Cytological studies on few *Berberis* L. species

Kumkum Mishra 1, Varsha Srivastava 2*, Tariq Hussan 3 and Priyanka Aghnihotri 4

1,2 Unit of Plant Genetics, Department Of Botany, Lucknow University, Lucknow, India.
3,4 Plant Diversity, Systematics & Herbarium Division, CSIR-National Botanical Research Institute, Lucknow-226001, India.

ABSTRACT

In India the genus *Berberis* is represented by 55 species distributed predominantly in the Himalayan region. They have also been reported from Bihar and Madhya Pradesh where one species i.e. *B. hainesii* is endemic. The taxa have time and again faced taxonomical ambiguity owing to number of variations in morphology. It has been also revealed that there is need for biosystematics studies to place the taxa their respective group. The genus has been studied in the area of floral anatomy, palynology and DNA bar coding, the studies having assisted in solving taxonomical problems to quite an extent however may still remains unresolved, for which one has to fall back on cytological studies.

In the present study cytological analysis was carried out on squash preparations of shoot tips and buds of *B. asiatica*, *B. glauccarpa*, *B. lycium* and *B. hainesii* collected from different parts of India. The observations exhibited several mitotic and meiotic aberrations such as fragmentations, bridges, condensation, clumping, tripolar and multinucleate conditions, micronuclei, laggards and stray owing to unstable behaviour of chromosome and malfunctioning of spindle fibers. Significant differences in the mitotic index of *B. asiatica* and *B. hainesii* and also in *B. asiatica* and *B. lycium* were observed. The findings related to the chromosomal behaviour points to the fact that the ambiguity exists not only due to extensive variations at morphological level but also at cytological level too. Further detailed karyotypic studies are required to unveil the cause for these variations and use these variations for appropriate placement of this genus.

Key word: Berberis, Kumaon Himalaya, Cytology, Mitotic anomalies, Meiotic anomalies.

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INTRODUCTION

The genus *Berberis* was first described by Linnaeus [1] and family Berberidaceae was first established by Jussieu [2] as Berberides. It was considered to be one of the most important primitive angiospermous family. The main alkaloid Berberine is obtained from alcoholic extracts of the root apex has a great economic importance. It is one of the important constituent in antibacterial medicines used for treatment of oriental sore and dysentery [3]. The taxonomic account of the family Berberidaceae from India was first published by Hooker [4]. In India the genus is represented by 55 species distributed predominantly in the Himalayan regions [5]. There are taxonomical and nomenclatural problems related to these taxa. Added to this perhaps, is of the immense difficulty in their collection, therefore this family remains most neglected even by other experimental botanists in India. Morphological taxonomists [6,5,7] revised the genus based entirely on the morphological characters like leaves, arrangement of spines, deciduous evergreen nature and colouration of berries. It was observed that these morphological characters vary not only from population to population but even within the same population and within the same plant. The efforts were taken up in the field of taxonomy [6], floral anatomy, palynology [8] and DNA barcoding [9] but the taxonomic loopholes exist in the genus owing to lack of proper information because workers have primarily based their observations on solitary collection, scattered in different herbaria. Also study of live plant in natural habitat along with cytology has rarely been attempted. As a result no such detailed information is available on the genetics of the genus. Rao et al. [5] suggested the use of biosystematics approaches for solving the taxonomical problems in this group. Earlier studies has also been revealed the taxonomic clarification of the genus on the basis of cytological studies viz; bryophytes [10], *Fagaceae* [11], *Cruciferae* [12]. The present study aims at elucidating the cytological variations that exist in a given population and even within a single plant with the objective of solving taxonomical clutter that exists in this genus.
In the present study the detailed cytological studies were conducted in *Berberis asiatica*, *B. glaucocarpa*, *B. lycium* and *B. hainesii* collected from different parts of India like Kumaon Himalayan region, Madhya Pradesh and Himachal Pradesh with the above mentioned objectives in mind.

**MATERIALS AND METHODS**

**Taxonomical analysis**

Taxonomical work was done by intense survey of entire vegetation from Kumaon Himalayan region (Nainital and Almorah) altitude ranges from 1460m to 2614 m, M.P. (Pachmarhi) altitude ranges from 1110m to 1488 m, respectively. Fifteen plant excisions were collected along with 78 samples belonging to four species of *Berberis* taken from Kaichi Dham (alt. 1450 m), China Peak (alt. 1450 m), China Peak (alt. 2025 m), M.P., and Patnitop (alt. 2025 m), H.P. etc. Herbarium specimens were prepared using standard Herbarium technique [5] for correct identification and were deposited in LWG for further consultation.

