Cytogenetic Variation among Populations of Aster thomsonii C. B. Clarke from District Sirmaur, Himachal Pradesh (India)

Raghbir Chand Gupta and Vijay Singh*
Department of Botany, Punjabi University, Patiala 147002, Punjab, India

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Summary  Presently, cytomorphological investigations have been carried out in five populations of Aster thomsonii from different altitudinal areas of district Sirmaur (H.P.). The investigations revealed three cytomorphotypes: diploid (2n=18), tetraploid (2n=36) and hexaploid (2n=54). The chromosome count of 2n=54 (hexaploid cytotype) has been reported for the first time at world level. All the populations have anomalous meiotic course and reduced reproductive potential of 72, 81 and 59% for diploid, tetraploid and hexaploid, respectively.

Key words  Cytomorphotype, Aster thomsonii, Sirmaur, Intraspecific polyploidy.

Genus Aster, a member of family Asteraceae, was once thought to contain 600 species in Eurasia and North America. However, due to reduction by morphologic and molecular research (Li et al. 2009), only 250 species were retained within the genus (Bibi et al. 2011). The center of the diversity of the genus is North America. In India, 23 species (Hajra et al. 1995) have been reported. The species of the genus are medicinal and have shown diuretic, antitumor, antibacterial, antiviral and antiulcer activities (Morita et al. 1996, Shao et al. 1997, Shirota et al. 1997, Wang and Yu 1998). Aster thomsonii C. B. Clarke, commonly called Thomson’s Aster, is a 30–90 cm tall herb with marginate leaves, distributed in Himalaya up to 2100–3500 m. It bears pale yellow disc florets and white/light yellow/purplish white ray florets in the months of July–August. The infusion of the aerial parts is used for anti-diarrhoeic effect because it increases the intestinal absorption of water and reduces gastrointestinal propulsion (Almeida et al. 1995). Polyploidy (especially allopoloids) can affect overall increase in enzyme activity, isozyme diversity, alteration in flavonoid profile, and may lead to enhanced production of secondary metabolites (Dhawan and Lavania 1996). Hence, in view of polyploidy and its impact on chemical constituents, this is an attempt to understand the meiotic behaviour, microhabitat distributions and reproductive potential of these sympatric cytomorpho variants to find the best chemotype.

Materials and methods

Four populations of Aster thomsonii have been collected during botanical surveys, made from high altitudinal sites (Fig. 1, Table 1) of district Sirmaur (H.P.). The appropriate sized flower buds were collected and fixed in Carnoy’s fixative (ethanol/chloroform/acetic acid 6 : 3 : 1, v/v/v) for 24 h. These buds were washed and placed in 70% ethanol for preservation at 10°C until used. Detailed meiotic course had been studied by preparing smears using standard aceto-carmine technique (Belling 1921). The difference of ploidy between the three morphotypes has been confirmed by observing around 50 PMCs of different stages of meiosis (preferably, at diakinesis, metaphase-I–II,
and anaphase-I–II). Pollen fertility was estimated by staining in 1% glycerol-acetocarmine (Marks 1954). Well-stained pollen grains were considered as fertile, and shriveled, unstained pollen grains were regarded as sterile. For stomatal studies, leaves were immersed in 10% KOH for 10–15 min and peels were examined under a microscope. Photomicrographs of PMCs, pollen grains and stomata were made by using a Nikon 80i Eclipse Digital Imaging System. Voucher specimens are deposited in Herbarium, Punjabi University, Patiala (PUN).

Table 1. Comparison of morphological characters, voucher data of diploid, tetraploid and hexaploid cytotypes of *Aster thomsonii*.

