Cytogenetic Analysis of Induced Translocation Heterozygote in *Trachyspermum ammi* (L.) Sprague

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**Summary** Numerous naturally occurring mutations are heterozygous, and thus, a contributing source of variation. Their crucial fate rests on the gametic fertility and viability. Translocations have been reported in many plant species; however, no previous report is available in this regard in *Trachyspermum ammi* (L.) Sprague (ajwain). During meiotic analysis, a heterozygote for reciprocal translocation has been isolated from the population through EMS treated set in ajwain var. AA-1. The results of the cytological studies showed the predominance of ring quadrivalents along with seven bivalents. Some extent of univalents and bivalents with multiple chromosomal associations along with rings and chains were also observed at metaphase I. Heterozygous plant showed reduced pollen fertility up to 36.23% due to the predominance of adjacent orientation over alternate. At anaphase I, some pollen mother cells (PMCs) showed normal 9:9 separation but simultaneously, abnormal 8:10 chromosomal separation was also observed. This study deals with the cytological behavior of heterozygote and its occurrence and consequences in the present crop.

**Key words** *Trachyspermum ammi* (L.) Sprague (ajwain), Heterozygote, Ring, Chain, Quadrivalent, Pollen fertility.

Chromosomal interchanges commonly referred to as 'translocations' are important in constructing chromosome maps and offer the possibilities for breaking gene blocks in the chromosomal regions where chances of recombination are rare (Minocha *et al.* 1982). In a translocation heterozygote, the two haploid sets of chromosomes are heterologous (do not carry the same arrangement of genetic information); furthermore, it will show linkage among those genes that would normally segregate independently (Sturtevant and Beadle 1940). Therefore, heterozygosity could affect the genetic recombination by generating new linkages.

Translocation heterozygosity, with the potential for creating and conserving specific gene combinations, is generally identified by the reduced reproductive capacity and the presence of multivalents during reduction division (Sharma and Gohil 2011). The fertility would be reduced by the formation of sterile pollen in case of male plants and seed abortion in case of female. The fertility of an interchanged heterozygote depends on the frequency of segregation patterns in meiosis I (adjacent and alternate). In absence of crossing-over in the interstitial segment, alternate orientation results in balanced chromosome combinations, adjacent orientation results in unbalanced combinations (Sybenga 1967). Although the occurrence and consequences of chromosomal heterozygosity have been studied in almost all animals, plants have much to suggest in this regard. Various reports are available related with the heterozygosity for reciprocal translocations in several plants from time to time such as *Tradescantia* (Sax and Anderson 1932), maize (Burnham 1949), rice (Hsieh 1961, Siddiq 1973), *Secale* (Sybenga 1967), pearl millet (Koduru 1984, Kumar and Singh 2003),...
Egyptian henbane (Tyagi and Bahl 1992), soybean (Mahama et al. 1999), and grass pea (Tripathi and Kumar 2009, Talukdar 2010).

Translocation affects the fertility of the plants but there are some positive attributes related with it. A viable heterozygote has significant traits as to provide new linkage groups within the
chromosome and to introduce it without changing the genetic complement of the individual plant. This has immense value in the breeding practices of the crop. Chromosome engineering through translocation has proved to be the most efficient tool by various researchers to introduce desirable traits in wheat (Sears 1956, Driscoll 1965) and barley (Hagberg 1962, Gustafsson 1965) and also to generate aneuploids like different trisomics in numerous crop plants (Ramage 1955, Huang 1985, Ashraf and Bassett 1987).

*Trachyspermum ammi* is a plant of immense pharmaceutical importance, highly valued for its phytochemical constituents, such as thymol (a major constituent), *para*-cymene, *γ*-terpenine, *α*- and *β*-pinenes, *α*-terpinene, dipentene and carvacrol (as non-thymol fraction) (Dwivedi et al. 2012). The fruits have been used as remedy for indigestion and colic and also used in poultices to relieve asthma and arthritis (Ramaswamy et al. 2010). During the course of cytological study of EMS- (ethyl methane sulphonate) treated *T. ammi*, a translocation heterozygote was screened out. The present research makes an effort to understand the cytological behavior and peculiarities of translocation and its consequences in the fertility of the present crop.

**Materials and methods**

*Procurement of seeds*

Healthy and fresh seeds of *T. ammi* var. AA-1 were collected from National Research Centre for Seed Spices, Ajmer, Rajasthan, India.

*Seed treatment*

Seeds were treated with EMS solution prepared in potassium phosphate buffer with pH=7.0 at three different concentrations, viz. 0.1%, 0.3% and 0.5% for 5 h, with constant shaking. Thereafter thorough washing in tap water was done. After that, the seeds were then sown along with their respective controls.

*Meiotic analysis*

For meiotic studies, young floral buds were fixed in freshly prepared acetic acid–ethanol (1:3) solution for 24 h and stored in 90% ethanol in a refrigerator until use. Slides were prepared using anther squash technique with 2% acetocarmine. Slides were analyzed and suitable cells were photographed under a Nikon research photomicroscope. Observations were recorded on chromosome configurations at metaphase I with interchanged complex as a ring or chain along with multiple chromosomal associations. Pollen fertility was also estimated using 2% acetocarmine. Fertile pollen grains were recorded with stained nuclei whereas undersized and unstained pollen grains without nuclei were considered sterile. Computer software SPSS16.0 for Windows was used for statistical analysis.

**Results**

The meiotic configuration of the control plant was found to be standard showing nine bivalents at metaphase I (n=9) and 9:9 separation at anaphase I. However, among the plants of 0.1% EMS-treated population, one translocation heterozygote was isolated, with reduced number of seed formation as compared to control plants. Cytogenetic analysis of PMCs of translocation heterozygote at diakinesis and metaphase I showed occurrence of an array of aberrant chromosomal behavior with multiple associations by forming rings and chains. Table 1 gives an account of major chromosomal associations at metaphase I in the translocation heterozygote.

