Cytogenetics of Four Species of Genus *Berberis* L. (Berberidaceae Juss.) from the Western Himalayas

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Summary The genus *Berberis* belongs to the family Berberidaceae and includes mostly wild, important medicinal plants. Meiotic studies have been carried out for analyzing the genetic diversity in 11 populations covering four species from different selected parts of the Western Himalayas, such as Kashmir (Jammu and Kashmir) and the districts of Kangra and Sirmour (Himachal Pradesh). The species being cytologically worked out for the first time worldwide include *B. ceratophylla* ($2n=28$). Similarly, *B. vulgaris* ($2n=28$), although worked out earlier from other countries, is being reported cytologically for the first time from India. The meiotic course in most of these populations has been observed to be normal except for a single population each of *B. asiatica*, *B. ceratophylla* and *B. vulgaris* marked with abnormal meiosis. Out of these three species, two (*B. asiatica* and *B. vulgaris*) are marked with cytotoxicity. These meiotic abnormalities lead to the production of heterogenous-sized fertile pollen grains and reduced pollen fertility.

Key words *Berberis*, Chromosome number, Meiotic abnormality, Western Himalayas.

Berberis is popularly known as barberry and is the largest genus of the family containing about 500 species native to the temperate and subtropical regions of Europe, Asia, Africa, and North and South America (Ahrendt 1961). It includes 55 species from India (Rao *et al.* 1998). Taxonomically, *Berberis* is considered a very complex genus with variable characters in its species. The genus is characterized by deciduous or evergreen shrubs with thorny yellow stems, simple leaves and sepals usually in two series or whorls.

Many species of *Berberis* are known for their ethnobotanical properties. For example, the root extracts of *B. asiatica*, *B. lyceum* and *B. chitria* are used as an antidiarrhoeal, laxative, antiseptic, intestinal astringent, cough medicine and a blood purifier (Khare 2007, Qureshi *et al.* 2007, Gangwar *et al.* 2010). Other species of *Berberis*, such as *B. holstii* and *B. vulgaris*, are medicinally important due to the presence of alkaloids with different pharmacological properties (Maliwichi-Nyirenda *et al.* 2011).

Several cytological studies have been made in the genus from different parts of the world by different researchers (Pogan *et al.* 1980, Sheng *et al.* 2010). However, most of these studies are concentrated on the evaluation of chromosome numbers with only a few studies carried out for the study of meiotic behavior (Yan-Jun *et al.* 2006, Rounsaville and Ranney 2010) and a single cytomorphological study (Heidary *et al.* 2009). Some of the cytological contributions from India

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include those by Mehra and Sareen (1969), Singhal *et al.* (1980), Gill *et al.* (1984) and Sandhu and Mann (1988), but they all represent the same chromosome number evaluation. In this regard, the present study is the first attempt to look for genetic diversity in the different species of the genus from the Western Himalayas to segregate the variants for further exploitation along with adding information to the chromosomal database of Indian plants. Furthermore, we study the effects of different meiotic abnormalities on pollen fertility and pollen size in the abnormal populations/species.

Materials and methods

During the present investigations, materials for cytomorphological studies were collected from different populations of the wild plants growing in different climatic zones in different seasons. For meiotic studies, young flower buds of an appropriate size were collected during the peak flowering period. These buds were fixed in Carnoy's fixative (six parts ethyl alcohol, three parts chloroform and one part glacial acetic acid) for 24–48 h. Then, the materials were transferred to 75% ethyl alcohol and stored in a refrigerator at about 4–10°C. For chromosomal preparations, smears of the young anthers were made in 1% acetocarmine following the technique of Belling (1921). Pollen fertility was determined through the stainability of pollen grains in 50% glyceracetocarmine (Marks 1954). Only well-filled pollen grains and those with well-stained nuclei were taken as apparently fertile and viable. The pollen grain size was measured with an ocular micrometer. Photomicrographs of PMCs for chromosomal counts, meiotic irregularities, sporads and pollen grains were made from the freshly prepared slides using a Nikon Eclipse 80i microscope (X330, X1340 and X3400). Voucher specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

Results

Detailed population-based meiotic studies have been carried out on 11 populations covering four species belonging to the genus *Berberis* of the family Berberidaceae from different localities with an altitudinal range of 1,800–3,000 m in the districts of Kangra and Sirmaur of Himachal Pradesh and 1,800–2,300 m in Kashmir. The data regarding locality, altitude, voucher specimen number (PUN), and present and previous chromosome number reports of the presently studied species have been presented in Table 1.

Based on $x=14$, all the 11 populations of the four species, namely *B. asiatica*, *B. ceratophylla*, *B. chitria* and *B. vulgaris*, invariably depict the same diploid chromosome number ($2n=28$) (Figs. 1–4). The present chromosome number, $2n=28$, for *B. ceratophylla* is the first cytological report for the species in the world, and for *B. vulgaris* ($2n=28$), it is the first report from India.