<table>
<thead>
<tr>
<th>Cytotype</th>
<th>Voucher data (PUN)*</th>
<th>Average plant height (cm)</th>
<th>Average lamina size (cm)</th>
<th>Average petiole length (cm)</th>
<th>Leaf shape</th>
<th>Flower colour</th>
<th>Stomatal size (μm)</th>
<th>Pollen fertility (%)</th>
<th>Pollen size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid (2x)</td>
<td>Shilai/58301</td>
<td>24±5</td>
<td>3.2±0.8</td>
<td>0.3</td>
<td>Lanceolate</td>
<td>White</td>
<td>28.01±0.52×23.91±1.20</td>
<td>72</td>
<td>21.44±0.89×23.52±1.23</td>
</tr>
<tr>
<td>Tetraploid (4x)</td>
<td>Nauradhar/55474</td>
<td>32±3</td>
<td>3.1±1.4</td>
<td>0.4</td>
<td>Ovate-lanceolate</td>
<td>Light yellow</td>
<td>30.12±1.56×22.68±1.32</td>
<td>81</td>
<td>26.01±0.67×21.93±1.82</td>
</tr>
<tr>
<td>Hexaploid (6x)</td>
<td>P1 Churdhar/58302</td>
<td>74±9</td>
<td>4.9±3.8</td>
<td>0.8</td>
<td>Ovate-lanceolate</td>
<td>White</td>
<td>30.34±0.58×26.53±0.34</td>
<td>59</td>
<td>32.56±0.87×17.29±1.51</td>
</tr>
<tr>
<td></td>
<td>P2 Tisri/58303</td>
<td>75±8</td>
<td>4.8±3.5</td>
<td>0.9</td>
<td>Ovate-lanceolate</td>
<td>White</td>
<td>31.32±0.58×26.53±0.39</td>
<td>59</td>
<td>32.26±0.87×17.98±1.51</td>
</tr>
<tr>
<td></td>
<td>P3 Nauradhar/52270</td>
<td>60±5</td>
<td>4.6±3.2</td>
<td>1.0</td>
<td>Ovate-lanceolate</td>
<td>Purplish white</td>
<td>26.01±0.67×27.0±0.89</td>
<td>67</td>
<td>26.09±0.7×21.01±0.3</td>
</tr>
</tbody>
</table>

* Herbarium, Punjabi University, Patiala.

Fig. 1. (a) Map of India showing location of Himachal Pradesh (highlights), (b) Map of Himachal Pradesh showing location of Sirmaur district (in circle), (c) Map of Sirmaur locating collection areas of diploid (2x), tetraploid (4x) and hexaploid (6x) cytotypes with respective altitudes (in parenthesis).

Results

The five seemingly morphologically different populations of *Aster thomsonii*, collected from different localities of district Sirmaur (H.P.), prompted us to examine the cytology in detail. The detailed meiotic analysis of the four morphovariants revealed cytological variants on the basis of ploidy level. All the accessions showed abnormal meiotic course. The first population (Table 1)
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comes out as diploid ($2n=18$, Fig. 2a) and predominantly showed chromatin stickiness (23.80%), un-oriented bivalents (9.92%) at metaphase-I (Fig. 2b). The segregation of chromosomes at anaphase-I (Fig. 2c) was irregular; cytomixis (7.29%) and chromatin bridge formation (Fig. 2c, Table 2) were also observed in a few PMCs (Table 2), resulting in reduced pollen fertility (72%). The second one was tetraploid ($2n=36$, Fig. 2f) and shows chromatin transfer (11.53%) at prophase-I and metaphase-I (Fig. 2g), and heterogeneous sized pollen grains were observed (Fig. 2h). Besides this, tetraploids show normal pollen fertility (81%). Last two accessions (Table 1) come out as hexaploid ($2n=54$, Fig. 2i), and are highly abnormal (Table 2). Both the populations

