromosomal stickiness at M I (%)	PMCs with unoriented bivalents at M I (%)	PMCs with bridges (at A I/T I) (%)	PMCs with laggards (at A I/T I) (%)
<i>P. gracilis</i>				
P-1 60053	5.5 (6/109)	2.3 (3/130)	4.3 (6/139)	1.5 (2/129)
<i>P. heterodonta</i>				
P-1 59664	24.6 (31/126)	19.1 (25/131)	5.4 (6/110)	0 (0/129)
P-2 60054	22.1 (29/131)	0.1 (1/101)	5.4 (6/110)	0 (0/105)
<i>P. hoffmeisteri</i>				
P-1 58623	0 (0/101)	0 (0/114)	0 (0/95)	0 (0/117)
<i>P. oederi</i>				
P-1 59502	11.3 (1/97)	5.5 (5/90)	7.2 (7/97)	2.1 (2/97)
P-2 60050	11.3 (11/97)	9.3 (9/97)	7.2 (7/97)	0 (0/105)
<i>P. pectinata</i>				
P-1 59784	0 (0/110)	0 (0/104)	3.0 (3/100)	1.8 (2/109)
P-2 60055	0 (0/121)	0 (0/103)	2.7 (3/110)	3.0 (3/100)
<i>P. punctata</i>				
P-1 58622	0.9 (1/107)	0 (0/111)	3.6 (4/111)	2.7 (3/109)

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

Fig. 2. PMC's with Interbivalent connections in *P. gracilis* and *P. punctata*, respectively (A, B), a PMC showing chromatin bridge in *P. heterodonta* (C), a PMC showing laggards in *P. oederi* (D), tetrad with micronucleus (E), heterogeneous sized pollen grains in *P. punctata* (F). Scale bars=10 μ m.

etc. by Levin (2002).

P. punctata reveals a diploid cytotype with $n=8$ (Fig. 1F), which is reported for the first time.

The chromosomal numbers reported in 122 *Pedicularis* species are ranging from $2n=12$ to $2n=36$ with a maximum frequent number of $2n=16$ as the number is reported in 102 species (see Rice *et al.* 2015). The genus is monobasic on $x=8$. But, there are few reports on $x=6$, 7 and 15, which need further investigations to clear evo-

lution of basic number in the genus.

Meiotic abnormalities

Meiotic abnormalities viz. chromatin stickiness, un-oriented bivalent, interbivalent connections (Fig. 2A, B), chromatin bridges (Fig. 2C), laggards, and abnormal microsporogenesis along with heterogeneity in the size of pollen grains have been reported in most of the studied populations (Table 2). *P. heterodonta* reveals the maximum frequency of un-oriented bivalents (Table 2), that arise due to impaired kinetochore attachment or late terminalization of chiasmata (Pagliarini 2000) and results in the laggards formation (Table 2, Fig. 2D), which might result into micronuclei (Fig. 2E) and un-even gametes. These laggards may further lead to heterogeneity in pollen grain size (Fig. 2F).

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