Analysis of Meiotic Behavior in *Eremurus himalaicus* Baker (Liliaceae):
A Rare Endemic Perennial from Kinnaur, Himachal Pradesh, India

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Received February 25, 2016; accepted September 3, 2016

**Summary**
Detailed meiotic studies have been carried out in 11 accessions of *Eremurus himalaicus* Baker belonging to family Liliaceae—an endemic medicinal plant of the Northwestern Himalayas. *E. himalaicus*, due to its excessive exploitation for edible uses, is living under stress and has therefore been listed in the Red Data Book of Indian Plants as a ‘rare’ species. Considering \( x=7 \) as the basic chromosome number for the species, *E. himalaicus* revealed the diploid chromosome count of \( 2n=14 \). Out of 11 populations worked out cytologically, 3 populations of the species were normal in their meiotic behavior while the rest of the populations showed abnormal meiotic behavior. The phenomenon of cytomixis, presence of two to five nucleoli and various other meiotic abnormalities were observed in the form of unoriented bivalents, chromosome stickiness, late disjunction, laggards and bridges in eight populations. Earlier studies were limited to the counting of chromosome number, so these meiotic abnormalities were reported for the first time in the studied species. Further, the microsporogenesis was also abnormal leading to the formation of monad, dyads, triads and abnormal tetrads with the fusion among tetrads which ultimately leads to reduced pollen fertility.

**Key words** *Eremurus himalaicus*, Medicinal plant, Cytomixis, Abnormal meiosis, Himachal Pradesh, Kinnaur.

*Eremurus himalaicus* Baker (Himalayan Desert Candle) is a herbaceous perennial with long leaves, hundreds of white flowers held in long 30–40 cm dense racemes which resemble the tail of the fox and therefore is called the Himalayan foxtail Lily. Petals are oblong blunt with a brown line on the outside, and the leaves are all at the base, narrow, 30–40 cm long and erect. The young leaves of *E. himalaicus* locally named as 'Tache' are used as vegetables by the tribals of Kinnaur during summer, and the local people dry the leaves and cook them during winter time when no green vegetable is available due to heavy snow fall. The plant has medicinal importance. Powdered roots and boiled leaves are used by tribes to cure fever, dysentery and diabetes. The leaves are used to cure anemia (Shailja 2011) and they also possess antibacterial, antibiotic and antidiabetic properties (Zhou et al. 2010).

*Eremurus himalaicus* is endemic to the Northwestern Himalayas and is found in the rocky slopes of the drier and cold desert areas of the Himalayas from Afghanistan to Himachal. On account of its widespread exploitations for edibility and medicinal leaves, the species is living under stress and has therefore been listed in the red data book of Indian plants as a rare species (Nayar and Sastry 1987). The process of meiosis is a programmed genetic event and is pivotal in understanding reproduction, fertility, genetics and breeding in crop plants (Armstrong and Jones 2003). Till now very few attempts have been made to study this endemic species for chromosome counts from India (Pandita 1979, Mehra and Sachdeva 1976) and outside India (Oksala and Therman 1977), and no major investigation has been carried out.

Biodiversity conservationists are advocating that the rare endemic species be given priority for conservation (St. Clair and Howe 2011). Before finding the strategy for conservation of any plant, it is essential to have detailed knowledge about reproductive parameters of the endemic species. Presently, *E. himalaicus*, a rare endemic species of the Northern Himalayas, was studied cytologically. The main objective of the present research was to study the detailed meiotic course, to mark out the variability in meiotic abnormalities, microsporogenesis and the effects of these meiotic abnormalities on pollen fertility in the accessions of *E. himalaicus*, collected from Hangrang Valley of Kinnaur, Himachal Pradesh, India.

**Materials and methods**

Materials for male meiotic studies were collected...
from wild accession, and the meiotic behavior of 11 populations of *E. himalaicus* has been analyzed. Systematic surveys were conducted during the snow-free periods of April 2012 to September 2015 to cover different altitudinal zones between 3000–3700 m. The plant specimens were identified at Botanical Survey of India (BSI) herbarium, Dehradun. Voucher specimens of the cytologically studied plants were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). The young floral buds of suitable sizes were fixed in Carnoy’s fixative for 24 h and then stored in 70% alcohol at 4°C until use. For meiotic studies anthers were squashed in 2% acetocarmine. A number of freshly prepared slides were examined for meiotic analysis and chromosome counts. Pollen fertility was estimated through stainability test for which mature anthers were squashed in glyceroacetocarmine (1:1) mixture. Well-filled pollen grains with a uniformly stained cytoplasm were considered fertile, while shriveled pollen grains with no or lightly stained cytoplasm were counted as sterile. Photomicrographs were made from the temporary mounts using a Nikon 80i Eclipse Microscope.

