Structural Heterozygosity for Reciprocal Translocation in
*Tanacetum artemisioides* Sch. Bip. ex Hook. f.
from Ladakh Division of Jammu and Kashmir

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**Summary** Male meiotic studies have been carried out on the wild plants of *Tanacetum artemisioides* Sch. Bip. ex Hook. f. collected from the cold desert regions of Ladakh Division of Jammu and Kashmir. The studied individuals, which existed at the 2x level (2n=18), showed structural heterozygosity for reciprocal translocations as indicated by the presence of multiple chromosomal associations of four to six chromosomes. This is the first report of structural heterozygosity for the species. The structural heterozygotes showed considerable amount of pollen sterility (30–35%) which could be attributed to the presence of ring/chain shaped multivalent in PMCs.

**Key words** Structural heterozygosity, Reciprocal translocation, *Tanacetum artemisioides*, Pollen sterility.

*Tanacetum* L. (Tribe: Anthemideae; Family: Asteraeae) with 160 species is native to temperate zones of Europe, Asia, North Africa, and North America. In India, the genus is represented by 12 species, and nine are reported from Jammu and Kashmir. *T. artemisioides* Sch. Bip. ex Hook. f. also treated as *Chrysanthemum karakoremense* Kitam., grows as a tall perennial herb in the dry arid regions of Northwest Himalayas between 2400–2700 m above sea level. The species is characterized by having a sparse leafy stem and conical receptacle. It is used locally as an analgesic due to its anti-inflammatory effects (Bukhari et al. 2007). The whole plant is used to control blood pressure, diabetes, abdominal disorders, headache, and fever (Khan and Khatoon 2008). Dried plants are used for chest problems and are useful for hepatitis (Hussain et al. 2013). Perusal of existing cytological literature reveals that the species has been described previously from the Kashmir Valley by Jee et al. (1987, 1989) and exists at the diploid level (2n=18). The same diploid chromosome number has been reported from the Republic Armenia by Khandjian (1975).

As a part of a project to explore the cytogenetical diversity in the plants of Indian cold deserts, the present studies have been carried out in *T. artemisioides* on an individual plant basis from the Ladakh Division of Jammu and Kashmir. These individuals exist at the diploid level but showed evidence of reciprocal translocations. The present communication covers detailed meiotic course, microsporogenesis, and pollen fertility in these individuals.

**Materials and methods**

Materials for male meiotic studies were collected from the Kargil District of Ladakh Division (Poyen Village, 2790 m) during July 2014. The young capitula of variable sizes were fixed in freshly prepared Carnoy’s fixative (6 ethanol : 3 chloroform : 1 acetic acid v/v/v) for 24h and stored in 70% ethanol in a refrigerator at 4°C. Developing anthers from florets were squashed in 1% acetocarmine and a number of slides of pollen mother cells (PMCs) were prepared and carefully examined for chromosome counts during late Prophase-I, Metaphase-I (M-I), and Anaphases (A-I/A-II). A total of 50 PMCs were examined for determining the chromosome counts while 10–30 slides were prepared from different florets/capitula for analysis of chromosomal associations. Sporad analysis was made by examining 400–500 PMCs by squashing the anthers in 1% acetocarmine. Pollen fertility was estimated through stainability tests for which mature florets were squashed in glyceracetocarmine mixture (1 : 1) and aniline blue dye (1%). The pollen grains with fully stained cytoplasm and nuclei were counted as viable or fertile while those with partially stained or unstained cytoplasm as non-viable or sterile. The specimens were identified by consulting the Flora of India (Hajra et al. 1995) and Flora of Ladakh (Kachroo et al. 1977). The plants were also compared with the specimens at the Herbarium, Northern Circle, Botanical Survey of India, Dehra Dun. The identified specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN 59771, 59772).

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Results

On the basis of detailed male meiotic studies, it has been found that the studied accessions depicted a diploid chromosome count of \( n=9 \) (based on \( x=9 \)) as confirmed from the presence of nine large-sized bivalents during diakinesis and M-I (Fig. 1a, b). Additionally, a considerable number of PMCs also depicted the presence of multiple chromosomal associations (hexavalents and quadrivalents). Analysis of various chromosomal associations on the basis of well-spread chromosomes in 75 PMCs (Table 1) revealed that 35 PMCs (46.67%) showed

![Image of meiotic stages and chromosomal associations](image-url)

**Fig. 1.** (a–i). Meiosis in *Tanacetum artemisioides*. a) A PMC showing nine bivalents at diakinesis. b) A PMC showing nine bivalents at M-I. c) A PMC showing \( 7_{II}+1_{IV} \) (ring type) at diakinesis (arrow). d) A PMC showing \( 7_{II}+1_{IV} \) (chain type) at diakinesis (arrow). e) A PMC showing \( 7_{II}+1_{IV} \) (zigzag type) at diakinesis (arrow). f) A PMC showing \( 6_{II}+2_{II} \) (chain type) at diakinesis (arrow). g) A PMC showing \( 8_{II}+2_{II} \) at diakinesis (arrow). h) Normal tetrads. i) Stained/fertile and unstained/sterile pollen grains. Scale bars=10 \( \mu \)m.

