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 cytomixis. 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% glycerol-acetocarmine (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-disjuncting 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.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Taxa</th>
<th>Locality/Altitude (m)/Accession number</th>
<th>Chromosome number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Berberis asiatica</em> Roxb. (=B. lycium Royle)</td>
<td>Bara-gran, District/Kangra (H.P); 3,000/55133 Nauradhar, District/Sirmaur (H.P.); 1,800/57334 Batnoor-Tral, District/Pulwama (J&amp;K); 2,200/57291 Tangmarg, District/Baramulla (J&amp;K); 2,300/57258</td>
<td>(2n=28)</td>
</tr>
<tr>
<td>2</td>
<td><em>B. ceratophylla</em> G. Don</td>
<td>Chotta-bhangal/District Kangra (H.P); 2,300/55080 Triund, District/Kangra (H.P); 3,000/55899</td>
<td>(2n=28)</td>
</tr>
<tr>
<td>3</td>
<td><em>B. chitria</em> Lindl.</td>
<td>Shillai, District/Sirmaur (H.P.); 2,000/55991 Chapdhar, District/Sirmaur (H.P.); 2,400/56026</td>
<td>(2n=28)</td>
</tr>
<tr>
<td>4</td>
<td><em>B. vulgaris</em> L.</td>
<td>Boh, District/Kangra (H.P); 1,900/55129 Dachigam, District/Srinagar (J&amp;K); 1,800/52474 Tral, District/Pulwama (J&amp;K); 1,900/56221</td>
<td>(2n=28)</td>
</tr>
</tbody>
</table>

*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%
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.

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

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.

<table>
<thead>
<tr>
<th>Taxa/Accessions</th>
<th>Microsporogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dyads</td>
</tr>
<tr>
<td></td>
<td>WMN</td>
</tr>
<tr>
<td><em>B. asiatica</em> / 55133</td>
<td>0.99 (1/101)</td>
</tr>
<tr>
<td><em>B. ceratophylla</em> / 55080</td>
<td>−</td>
</tr>
<tr>
<td><em>B. vulgaris</em> / 55129</td>
<td>0.96 (1/104)</td>
</tr>
<tr>
<td>52474</td>
<td>−</td>
</tr>
<tr>
<td>56221</td>
<td>−</td>
</tr>
</tbody>
</table>

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.
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 Fadael 2005, Sheidai 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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