Distribution Pattern, Variations of Morphology and Chromosome Numbers of *Sium latijugam* C. B. Clarke (Apiaceae) from the Kashmir Himalayas

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**Summary**  The present study was performed for the Himalayan medicinal species *Sium latijugam* on male meiosis, morphological parameters and distribution pattern of diploid as well as tetraploid cytotypes. Both diploid (2n=12) and tetraploid (2n=24) cytotypes were found in Kashmir, however, the tetraploid cytotype is new addition to the species for the first time. The tetraploids were restricted to higher altitudes and characterized by abnormal meiosis leading to high pollen sterility and formation of variable sized pollen grains. Additionally tetraploids were morphologically more luxuriant in comparison to their diploid counterparts.

**Key words**  Cytotypes, Distribution, Abnormal meiosis, Kashmir Himalayas, *Sium latijugam*.

The species of family Apiaceae are mostly aromatic in nature. However, many of these are known for their ethnobotanical and medicinal properties (Sharma *et al.* 2005, Qureshi *et al.* 2007). The genus *Sium* L. also includes such species with great medicinal value. It contains 15 species including one from India (Aswal and Mehrotra 1994) is wide spread in Africa, Asia, Europe and North America includes perennial glabrous herbs, generally occurring in aquatic habitats.

*Sium latijugam* C. B. Clarke is abundant in the water channels and found throughout the Kashmir valley between 1600–3000 m, characterized as glabrous, up to 1.5 m tall, much branched herb with lower leaves petiolate and upper sessile to sub-sessile, flowers small white, fruit oblong, furrowed. The flowering and fruiting is seen from July–September.

The survey of literature shows that there exists a lot of variation in the quantity and quality of active principles in different morphotypes/cytotypes of the same species (Bahuguna *et al.* 2000, Berkov 2001), so the present study was performed in the same direction to look for cytomorphic diversity in the different populations of *S. latijugam* from different areas of Kashmir Himalayas to ultimately mark out the better performing variants for further exploitation.

### Materials and methods

**Materials**

For meiotic studies, flower buds were collected from 15 randomly selected plants of each population growing in different localities of Kashmir Himalayas (Table 1).

**Morphological study**

A total of 10 morphometric characteristics (see Table 2) were studied for each cytotype to have proper insight of morphological variation. For stomata studies, mature leaves were treated with 10% aqueous solution of potassium hydroxide (KOH) at room temperature for 10–15 min and then epidermal peels so obtained were stained with 10% safranin in 95% ethanol.

**Cytological study**

For population-based meiotic studies, flower buds were collected from several populations of each species from different localities in the Kashmir Himalayas. The smears of appropriate-sized flower buds were made after fixing these flower buds in Carnoy’s fixative by using the standard acetocarmine technique. To confirm the chromosome number in the case of normal meiosis, around 50 pollen mother cells (PMCs) were observed at different stages of meiosis, preferably at diakinesis/metaphase-I/anaphase-I or anaphase-II. In the case of abnormal meiosis, however, more than 300 PMCs were considered to ascertain the type and frequency of various abnormalities per plant. Pollen fertility was estimated by mounting mature pollen grains in glycerol–aceticarmine (1:1) mixture. Approximately 400–500 pollen grains were counted at random.
The tetraploids show mild gigantism in vegetative and some significant qualitative and quantitative differences. Morphological comparison of diploids and tetraploids reveals the species, six are diploids, and three are tetraploids. The morphological comparison of diploids and tetraploids reveals some significant qualitative and quantitative differences. The tetraploids show mild gigantism in vegetative and reproductive characters, as is apparent from increased plant height, number of leaves/plant, size of leaflets, stomata and pollen grain size as well as number of umbels/plant (Table 2).

