Effect of B-chromosomes on meiotic behaviour and genetic recombination in *Artemisia nilagirica* (C. B. Clarke) Pamp.

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**ABSTRACT.** The cytological studies revealed a population of *Artemisia nilagirica* (C. B. Clarke) Pamp. from Sirmaur, Himachal Pradesh, India was found to be diploid (2n=18) with 0-4B chromosomes. The number of Bs was almost fixed to 4 and found in 84.44% cells. The B-chromosomes showed regular bivalent formation in most of the PMCs. In spite of these, meiotic course analysis showed the presence of various irregularities in the form of laggards, bridges, cytomixis, and failure of cytokinesis, which resulted in formation of monads, dyads, triads and micronuclei imparting overall 17% abnormality. Analysis of chiasma frequency revealed that cells without Bs showed more chiasma frequency (14.06) as compared to cells with Bs (9.2-13.73). Pollen fertility was found to be quite high (96%). Presence of B-chromosomes and other meiotic anomalies had no impact on pollen fertility.

**KEYWORDS:** *Artemisia nilagirica*, B-chromosome, Chiasma frequency, Pollen fertility.

*Artemisia* L. of the family Asteraceae is the largest and diverse genus of the tribe Anthemideae (Martin et al. 2003). It comprises of 500 species (Valles and Garnatze 2005) and ca 32 in India (Hajra et al. 1995). The present study focused on *Artemisia nilagirica* (C. B. Clarke) Pamp. It is an aromatic herb found throughout the mountainous and hilly districts of India, common along roadsides and waste places up to 600-2,700 m in the Western Himalayas. It is commonly known as Indian wormwood. Leaves are large prominently lobed, heads crowded in leafy panicles. The plant shows good anticancer potential (Devmurari and Jivani 2010).

The presence of Bs has been previously reported in 177 species of the Asteraceae (Jones and Reese 1982), in many species of *Artemisia* (Bakshi et al. 1987; Valles and Garnatje 2005). During the present study B-chromosomes were found for the first time in *A. nilagirica*. Further behavior of Bs during course of microsporogenesis was studied in detail.

**Materials and Methods**

*Collection and preservation of sample* Aerial parts of wild populations of *Artemisia nilagirica* were collected in July, 2009 from Rajgarh, alt.1637 m, Sirmaur, Himachal Pradesh, India. The plant was identified and confirmed at the Botanical Survey of India, Dehradun and its voucher specimen (PUN-52264) was deposited in the Herbarium, Department of Botany, Punjabi University, Patiala. For meiotic studies appropriate sized young capitula were collected and fixed in Carnoy’s fixative (6:3:1=absolute alcohol:chloroform:glacial acetic acid v/v) for 24 h. and then preserved in 70% alcohol at 4°C.

*Meiotic analysis* For meiotic studies the appropriate sized anthers were squashed in 1% aceticarmine. Pollen fertility was estimated by mounting mature pollen grains in glycerco-acetocarmine (1:1). Normal well filled and deeply stained pollens were taken as fertile while shriveled up and unstained pollens as sterile. Photomicrographs of chromosome counts were made from freshly prepared slides using Nikon Microscope Eclipse 80i system.

**Results**

Meiotic analysis revealed the presence of regular nine bivalents at diakinesis to metaphase I in some pollen mother cells (Table 1; Fig. 1A). In some PMCs, one bivalent showed tendency towards early separation as a result of which two univalents were clearly observed (Fig. 1B). Besides these normal bivalents, up to four B-chromosomes were reported in 92 PMCs (84.41%) out of total 109 PMCs observed at diakinesis to metaphase I. PMCs with four B-chromosomes have been found in maximum % (63.60), while 1B was reported in 23.01% PMCs (Fig. 1C). These Bs may be sometimes included to the poles (Fig. 1D) and may lag behind. The PMCs with 4 Bs tend to pair themselves to form two bivalents (Fig. 1E) and show normal segregation at anaphases and telophases stages or in some cases only two Bs pair and rest two Bs remain as univalents (Fig. 1F). Data on various meiotic configurations of A and B-chromosomes at diakinesis to metaphase I and number of chiasmata per PMC has been counted for number of cells and data was compiled in table. The number of chiasmata per PMC in A-chromosomes was found to be more in cells without Bs (14.06) and vary from 9.2 in cells with 4Bs to 13.75 in cells with 1B. Whether the Bs were in paired form or lie as univalents tend to affect the chiasmata frequency.

During anaphase I, in some PMCs B-chromosomes move towards pole with A-chromosomes (Fig. 2A). The subsequent course of meiosis is found to be abnormal with presence of laggards (1-4 per PMC) at Anaphase I/ Telophase I (10% PMCs) and Anaphase II/ Telophase II
Fig. 1. Photomicrographs of meiosis in *Artemisia nilagirica*.
A. Metaphase I showing 9II; B. Metaphase I showing 8II + 2I; C. Diakinesis showing 9II + 1B chromosome; D. Metaphase I showing 9II + 4B chromosomes and early separation of 1 bivalent (▲); E. Metaphase I showing 9II + 2BII; F. Metaphase I showing 9II + 1BII + 2BI and early separation of 1 bivalent (▲). Scale Bar = 10 μm

Table 1. Data on various meiotic configurations at diakinesis/metaphase I and chiasmata frequency.