**Figs. 1–12.**
1) A PMC at diakinesis showing seven bivalents (P-2). 2) A PMC showing seven bivalents at M-I (P-11). 3) A PMC showing 7:7 distribution of chromosomes at A-I (P-9). 4) A PMC with two nucleoli at diakinesis (arrowed) (P-1). 5) A PMC with three nucleoli at diakinesis (P-2). 6) A PMC with four nucleoli at diakinesis (P-2). 7) A PMC at M-I showing interbivalent connections (P-4). 8) A PMC showing interbivalent connection in distantly placed bivalents at M-I (P-1). 9) A PMC showing stickiness of chromosomes at M-I (P-2). 10) A PMC showing stickiness of chromosomes at A-II (P-2). 11) A PMC showing late disjunction of chromosomes at A-I (P-11). 12) A PMC with late disjunction at A-II (P-6).
Results

The accession studied showed the meiotic chromosome number \(2n=14\) (based on \(x=7\)) at different stages like diakinesis and metaphase I, and 7:7 chromosome distribution at anaphase I (Figs. 1–3) which is in accordance with the earlier meiotic reports. Cytological investigations of the three accessions of \(E. \) himalaicus (Gonba 3500 m, Hango 3600 m, Maling 3600 m) showed normal meiosis while the remaining accessions showed one or more types of meiotic abnormalities. The data for different populations, total number of PMCs analyzed, percentage of cytomixis, number of nucleoli and other different meiotic abnormalities from diakinesis to telophase II and percentages of irregularities at each meiotic phase and interpopulation differences in the meiotic abnormalities are represented in Table 2.

In four populations of \(E. \) himalaicus (Pooh Gonba 2700 m, Thul 3600 m, Chalong 3550 m, Gonba 3500 m), the persistence of one to five nucleoli of variable sizes has been observed at different stages of meiosis from early prophase to late anaphases (Figs. 4–6, 22–23). Earlier persistent nucleoli have been noted in certain species of plants as a normal feature during mitosis by Brown and Emery (1957). Besides these nucleoli, interbivalent connection between two to three bivalents was seen in five populations (Thul 3650 m, Rishing 3650 m, Chuling 3510 m, Sholing 3000 m, Pooh Gonba 2700 m) at metaphase between closely associated bivalents (Fig. 9) and distantly placed bivalents (Fig. 10). Late disjunction was observed in populations collected from three different regions (Thul 3650 m, Pooh 2662 m, Pooh Gonba 2700 m), which is the failure of homologous chromosomes or sister chromatids to separate properly.

### Table 1. The data showing populations collected, their accession number, microsporogenesis, pollen fertility and pollen sizes.

<table>
<thead>
<tr>
<th>Population</th>
<th>Locality/Altitude (m)</th>
<th>Accession number (PUN)</th>
<th>Microsporogenesis (%PMCs)</th>
<th>Pollen fertility (%)</th>
<th>Pollen size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Monad</td>
<td>Dyad</td>
<td>Triad</td>
</tr>
<tr>
<td>P-1</td>
<td>Thul (3650 m)</td>
<td>59594</td>
<td>11.21</td>
<td>13.67</td>
<td>23.84</td>
</tr>
<tr>
<td>P-2</td>
<td>Chalong (3080 m)</td>
<td>59595</td>
<td>14.56</td>
<td>11.53</td>
<td>—</td>
</tr>
<tr>
<td>P-3</td>
<td>Rishing (3650 m)</td>
<td>59596</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-4</td>
<td>Chuling (3510 m)</td>
<td>59597</td>
<td>19.60</td>
<td>21.17</td>
<td>—</td>
</tr>
<tr>
<td>P-5</td>
<td>Gonba (3500 m)</td>
<td>59598</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-6</td>
<td>Pooh (2626 m)</td>
<td>59599</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-7</td>
<td>Hara (3000 m)</td>
<td>59600</td>
<td>21.60</td>
<td>—</td>
<td>17.85</td>
</tr>
<tr>
<td>P-8</td>
<td>Maling (3600 m)</td>
<td>59601</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-9</td>
<td>Sholing (3000 m)</td>
<td>59618</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-10</td>
<td>Hango (3600 m)</td>
<td>59619</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-11</td>
<td>Pooh Gonba (2700 m)</td>
<td>59602</td>
<td>16.66</td>
<td>17.54</td>
<td>15.62</td>
</tr>
</tbody>
</table>