Fig. 2. (a–s) Detailed meiotic course in Aster thomsonii. (a–c,q) Diploid cytotype ($2n=18$); (a) PMC at M-I, (b,c) chromatin stickiness at M-I and A-I, (d) chromatin bridge at A-I, (e) fertile and sterile pollen grains, (q) stomata. (f–h,r) Tetraploid cytotype ($2n=36$); (f) 18 bivalents at M-I, (g) PMCs involved in chromatin transfer, (h) heterogeneous sized pollen grains, (r) stomata. (i–p,s) Hexaploid cytotype ($2n=54$); (i) 27 bivalents at M-I, (j) PMCs involved in cytomixis, (k) lagard at A-I, (l) chromatin bridges at A-I, (m) diads with two micronuclei, (n) triad with micronucleus, (o) triad, (p) heterogeneous sized sterile and fertile pollen grains, (s) stomata. PMC=Pollen mother cells; M-I=metaphase-I; A-I=anaphase-I (scale 10μm).
showed cytomixis (Fig. 2j), stickiness with unoriented bivalents at metaphase-I, laggards (Fig. 2k) and chromatin bridge (Fig. 2l) during segregation (Table 2). Subsequent microsporogenesis is abnormal (Table 3), and shows diads (Fig. 2m), triads (Figs. 2n, o) with or without micronuclei and low pollen fertility (59%) with heterogeneous sized sterile or fertile pollen grains (Fig. 2p).

The difference in morphology of diploid and tetraploid accessions (Table 1) are infinitesimal as average plant height show little variation, but tetraploids have slightly wider lamina and flower colour varies from white to light yellow, respectively. The hexaploid accessions exceed diploid and teraploid in lamina size, petiole length, and height of plant, and flower colour varies to purplish blue (Table 1). The stomata in all the accessions are diacytic type (Figs. 2m–o). The information regarding morphological characters along with stomatal and pollen features of three cytomorphotypes showing significant differences are shown in Table 1.

**Discussion**

The species is known to exist as diploid, 2n=18 (Mehra et al. 1965, Jee et al. 1987), and as tetraploid, 2n=36 (Mehra and Remanandan 1974), from India. B-chromosomes (2n=18+0–4B) have also been reported in the species by Gupta et al. (1989) from the Western Himalayas. The hexaploid (2n=54) cytotype is the first ever report for the species. The perusal of cumulative cytological literature (cf. Fedorov 1969, Kumar and Subramaniam 1986, Khatoon and Ali 1993, etc.) and present investigations reveal 221 species from the world and only seven species from India are cytologically known. The chromosome numbers vary considerably from 2n=8 to 2n=122 with 2n=18 (48.81%) being the most common, followed by 2n=32 (14.47%), 2n=16 (12.66%), 2n=20 (6.78%) and 2n=26 (5.34%). Rest of the chromosomal reports is with reduction in number. The genus is polybasic (x=4, 5, 7, 8, 9, 13), and among these x=9 is most common. Chromatin transfer was first recorded by Kornicke (1901) in *Crocus sativus*. This phenomenon has been reported in a wide range of naturally occurring flowering plants and artificially synthesized interspecific/generic hybrids (Li et al. 2009). As per the review of literature, cytomixis is caused by temperature (Narain 1976), stress factors coupled with genetic control (Baptista-Giacomelli et al. 2000, Malallah and Attia 2003) and direct genetic control (Haroun et al. 2004). Cytomixis plays a

### Table 2. Data on cytomixis and meiotic course in the studied populations of *Aster thomsonii* from Sirmaur district.

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Cytomixis</th>
<th>Meiotic course</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMCs involved</td>
<td>No. of PMCs involved</td>
</tr>
<tr>
<td>58301</td>
<td>3.64% (5/137)</td>
<td>2–3</td>
</tr>
<tr>
<td>55474</td>
<td>11.53% (15/130)</td>
<td>2–4</td>
</tr>
<tr>
<td>58302</td>
<td>19.56% (27/138)</td>
<td>2–6</td>
</tr>
<tr>
<td>58303</td>
<td>20.15% (26/129)</td>
<td>2–6</td>
</tr>
<tr>
<td>52270</td>
<td>16.27% (21/129)</td>
<td>2–6</td>
</tr>
</tbody>
</table>