<table>
<thead>
<tr>
<th>Meiotic configurations</th>
<th>Total PMCs involved</th>
<th>Chiasmata frequency per PMC</th>
</tr>
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<tbody>
<tr>
<td>8II + 2I + 2BII</td>
<td>29</td>
<td>09.75 ± 0.95 0.48</td>
</tr>
<tr>
<td>8II + 2I + 4B</td>
<td>5</td>
<td>09.20 ± 0.44 0.19</td>
</tr>
<tr>
<td>8II + 2I + 1BII + 2BI</td>
<td>19</td>
<td>10.00 ± 1.00 0.57</td>
</tr>
<tr>
<td>9II + 1BII + 2BI</td>
<td>6</td>
<td>10.50 ± 2.12 1.49</td>
</tr>
<tr>
<td>9II + 2BII</td>
<td>9</td>
<td>09.22 ± 0.44 0.14</td>
</tr>
<tr>
<td>9II + 1B</td>
<td>24</td>
<td>13.75 ± 0.95 0.48</td>
</tr>
<tr>
<td>9II</td>
<td>17</td>
<td>14.06 ± 2.95 0.73</td>
</tr>
</tbody>
</table>

(11% PMCs) (Figs 2B, C). Paired Bs show late separation in most of the PMCs (Fig. 2D). Further some of the PMCs with chromatin bridges were observed at Anaphase I/ Telophase I (7% PMCs) (Fig. 2E). Chromatin transfer during Anaphases/Telophases were observed in 4.30 percent PMCs (Fig. 2F) as a result cells with more than four
poles at telophase II were reported (Fig. 2G). Microsporogenesis is also abnormal with formation of monads (1.74%), dyads (4.15%), triads (5%) and tetrads with (1.59%) or without micronuclei (87.52%) (Figs 2H-L), besides the normal tetrads which lead to heterogeneous sized pollen grains. In some microsporads, micronuclei were included within nuclei or some times form separate nuclei within tetrad. In spite of all these irregularities, the apparent pollen fertility was found to be quite high (96%).

**DISCUSSION**

The present chromosome report reveals the species to be diploid (2n=18) based on x=9, most common base number in the genus (Valles and Garnatje 2005) and is in line with the previous reports by Mehra and Remanandan (1974) and Mathew and Mathew (1988). Presence of 0-4B chromosomes is the first report for the species. Bs may be present in high frequencies and their frequency mainly depends up on the degree of tolerance of the plant and accumulation strength. Number and its distribution are not definite for a species.

There is evidence that chiasma frequency, phenotype and pollen fertility are influenced by B-chromosomes but their behavior also show lot of variation. Presence of B-chromosomes either enhances, decreases or has negligible effect on these parameters. The influence of Bs on chiasma frequency is cited in number of plants such as Rye (González-Sánchez et al. 2004), Pearl Millet (Kumar et al. 2005), etc.
and Singh 2005) and Cirsium spp. (Nouroozi et al. 2011). In Trigonella foenum-graecum, the chiasma frequencies are uneven, and the total chiasmata frequency of Bs carrier plants is not proportional to the frequency of B chromosomes due to statistically non-significant differences. Thus concluded that B-chromosomes do not alter the chiasma frequency in this species. Three types of effects on B-chromosome carriers have been reported such as decrease in chiasmata frequency (Simchen et al. 1971; Bakshi et al. 1986), increase in chiasmata frequency (Ghaffari and Bidmeshkipoor 2002; Kumar and Singh 2004; Barlow and Vosa 1970) and no effect has been observed by Carter and Smith-White (1972). In present case, Bs decreases the number of chiasma per PMC thus decreases genetic recombination in A-chromosomes.

B-chromosomes known to exhibit both qualitative as well as quantitative effects on carrier plants (Jones and Rees 1982). Quantitative effect was documented in two genera. Plantago coronopus show complete male sterility (Paliwal and Hyde 1959) due to presence of only 1B chromosome. Similarly qualitative effect was documented to show decrease in the essential oil yield in the natural population of Salvia coccinea (Mani and Thoppil 2005) and change in achene color in Haplopappus gracilis (Jackson and Newmark 1960) due to 1B chromosome. Origin and function of Bs are not known (Palestis et al. 2004), though their presence does not necessarily damage the viability of the species as the case reported presently which shows that the presence of 4 B-chromosomes does not affect the male fertility. In case of maize, a high number of Bs show significant reduction in pollen fertility and plant vigor (Randolph 1941). A decrease in pollen fertility percentage has been observed in Maize (Webert et al. 2007); Safflower (Kumar and Srivastava 2010). The presence of laggards at anaphases and telophases clearly points out the possibility of unpaired b-chromosomes lie on spindle as laggards. In paired form Bs can show regular meiosis but as univalents they suffer elimination (Jones and Houben 2003). Bs mainly inherited in non-Mendelian way, in unpaired form behave as laggards and further form micronuclei. This idea supports the occurrence of tetrads with micronuclei in present case. Further some other genetic factors may be responsible for other meiotic abnormalities.

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