All localities in Hangrang Valley Kinnaur (H.P)

### Table 2. The data showing different populations, total number of PMCs analyzed, percentage of cytomixis, number of nucleoli and other different meiotic abnormalities from diakinesis to telophase II and percentages of irregularities at each meiotic phase.

<table>
<thead>
<tr>
<th>Population</th>
<th>Total number of PMCs analyzed</th>
<th>Cytomixis (%)</th>
<th>Number of nucleoli</th>
<th>Total number of PMCs analyzed</th>
<th>Interbivalent connection at M-I</th>
<th>Stickiness (%)</th>
<th>Late disjunction at A-I</th>
<th>Unoriented bivalents at M-I</th>
<th>Laggards at A-I/A-II</th>
<th>Bridges at A-I/A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-2</td>
<td>—</td>
<td>—</td>
<td>1–4</td>
<td>193</td>
<td>—</td>
<td>17/66</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>171</td>
<td>5/48</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-4</td>
<td>142</td>
<td>10/142 (7.04%)</td>
<td>—</td>
<td>247</td>
<td>4/47</td>
<td>17/72</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3/37</td>
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<tr>
<td>P-5</td>
<td>—</td>
<td>—</td>
<td>1–2</td>
<td>191</td>
<td>—</td>
<td>16/63</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P-6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>77</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4/28</td>
<td>3/49</td>
</tr>
<tr>
<td>P-7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>237</td>
<td>4/66</td>
<td>35/126</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4/45</td>
</tr>
<tr>
<td>P-8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>P-10</td>
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<td>1–3</td>
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<td>P-11</td>
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</table>
during meiosis I and meiosis II. Late disjunction of chromosomes during their segregation is generally due to interlocking of chiasmata. This type of chromosome behavior may result in laggard formation during anaphase/telophase and consequently reduced pollen fertility (dela Vina and Ramirez 1995).

Further, the cytomixis involving transfer of chromatin material among proximate PMCs at early stages of prophase, diakinesis and telophase of meiosis was observed in two populations collected from Thul (3650 m) and Chuling (3510 m) (Figs. 14–16). Due to the presence of cytomixis some PMCs become either hypoploid or hyperploid (Figs. 17–18). Figure 19 shows a PMC with hypoploid chromosome with six bivalents at metaphase I, and Fig. 20 shows hyperploid PMCs with 10 bivalents during metaphase I. Cytoplasmic connections were also present among the microspores of a tetrad giving the appearance of a ring-like structure (Fig. 28). According to some workers (Maheshwari 1950, Kundu and Sharma 1988, Sen and Bhattacharya 1988), early stages of meiosis are more favorable for cytomixis.

The majority of the PMCs observed showed irregular spindle formation, which resulted in PMCs showing the presence of a few out-of-plate bivalents at metaphase I
An analysis of meiotic behavior in *Eremurus himalaicus* Baker (Liliaceae)

(Fig. 13) and a high amount of chromatin stickiness at metaphases and anaphases of meiosis which involved clustering of chromosomes (Figs. 9–10). Due to severe chromatin stickiness, the presence of lagging chromosomes at A-I/A-II (Figs. 19–20) and chromosome bridges at A-I/A-II and T-II (Figs. 21–24) were observed in a majority of the populations studied, and various forms of bridges like single bridge or a couple of bridges with variable thickness were observed when most of the chromosome complements began to move to the poles.

Due to abnormal chromosomal behavior during the different stages of meiosis, abnormal microsporogenesis was observed in five populations (Thul 3650 m, Chalong 3080 m, Chuling, 3510 m, Pooh Gonba 2700 m, Hara 3000 m) which revealed the presence of monads, dyads, triads and cytoplasmic connections in tetrads (Figs. 25–28). The data regarding populations collected, their accession number, microsporogenesis, pollen fertility and pollen sizes are provided in Table 1.