Meiotic abnormalities have been recorded in some populations of *B. asiatica* and *B. ceratophylla* and all the populations of *B. vulgaris* except *B. chitria*, which showed a normal course of meiosis. In such populations, abnormalities in the form of cytomixis, chromatin stickiness, unoriented bivalents, bridges and laggards as well as multipolarity have been observed at different stages of meiosis (Figs. 5–10; Table 2). Cytomixis in these species results in the production of hyper- and hypo-ploid PMCs (Figs. 5, 6; Table 2), except in *B. ceratophylla* where no cytomixis is observed. It is seen that late or non-disjoining bivalent bridges and chromosomal laggards are most common. Chromatin stickiness involving few bivalents or the whole complement is seen from prophase-I to metaphase-I. This results in abnormal microsporogenesis leading to the formation of diads, triads and polyads (Figs. 10, 11, 14). Further, micronuclei have also been observed in most of these species (Figs. 11–14; Table 3). These meiotic abnormalities along with abnormal microsporogenesis lead to the formation of heterogeneous-sized fertile pollen grains and reduced

Table 1. Data showing locality, altitude with accession number (PUN) and $2n$ chromosome number (present and previous reports) of the presently studied species of genus *Berberis* from the Western Himalayas.

S. No.	Taxa	Locality/Altitude (m)/ Accession number	Chromosome number	
			Present report	Previous reports*
1	<i>Berberis asiatica</i> Roxb. (= <i>B. lycium</i> Royle)	Bara-gran, District/ Kangra (H.P.); 3,000/ 55133	$2n=28$	$2n=28$ Mehra 1976, Sandhu & Mann 1988, Malla <i>et al.</i> 1975, Gill <i>et al.</i> 1984, Singhal and Gill 1984.
		Nauradhar, District/ Sirmaur (H.P.); 1,800/ 57334	$2n=28$	
		Batnoor-Tral, District/ Pulwama (J&K); 2,200/ 57291	$2n=28$	
		Tangmarg, District/ Baramulla (J&K); 2,300/ 57258	$2n=28$	
2	<i>B. ceratophylla</i> G. Don	Chotta-bhangal/ District Kangra (H.P.); 2,300/ 55080	$2n=28$	No Reports
		Triund, District/ Kangra (H.P.); 3,000/ 55899	$2n=28$	
3	<i>B. chitria</i> Lindl.	Shillai, District/ Sirmaur (H.P.); 2,000/ 55991	$2n=28$	$2n=28$ Singhal <i>et al.</i> 1980.
		Chapdhar, District/ Sirmaur (H.P.); 2,400/ 56026	$2n=28$	
4.	<i>B. vulgaris</i> L.	Boh, District/ Kangra (H.P.); 1,900/ 55129	$2n=28$	$2n=28$ Langlet 1928, Tischler 1928, Dermen 1931, Giffen 1936, Delay 1947, Magulaev 1984, Verlaque <i>et al.</i> 1987, Pogan <i>et al.</i> 1980, Měsíček 1992.
		Dachigam, District/ Srinagar (J&K); 1,800/ 52474	$2n=28$	
		Tral, District/ Pulwama (J&K); 1,900/ 56221	$2n=28$	

* Previous chromosome number reports are based on literature [Chromosomal Atlases by Fedorov (1969), and Kumar and Subramanian Vol. I (1986)]; Index to Plant Chromosome Number Reports from 1968 onwards; various journals, proceeding volumes and the Internet.

pollen fertility (Figs. 15, 16; Table 2).

Three populations (57334, 57291 and 57258) of *B. asiatica*, one population (55899) of *B. ceratophylla* and all two populations (55991 and 56026) of *B. chitria* have been observed to exhibit normal meiotic course, and their pollen fertility was nearly 100%.

Discussion

A perusal of the cytological collective literature shows that 110 species/110 cytotypes of the genus *Berberis*, including 15 species from India, have been cytologically examined and found to have $2n=28$ (90.90%), $2n=42$ (1.82%) or $2n=56$ (7.27%) (cf. Fedorov 1969, Kumar and Subramanian 1986, Index to Plant Chromosome Numbers, web, *etc.*). However, the genus is monobasic, *i.e.*, $x=14$, as suggested earlier by Bottini *et al.* (1999). Polyploidy is noted to be 8.18%

Figs. 1–16. 1. *B. asiatica* ($2n=28$) Diakinesis. 2. *B. ceratophylla* ($2n=28$) Anaphase-I. 3. *B. chitria* ($2n=28$) Diakinesis. 4. *B. vulgaris* ($2n=28$) Metaphase-I. 5, 6. PMCs (Pollen Mother Cells) showing chromatin transfer at Anaphase-I with hyperploid (double headed arrow) and hypoploid cells (single headed arrow). 7. PMC shows bridge (arrowed) at Anaphase-I. 8. PMC showing laggards (arrowed) at Anaphase-I. 9. PMC showing laggard (arrowed) at Anaphase-I. 10. Diad with micronucleus (arrowed). 11. Triad. 12, 13. Tetrad with micronucleus (arrowed). 14. Polyad. 15. Heterogeneous-sized fertile pollen grains. 16. Fertile and sterile pollen grains. Scale 10 μ m.

compared to 6% as previously reported by Hong (1990) for species with only diploids known from India.