### Table 3. Data on abnormal microsporogenesis in hexaploid accessions of *Aster thomsonii*.

<table>
<thead>
<tr>
<th>Total no. of PMCs observed</th>
<th>Monads</th>
<th>Dyads</th>
<th>Triads</th>
<th>Tetrads</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WMN</td>
<td>WM</td>
<td>WMN</td>
<td>WM</td>
</tr>
<tr>
<td></td>
<td>WMN</td>
<td>WM</td>
<td>WMN</td>
<td>WM</td>
</tr>
<tr>
<td></td>
<td>WMN</td>
<td>WN</td>
<td>WMN</td>
<td>WN</td>
</tr>
<tr>
<td>210</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>0</td>
<td>0</td>
<td>5.71%</td>
<td>3.34%</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>15</td>
<td>8.09%</td>
<td>7.14%</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>138</td>
<td>10</td>
<td>65.71%</td>
</tr>
</tbody>
</table>
vital role in chromosomal diversity, as it forms hypo-/hyperploid cells and gametes afterward (Levan 1941, Zheng et al. 1987, Kim et al. 2009), and seemingly these results are observed in the presently investigated populations. Another unusual behavior is chromosome stickiness, which has been prominently observed in diploid cytotype (45%). Chromosome stickiness has been introduced for the first time in maize by Beadle (1932), who regards it as a mutation caused by sticky (st), a recessive gene. Afterward, this concept was promoted by Utsunomiya et al. (2005), Risso-Pascotto et al. (2006, 2009) and Pagliarini et al. (2008). According to Ranjbar et al. (2011), sticking chromatin may hold during segregation and results in chromatin bridges. Such chromatin bridges have been prominently observed in tetraploid and hexaploid cytotypes (Table 1), and may be a result of paracentric inversions, or due to malfunctioning of non-histonic chromosome proteins (Gaulden 1987). Unoriented bivalents have also been reported at metaphase-I, along with the chromatin stickiness which may be related to impaired attachment of kinetochore to the spindle fibre or due to late chiasma terminalization and may thus result in laggards (Nicklas and Ward 1994, Pagliarini 2000). The laggards fail to reach the poles, generally form micronuclei (Utsunomiya et al. 2002), or form micro-pollen/uneven gametes. According to Dowd et al. (1986), irregularity in microsporogenesis (formation of micronuclei at telophase) could be due to patchy breakage of chromatin bridge at anaphase-I. Heterogeneous sized pollen grains and reduced pollen viability in tetra- and hexaploid cytotypes (Figs. 2f, l) may be due to the formation of aneuploid gametes (lacking one or more chromosome or chromosome segments) by cytomixis (Falistocco et al. 1995, Ghaffari 2006, Singhal et al. 2011, Jeelani et al. 2012), or may be attributed to some other genetic reasons as reported in Avena sativa and Cereale secale (Baptista-Giacomelli et al. 2000).

During normal meiosis, the formation of stable bivalents at metaphase-I is required for correct segregation of chromosomes. But in polyploids, a high frequency of quadrivalents or multivalent at diakinesis/metaphase-I hinders normal meiosis. Nowadays, such phenomenon may be absent in polyploids due to their cytological diplodization (Cifuentes et al. 2010, Ramsey and Schemske 2002), a gradual process of bivalent formation to overcome irregularity in meiotic course altered by multivalents. It seems to be applicable in hexaploid accessions.

All the cytotypes differ significantly in their morphology, and hence can be regarded as cytomorphotypes. Previous studies suggest the gigas effect of polyploids (Stebbins 1971, Levin 2002), as this is revealed in the present investigation (Table 1). The difference in the qualitative traits like average plant height, lamina size and petiole length are in accordance with Srivastava and Srivastava (2002) and Malik et al. (2012).

Polyploids usually have different geographical ranges than their diploid progenitors (Lewis 1980), as they increase with increasing altitude (Brochmann et al. 2004) and are prominently distributed in meadows and wet soils (Grant 1981), and the increase in chromosome seems to be a suitable reason for hexaploid accessions which were collected from damp slopes.

Ecologically, the polyploids have greater amplitude, establish better adaptation than their ancestors and sustain the rigours of changing environment over an evolutionary time scale, as they have the collective feature of the parent genome (Brochmann et al. 2004).

Conclusion

The intraspecific variability in morphological character (Table 1) and meiotic course based on chromosome behavior (Table 2) explain the cytogenetic diversity in the species. Such studies can be helpful in germplasm evaluation, chromosome database cataloguing, and understanding the role of intraspecific variability in evolution in medicinally important plant species from this area.
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References