Pollen fertility was also lower in a majority of the accessions, and it varied from 63.05 to 83.69% which may be due to a physiological factor, but the size of the pollen grains was found to be almost the same in all populations and no micronuclei were observed in tetrads (Fig. 29). The abnormal meiotic course due to irregular spindle activity, chromatin transfer among neighboring PMCs and chromatin stickiness which results in abnormal sporads and reduced pollen fertility has been reported in a number of flowering plants (Baum et al. 1992).

### Discussion

Detailed meiotic abnormalities including cytomixis and diploid chromosome count (2n=14) were studied in the species of *E. himalaicus*. Cytologically, the species *E. himalaicus* has been studied little, and the studies are limited to the counting of chromosome number only. In order to contribute to the better understanding of the species, the study reports the chromosome number and the various meiotic abnormalities including cytomixis through the analysis of various accessions. In the presently studied species of *Eremurus*, out of various meiotic abnormalities, chromosome stickiness, laggards and bridges were observed to occur at a high frequency and in a majority of the populations.

Chromosome stickiness involving the clustering of chromosomes during meiosis I was observed in six populations (Thul 3650 m, Chalong 3080 m, Chuling 3510 m, Pooh 2662 m, Sholing 3000 m, Pooh Gonba 2700 m). Earlier, Beadle (1932) reported chromosome stickiness in maize for the first time and attributed such irregularity due to a mutation caused by a recessive gene called sticky (st). After that, reports on chromosome stickiness on different species of grasses have been published like genus *Brachiaria* (Pagliarini et al. 2008), *Pennisetum* (Rao et al. 1990), wheat (Zanella et al. 1991) and maize (Caetano-Pereira et al. 1998). Stickiness may also have been caused by environmental factors such as X-rays, temperature and soil elements (Mendes-Bonato et al. 2001). Besides the presence of chromosomal stickiness, the spindle abnormalities resulted due to the environmental influence and dishar-
monious gene interaction (Nirmala and Rao 1996). The causes of bridges and laggard formation may also be due to interlocking of bivalents (Bhattacharjee 1953) or paracentric inversion (Sinha and Godward 1972) or delayed chiasma terminalization (Kumar and Tripathi 2007).

Cytomixis was observed during early prophase, diakinesis and T-I involving two to three PMCs (Figs. 16–18). This phenomenon of cytomixis involving transfer of chromatin material among proximate meiocytes was first recorded by Koernnkie (1901) in *Crocus sativus*. Since then, this phenomenon was reported by many workers in a wide range of flowering plants (Sarvella 1958, Omara 1976, Saggoo and Bir 1983, Sen and Bhattacharyya 1988, Bedi 1990, Datta et al. 2005, Lattoo et al. 2006, Kumar et al. 2010, Saggoo and Kumari 2013).

The possible causes suggested earlier include the effect of fixation (Heslop-Harrison 1966), pathological changes (Morisset 1978), chemicals and herbicides (Ajay and Sarbhoy 1987). Recent evidence suggests that it is a normal, genetically controlled phenomenon influenced by physiological and environmental factors (Omara 1976, Lattoo et al. 2006).

According to some workers, meiosis is the most sensitive stage in the life cycle of plants and is primarily influenced by various genetic and environmental factors (Ahmad et al. 1984). There are a number of reasons suggested by various workers for the cause of cytomixis and associated meiotic abnormalities in flowering plants. In the present study, we are here of the view that climatic conditions, particularly high altitude and the low temperature during flowering period, may be a possible cause of cytomixis and associated meiotic abnormalities.

It is thus concluded that due to various meiotic abnormalities during meiosis I and meiosis II including cytomixis and abnormal microsporogenesis, the pollen fertility was reduced in a majority of the accessions, but the size of the pollen grains was same in all the accessions.

Acknowledgements

The authors are grateful to the UGC, New Delhi for providing financial assistance under the UGC-BSR Fellowship and DRS SAP III and ASIST programme. Authors are also grateful to the resource people of tribal areas of Kinnaur for sharing valuable information. We are thankful to the Director Botanical Survey of India, Dehradun for helping in authentication of herbarium samples. Thanks are also due to the Head, Department of Botany, Punjabi University, Patiala for providing all the necessary laboratory facilities.

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