Results and discussion

Meiotic analysis was made on nine populations of *Sium latijugam* from different areas of Kashmir Himalayas along with previous chromosome reports. Among the nine worked out populations of the species, six are diploids, and three are tetraploids. The morphological comparison of diploids and tetraploids reveals some significant qualitative and quantitative differences. The tetraploids show mild gigantism in vegetative and

<table>
<thead>
<tr>
<th>Populations</th>
<th>Locality/Altitude (m)/Geographical coordinates</th>
<th>Observed chromosome number (2n)/Ploidy level (x)/Nature of meiotic course</th>
<th>Previous chromosome numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Dachigam/2500/34° 04′N, 74° 24′E</td>
<td>12/2x/N</td>
<td>2n=12</td>
</tr>
<tr>
<td>P2</td>
<td>Aru/2800/34° 05′N, 75° 15′E</td>
<td>24′ 4x/A</td>
<td>Naqshi and Koul 1974;</td>
</tr>
<tr>
<td>P3</td>
<td>Yusmarg/2600/33° 47′N, 74° 38′E</td>
<td>12/2x/N</td>
<td>Naqshi and Javeed 1976;</td>
</tr>
<tr>
<td>P4</td>
<td>Mahadev/3200/34° 10′N, 75° 01′E</td>
<td>24/2x/A</td>
<td>Ahmad and Koul 1980;</td>
</tr>
<tr>
<td>P5</td>
<td>Gurez/2900/34° 38′N, 74° 46′E</td>
<td>12/2x/N</td>
<td>Hamal et al. 1986;</td>
</tr>
<tr>
<td>P6</td>
<td>Chumnai/3000/34° 04′N, 75° 18′E</td>
<td>12/2x/N</td>
<td>Khatoon and Ali 1993;</td>
</tr>
<tr>
<td>P7</td>
<td>Liddervatt/3100/34° 04′N, 75° 14′E</td>
<td>12/2x/N</td>
<td>Kumar and Singhal 2011a</td>
</tr>
<tr>
<td>P8</td>
<td>Daksum/2400/33° 36′N, 75° 28′E</td>
<td>124x/N</td>
<td></td>
</tr>
<tr>
<td>P9</td>
<td>Thajwas/3300/34° 17′N, 75° 17′E</td>
<td>24/2x/A</td>
<td></td>
</tr>
</tbody>
</table>

"New varied report for the species; ***N=normal, A=abnormal

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Character</th>
<th>Diploids</th>
<th>Tetraploids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Plant height (cm)</td>
<td>60–80 (72.8±5.13)</td>
<td>100–120 (110.9±5.8)</td>
</tr>
<tr>
<td>2.</td>
<td>Stem surface</td>
<td>Smooth</td>
<td>Hairy</td>
</tr>
<tr>
<td>3.</td>
<td>Number of leaves/plant</td>
<td>6–10 (7.4±1.12)</td>
<td>12–14 (12.7±0.86)</td>
</tr>
<tr>
<td>4.</td>
<td>Leaf surface (upper/lower)</td>
<td>Smooth</td>
<td>Hairy (more/less)</td>
</tr>
<tr>
<td>5.</td>
<td>Leaf margins</td>
<td>Shallowly teethed</td>
<td>Deeply teethed</td>
</tr>
<tr>
<td>6.</td>
<td>Size of leaflets (cm)</td>
<td>1.2–1.7±0.3–0.5</td>
<td>4.0–4.5×1.5–2.2</td>
</tr>
<tr>
<td>7.</td>
<td>Average stomata size (µm)</td>
<td>18.78±0.82×15.65±0.67</td>
<td>21.65±1.19×17.67±0.70</td>
</tr>
<tr>
<td>8.</td>
<td>Average stomatal index (µm)</td>
<td>12.45±1.02</td>
<td>15.67±0.98</td>
</tr>
<tr>
<td>9.</td>
<td>Number of umbels/plant</td>
<td>4–6 (5.0±0.81)</td>
<td>10–12 (11.0±0.81)</td>
</tr>
<tr>
<td>10.</td>
<td>Pollen grains size (µm)</td>
<td>18.16–18.42×8.51–8.68</td>
<td>23.45±0.84×8.96±0.50ª</td>
</tr>
</tbody>
</table>

Each value based on minimum of 10 observations. *Larger; †Smaller

Grains were analyzed in each case for evaluating pollen fertility and pollen size. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shrivelled and unstained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Nikon 80i eclipse Digital Imaging System.