**Cytological analysis**

The shoot tips and buds were fixed in carnoy’s fluid for cytological studies and the squash were prepared according to method of Darlington and Lacour [13]. The mitotic index of three species i.e. *B.asiatica*, *B.hainesii* and *B. lycium* were calculated and several mitotic anomalies were scored from these three species (Table-1). Chromosome numbers of two species viz; *B.hainesii* and *B.glaucocarpa* were also counted. In *B.asiatica* various meiotic anomalies were scored.

**Table 1:** Comparative studies of Chromosomal and Mitotic aberrations in three species of *Berberis*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosomal aberrations</th>
<th>Mitotic aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of metaphase</td>
<td>Fragment</td>
</tr>
<tr>
<td><em>B. asiatica</em></td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td><em>B. hainesii</em></td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td><em>B. lycium</em></td>
<td>49</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>45</td>
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</tbody>
</table>

**Statistical analysis**

The significant differences among the species were measured by t-value analysis at p<0.05 [14].

**RESULTS**

During the present study it was observed that both the mitotic as well as meiotic divisions were not normal.

**Mitotic study**

Chromosome no. of 2 species viz. *B. hainesii* and *B. glaucocarpa* were counted from the c-metaphase cells and found same in both the mentioned species i.e. 2n=28(Fig.3 and Fig.4). Study revealed that there were significant difference in the mitotic index between *B. asiatica* (14.40±0.17) and *B. hainesii* (17.80±2.1) and between *B. asiatica* (14.40±0.17) and *B. lycium* (17.20±0.93) (Fig. 5) at p<0.05.

Several mitotic anomalies have also been seen like fragmentation, condensation, laggard, bridge, multipolar conditions, micronuclei and unequal separations (Fig.1).

**Meiotic study**

Various meiotic anomalies were observed in *B. asiatica* like fragmentations, laggards, condensation, bridges and polyploidy. (Fig.2). Among all these, most frequently encountered was polyploidy.
Figure 1: (A-C) *Berberis asiatica*: A. Fragmentation in metaphase, B. C-metaphase, C. Micronuclei and binucleate condition; (D-F) *B. hainesii*: D. Fragmentation in metaphase, E. Disorientation in anaphase, F. Laggard in anaphase; (G-I) *B. lycium*: G. Condensation in metaphase, H. C-metaphase, I. Stray in metaphase.

Figure 2: Various stages of meiotic division in *Berberis asiatica*: A. Fragmentation in metaphase, B. Condensation in anaphase, C. Tetrad.

Figure 3 Metaphase of *Berberis hainesii*  
Figure 4 Metaphase stage of *Berberis glaucocarpa*
DISCUSSION
The present study revealed that the 2n = 28 in B. hainesii and B. glaucocarpa. Similar results were obtained in other two species i.e. B. asiatica [15] and B. lycium [16] further there was no difference in the number of chromosomes in these species therefore studies were conducted to highlight cytological variations if any among them. Present study revealed that the cell divisions were not normal. The nature of chromosomes of these three species showed significant differences as depicted by mitotic index among them (Fig. 5). Various mitotic anomalies like condensation, bridges, fragmentations, laggards, disorientation, unequal separation, micronuclei were seen during cell division (Table 1). This may be the possible reason for high degree of variations in these taxa. In B. asiatica during meiotic cell division polyploidy was frequently seen and it was observed that the behaviour of chromosome is unstable. Levin and Funderberg [17], Bottini et al. [18,19] had earlier reported that environmental fluctuations were responsible for polyploidy however in the present study since samples were collected from the same environment polyploidy may be due to the unstable behaviour of chromosomes instead of environmental fluctuations. Bottini [18] revealed several important features of plants which promote polyploidy for instance: 1) a long life cycle generally associated with means of vegetative reproduction; 2) common occurrence of natural interspecific hybridization. Berberis fulfils the first condition specially because of predominant vegetative mode of reproduction. Hybridization is also common in some species of Berberis as observed by Bottini [18]. The condensation seen in mitotic as well as in meiotic divisions may arise due to improper folding of chromosomes. The fragmentations seen during the cell divisions may be due to chromosomal damage caused by fungi Puccinia graminis. Similar results were reported by Ahnstrom and Natrajin [20] and Kihlman et al. [21]. It was suggested that the chromosome damage was caused by an enzyme which actively removes nucleotide material from the already formed chromosomal DNA and unequal separation could be caused by malfunctioning of spindle fibers. It can be concluded from the above findings that genes govern the chromosomal behaviour and may be responsible for unstable behaviour of chromosomes. Additional karyotypic studies may help to solve the problems which are underway. The present study indicates that the morphological variations may have bearing or a correlation with variations at cytological level.

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REFERENCES


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