Cytomixis, chromosomal stickiness, unoriented bivalents, laggards and bridges among the presently studied species at the population level indicate the existence of intraspecific genetic diversity. Such genetic differences have been seen earlier in different plant species (Baptista-Giacomelli *et al.* 2000, Sheidai *et al.* 2008, Jeelani *et al.* 2012, Rani *et al.* 2012, 2013, Kumar *et al.* 2013). Cytomixis and chromatin stickiness are considered to result from genetic factors (Bellucci *et al.* 2003, Ghaffari 2006, Fadaei *et al.* 2010), environmental factors (Nirmala and Rao 1996) or genomic-environmental interactions (Baptista-Giacomelli *et al.* 2000). These factors all seem equally applicable to the presently investigated taxa. Cytomixis or the occurrence of multipolar cells and meiotic irregularities in anaphase segregation of chromosomes may be the 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 angiospermic plants

Table 2. Data on cytomixis, meiotic course, pollen fertility and pollen grain size in different species of *Berberis* marked with abnormal meiosis from the Western Himalayas.

Taxa/Accessions	Cytomixis		Meiotic course showing PMCs with						Pollen grain	
	% of PMCs involved at 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 (%)	Multipolarity at T-II (%)	Fertility (%)	Size (µm)	
<i>B. asiatica</i> 55133	2.00 (2/100)/–	2–3	3.63 (4/110)	–	3.47 (4/115)/–	3.03 (3/99)/–	–	65.87	30.78×27.78–26.78×28.89	
<i>B. ceratophylla</i> 55080	–/–	–/–	4.16 (5/120)	–	4.31 (5/116)/–	4.12 (4/97)/–	–	64.65	30.45×27.70–25.90×27.97	
<i>B. vulgaris</i> 55129	1.81 (2/110)/–	2–3	5.21 (6/115)	–	4.12 (4/97)/–	4.30 (4/93)/–	–	63.62	31.23×29.12–29.23×26.23	
52474	4.16 (3/72)/ 2.77 (2/72)	2–4	5.71 (4/70)	2.66 (2/75)	4.30 (4/93)/ 4.30 (4/93)	7.14 (6/84)/ –	2.38 (2/84)	64.65	30.05×27.18– 9.28×26.48	
56221	3.06 (3/98)/ 4.08 (4/8)	2–3	8.23 (7/85)	8.00 (6/75)	4.08 (4/98)/ 5.10 (5/98)	10.00 (7/70)/ 5.71 (4/70)	–	65.78	30.25×27.13–29.18×26.28	

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

Table 3. Data on abnormal microsporogenesis in different species of *Berberis* marked with abnormal meiosis from the Western Himalayas.

Taxa/Accessions	Dyads			Triads			Tetrads			Polyads	
	WMN	WN		WMN	WN		WMN	WN		WMN	WN
<i>B. asiatica</i> 55133	0.99 (1/101)	–		0.99 (1/101)	1.98 (2/101)		71.28 (72/101)	21.78 (22/101)		1.98 (2/101)	–
<i>B. ceratophylla</i> 55080	–	–		2.04 (2/98)	–		76.53 (75/98)	21.42 (21/98)		–	–
<i>B. vulgaris</i> 55129	0.96 (1/104)	–		0.96 (1/104)	–		70.19 (73/104)	26.92 (28/104)		0.96 (1/104)	–
52474	–	–		5.15 (5/97)/	6.19 (6/97)		69.07 (67/97)	19.59 (19/97)		–	–
56221	–	–		5.82 (6/103)/	4.85 (5/103)		66.01 (68/103)	21.35 (22/103)		–	–

Figures in parenthesis denote observed number of abnormal PMCs in the numerator and total number of observed PMCs in denominator. WMN=without micronuclei, WN=with micronuclei.

(Sheidai *et al.* 2008, Jeelani *et al.* 2011, 2012, Rani *et al.* 2012, 2013, Kumar *et al.* 2013). The production of hypo- and hyperploid PMCs due to cytomixis (Falistocco *et al.* 1995, Sheidai and Bagheri-Shabestarei 2007, Fadaei *et al.* 2010, Jeelani *et al.* 2012) accompanied by other meiotic abnormalities lead to anomalous microsporogenesis, resulting in the formation of variable-sized pollen grains, possibly with an aneuploid condition and low pollen fertility (Villeux 1985, Nirmala and Rao 1996, Sheidai and Fadaei 2005, Sheidai *et al.* 2003, Jeelani *et al.* 2012). The formation of large-sized pollen grains as seen at present is in accordance with previous information about the possibility of such pollen grains resulting from unreduced $2n$ pollen grains as has been observed in several angiosperms by Vorsa and Bingham (1979), Bertagnolle and Thomson (1995), Sheidai *et al.* (2008), Fadaei *et al.* (2010) and Jeelani *et al.* (2011).

The occurrence of limited variation of chromosome numbers but diversity in meiotic behavior at the intraspecific level of the presently studied species demands the need for extensive cytological exploration on a population basis in the genus *Berberis* from different geographical areas.

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