**Morphological variations**

Among the nine worked out populations of the species, six are diploids, and three are tetraploids. The morphological comparison of diploids and tetraploids reveals some significant qualitative and quantitative differences. The tetraploids show mild gigantism in vegetative and geographical distribution of diploids and tetraploids

*S. latijugam* has been cytologically worked out from different temperate regions. The distribution of the diploids and tetraploids in India and Kashmir Himalayas is shown in Fig. 1. Only diploids have been reported in the other parts of Himalayas, whereas both diploids and tetraploids are available in Kashmir Himalayas and the...
earlier being more common. However, the tetraploids are restricted to higher altitudes (2800–3300 m) than diploids and thus the species harbours maximum genetic diversity Kashmir Himalayas.

Cytological variations

Cytologically six populations are diploids revealing $2n=12$ at diakinesis and metaphase-I (Fig. 2a, b), whereas other three are tetraploids depicting $12:12$ distribution of chromosomes at anaphase-I (Fig. 3a) and other stages in different PMCs. The chromosome number $2n=12$ is in accordance with the previous reports from Kashmir (Naqshi and Javeid 1976), Lahul-Spiti (Kumar and Singhal 2011a) and Pakistan (Khatoon and Ali 1993), whereas $2n=24$ adds a new tetraploid cytotype indicating intraspecific polyploidy in the species from the area.

Cytological review brings to light that eight species/15 cytotypes are known for the genus. The species in the genus show chromosomal races as $2n=12, 18, 20, 22, 24, 40, 56$ describing genus to be polybasic ($x=6, 9, 10, 11$). Nevertheless, $x=6$ remains to be the most common one and others ($x=10, 11$) are secondarily evolved base numbers. Further, some authors have reported B-chromosomes in the genus from outside India e.g., in S. latifolium (Dmitrieva 2000).

Abnormal meiotic behavior in tetraploids

Only the tetraploids revealed abnormal meiosis in the form of meiotic anomalies as cytomixis involving two to three PMCs at meiosis-I/II, chromatin stickiness at metaphase-I, chromosomal laggards during meiosis-I/II and bridges during meiosis-I (Table 3; Fig. 3b–f). Microsporogenesis has also been seen to be abnormal with the formation of triads (Table 4; Fig. 3g) in low frequencies along with micronuclei, mostly associated with tetrads (Table 4; Fig. 3h). All these abnormalities induce variable sized fertile pollen grains (Fig. 3i) and reduction in pollen fertility (Table 4). In the present study, the occurrence of meiotic abnormalities in some populations only, indicate the existence of intraspecific genetic diversities. Such genetic differences have earlier been recorded in different plant species (Baptista-Giacomelli et al. 2000, Jeelani et al. 2010, 2011).

The diploids lack meiotic abnormalities and exhibit high pollen fertility (90–95%). Similarly, the diploid cytotypes with normal meiosis and the polyploid cytotypes with cytomixis and other abnormalities have already been shown in some other angiosperms (Sheidai and Fadai 2005, Kumar and Singhal 2011b, Rani et al. 2014).

According to Singhal and Kumar (2008), Fatemeh et al. (2010), Jeelani et al. (2011) and Rani et al. (2015) cytomixis results in the production of unreduced gametes in several angiosperms or leads to the production of aneuploidy plants.

Chromatin stickiness of few bivalents or whole complement has been seen from prophase-I to metaphase-I. Cytomixis and chromatin stickiness are considered to
be the result of genetic factors (Bellucci et al. 2003, Ghaffari 2006, Fatemeh et al. 2010) and environmental factors (Nirmala and Rao 1996) as well as genomic–environmental interaction (Baptista-Giacomelli et al. 2000) and applies equally to the presently investigated populations.