**Table 1.** Analysis of chromosomal associations at diakinesis/M-1 in *Tanacetum artemisioides*.

<table>
<thead>
<tr>
<th>PMCs observed</th>
<th>Hexavalent chain</th>
<th>Quadrivalents</th>
<th>Bivalents</th>
<th>Univalents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chain</td>
<td>Ring</td>
<td>Zigzag</td>
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<tr>
<td>35</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>1</td>
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<td>10</td>
<td>–</td>
<td>1</td>
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<td>7</td>
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<td>24</td>
<td>–</td>
<td>1</td>
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<td>7</td>
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<td>7</td>
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<td>–</td>
<td>–</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>1</td>
<td>11</td>
<td>25</td>
</tr>
</tbody>
</table>

Average/PMC

| % of chromosomes involved | 0.33 | 3.33 | 7.55 | 0.45 | 88.22 | 0.12 |
nine regular bivalents while the remaining 40 PMCs (53.33%) depicted the presence of quadrivalents (ring, chain or zigzag type, Fig. 1c–e) or hexavalents (chain type, Fig. 1f). In a few PMCs, two univalents (Fig. 1g) were detected, which could be due to precocious disjunction of a bivalent or is the consequence of asynapsis. Analysis of data revealed that the average chromosomal associations per PMC in the accession works out to 0.01IV+0.5IV+7.95II+0.01I. The most common type of chromosomal configuration consisting of 1IV (ring type)+7II, which was observed in 24 PMCs (32%), followed by 1IV (chain type)+7II (13.33%). Further analysis revealed that 11.66% of the chromosomes were involved in reciprocal translocations while the rest constitute a perfect nine bivalent formation. Additionally, the course of meiosis is observed to be regular as is apparent from the normal segregation and distribution of chromosomes during AI/AII. In spite of regular sporad formation (Fig. 1h) the studied individuals showed reduced pollen fertility (65–71%, Fig. 1i).

Discussion

Ever since the first report of structural heterozygosity for reciprocal translocations by Belling (1914) in ‘Florida velvet bean’ (Sitzolobium deerriingianum), heterozygosity has been reported to occur in a wide variety of flowering plants (Burnham 1956, Kaul 1977, Gohil and Koul 1978, Sharma and Gohil 2003, 2008, 2011, Talukdar and Biswas 2006, Kim et al. 2008, Ghaffari et al. 2009, Talukdar 2010, 2013, Kohli and Gohil 2011). From this laboratory, the naturally occurring reciprocal translocations have been reported in individuals of Artemisia parviflora (Gupta et al. 2010), Astragalus chlorostachys (Rana et al. 2012), Saxifraga diversifolia (Kumar and Singhal 2013), Achillea millefolium (Singhal et al. 2014), and Anemone rivularis (Kumar et al. 2015).

Kaul (1977) opined that breakage and exchange of heterologous chromosomes in a structural heterozygote are genetically conditioned and controlled, thereby eliminating the chance factor operating for the predominance of either ring or chain or both types of multiple associations of chromosomes. If the interchange segments of heterologous chromosomes are long, it is possible that the configurations at diakinesis and M-I will be rings. If both pieces of chromosomes are short or chiasmata are not formed in all the arms, chains instead of rings are formed (Burnham 1956). Ghosh and Datta (2006) reported that predominant occurrence of adjacent orientation in quadrivalents in heterozygotes is expected to induce high frequency of non-viable male gametes following the duplication and deficiencies of genes. In the presently studied accession of Tanacetum artemisioides, the majority of the PMCs showed the adjacent type of orientation of quadrivalents/hexavalents (ring or chain) and the alternate (zigzag) orientation was seen in only one PMC, resulting in a considerable amount of pollen sterility (30–35%), which appears to be the result of duplications and deficiencies of genes as mentioned by Ghosh and Datta (2006) in Nigella damascene. Similar effects of structural heterozygosity leading to non-viable pollen grains have been reported in a number of plants, namely, Citrus jambhiri (Singhal and Gill 1981), Chrysanthemum zawadskii (Kim et al. 2008), Artemisia parviflora (Gupta et al. 2010), Astragalus chlorostachys (Rana et al. 2012), Saxifraga diversifolia (Kumar and Singhal 2013), Tradescantia spathacea (Koul et al. 2013), Achillea millefolium (Singhal et al. 2014), and Anemone rivularis (Kumar et al. 2015). Gohil and Koul (1978) and Sharma and Gohil (2003) have reported that structural heterozygotes of Allium consanguineum and A. roylei depict complete gametic sterility due to reciprocal translocations.

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