The other presently observed meiotic abnormalities (chromosomal laggards and chromatin bridges seen at anaphases/telophases and aberrant spindle activity in the PMCs) also seem to be induced by cytomixis as in line with earlier suggestions made by Kumar et al. (2010) that laggards when failed to get included in telophasic

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**Table 3.** Data on abnormal meiotic course in different populations of *S. latijugum.*

<table>
<thead>
<tr>
<th>Population No.</th>
<th>Cytomixis at meiosis-I/meiosis-II</th>
<th>Meiotic course showing PMCs with</th>
<th>Laggards at meiosis-I/meiosis-II (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of PMCs involved % of PMCs involved</td>
<td>Chromosomal stickiness at metaphase-I (%)</td>
<td>Bridges at meiosis-I/meiosis-II (%)</td>
</tr>
<tr>
<td>P2</td>
<td>2–3</td>
<td>5.83/3.84</td>
<td>4.13</td>
</tr>
<tr>
<td>P4</td>
<td>2–3</td>
<td>2.70/0</td>
<td>0</td>
</tr>
<tr>
<td>P9</td>
<td>2–3</td>
<td>2.4/1.73</td>
<td>2.86</td>
</tr>
</tbody>
</table>

WMN=without micronuclei, WM=with micronuclei.

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**Table 4.** Data on abnormal microsporogenesis in tetraploid populations of *S. latijugum.*

<table>
<thead>
<tr>
<th>Population No.</th>
<th>Monads (%)</th>
<th>Diads (%)</th>
<th>Triads (%)</th>
<th>Tetrads (%)</th>
<th>Pollen fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WMN WM WMN WM</td>
<td>WMN WM WMN WM</td>
<td>WMN WM WMN WM</td>
<td>WMN WM WMN WM</td>
<td>WMN WM WMN WM</td>
</tr>
<tr>
<td>P2</td>
<td>0 0 0 0 0 0</td>
<td>0 0 0 0</td>
<td>2.04 0 0</td>
<td>0 0</td>
<td>76.53 21.42</td>
</tr>
<tr>
<td>P4</td>
<td>0 0 0 0 0 0</td>
<td>0 0 0</td>
<td>2.88 0 0</td>
<td>0 0</td>
<td>70.19 26.92</td>
</tr>
<tr>
<td>P9</td>
<td>0 0 0 0 0 0</td>
<td>0 0 0</td>
<td>5.86 0 0</td>
<td>0 0</td>
<td>71.28 21.78</td>
</tr>
</tbody>
</table>
nuclei, resulted in the formation of micronuclei at sporad stage. The presence of extra chromatin material in the recipient meiocytes due to chromatin transfer also contributed to the formation of micronuclei (Bhat et al. 2006).

All these meiotic abnormalities result in abnormal microsporogenesis, leading to the formation of monads, dyads, triads, or polyads. Furthermore, micronuclei have also been observed in most of these species. These meiotic abnormalities along with abnormal microsporogenesis lead to the formation of heterogeneous sized (large and small) fertile pollen grains and reduced pollen fertility. The occurrences of large pollen grains conforms to previous information about possibility of such pollen grains to be resulting from unreduced 2n gametes as has been seen in several angiosperms (Bretagnolle and Thomson 1995, Sheidai et al. 2008, Fatemeh et al. 2010, Jeelani et al. 2011).

Conclusion

The occurrence of variation in chromosome number in the form of euploid cytotypes (2x and 4x) and differences in meiotic behavior accompanied by different distributional pattern at intraspecific level in the species indicate further need for the extensive cytological exploration of this species at population basis. This will allow marking of different cytotypes/morphotypes/ecotypes, so as to mark the best chemotype for future medicinal use.

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