Some of the PMCs exhibited variation in the structure of rings and chains. Open rings and bi-rings (8-shaped) were screened out as rings whereas in the chains, the structure of chromosomal
arms was attached either side by side or in a zig-zag pattern. Sybenga (1967) classified diagrammatically, these four types of interchange patterns into two types of orientations. According to him, open ring and adjacently attached chromosomal arms were related with the adjacent orientation, while zigzag pattern and bi-rings (8-shaped) were a consequence of alternate orientation. A clear preponderance of rings (58.53%) over the chains was observed, in which open rings and bi-rings were approximately 33.82% and 24.71%, respectively (Table 2). Overall chain configuration was found to be 41.46%; though the incidence of adjacently oriented chains showed prevalence (28.69%) over the alternate chains (12.77%) (Table 2). Thus, the total occurrence of adjacent and alternate orientation was 62.51% and 37.48%, respectively.

These rings and chains have been found in the form of various multivalents such as trivalents, quadrivalents, pentavalents, hexavalents, heptavalents and octavalents which were involved in the formation of multiple chromosomal associations. Besides these multivalents, variable number of univalents and bivalents were also scored.

Although there were equally separated chromosomes observed at anaphase I, but a number of unequal separations (8 : 10) were also observed (Table 3). Chromosomal abnormalities at anaphase such as laggards and bridges were also reported. Pollen fertility was reduced to just 36.23% compared with 97.88% in the control set (Table 2).

**Table 1.** Metaphase I configuration of translocation heterozygote.

<table>
<thead>
<tr>
<th>Associations</th>
<th>No. of PMCs</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9II</td>
<td>16</td>
<td>11.18</td>
</tr>
<tr>
<td>7II+1IV</td>
<td>21</td>
<td>14.68</td>
</tr>
<tr>
<td>6II+1IV+2I</td>
<td>11</td>
<td>7.69</td>
</tr>
<tr>
<td>6II+1III+3I</td>
<td>6</td>
<td>4.19</td>
</tr>
<tr>
<td>5II+1III+1IV+1I</td>
<td>13</td>
<td>9.09</td>
</tr>
<tr>
<td>5II+2IV</td>
<td>7</td>
<td>4.89</td>
</tr>
<tr>
<td>4II+2IV+2I</td>
<td>15</td>
<td>10.48</td>
</tr>
<tr>
<td>4II+1IV+1VI</td>
<td>12</td>
<td>8.39</td>
</tr>
<tr>
<td>3II+1IV</td>
<td>10</td>
<td>6.99</td>
</tr>
<tr>
<td>3II+2VI</td>
<td>14</td>
<td>9.79</td>
</tr>
<tr>
<td>2II+2IV+1VI</td>
<td>8</td>
<td>5.59</td>
</tr>
<tr>
<td>2II+3IV+2I</td>
<td>10</td>
<td>6.99</td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Chromosomal orientations and pollen fertility of translocation heterozygote.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Rings (%)</th>
<th>Chains (%)</th>
<th>Adjacent orientation (%)</th>
<th>Alternate orientation (%)</th>
<th>Pollen fertility (mean±S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open</td>
<td>Bi-ring</td>
<td>Adjacent</td>
<td>Zig-zag</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>97.88±0.18</td>
</tr>
<tr>
<td>0.1% EMS</td>
<td>33.82</td>
<td>24.71</td>
<td>28.69</td>
<td>12.77</td>
<td>36.23±0.19</td>
</tr>
<tr>
<td>Total</td>
<td>58.53</td>
<td>41.46</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The formation of rings and chains of interchange heterozygote is correlated with pollen sterility due to the non-disjunction of chromosomal segments. In general, chromosomal rearrangements can be found in the whole fertility range from 0% to 100% (Searle et al. 1974). Fertility is positively correlated with the percentage of alternate orientations of translocation multivalent (van
Heemert and Wijnands-stäb (1975). An equal incidence of alternate and adjacent orientations contributes to 50% fertility but an increase in adjacent orientation would result in fertility less than 50%. Sax and Anderson (1932) stated that if adjacent chromosomes pass to the same pole, the resulting microspores should be sterile owing to deficiency of chromosome segment; but if adjacent chromosomes pass to the opposite poles, each microspore should have a complete complement and would be capable of further development. The spores of the heterozygote with these duplications and deficiencies do not give rise to viable gametes. Holsinger and Ellstrand (1984) stated that median position of the centromere may add to the flexibility of the translocation ring and aid the orientation of chromosomes in an alternate fashion at metaphase I of meiosis, which is necessary to avoid unbalanced gametes as a result of duplications and deficiencies. Thus, according to them, the position of centromere and size of chromosomes (small chromosomes that are approximately equal in size) are crucial traits for the translocation system. However, in 1962 Kurabayashi et al. conflicted this statement and suggested that “Apparently these traits ... are merely permissive to the development of such systems,” they are not necessarily absolute, or even fundamental, requirements for the evolution of translocation system (Holsinger and Ellstrand 1984).

The involvement of EMS in the formation of rings and chains is a point of interest, owing to limited knowledge available so far. An often cited hypothesis is that DNA bases ethylated by EMS (mostly the N-7 position of guanine) gradually hydrolyze from the deoxyribose on the DNA backbone leaving behind an apurinic (or possibly an apyrimidinic) site that is unstable and can lead to single-strand breakage of the DNA (Sega 1984), which might be the cause of breakage of chromosomal segments. The higher concentration of EMS should create more breakage and rejoining of chromosomal segments in comparison to lower concentrations. However, the results of the present study showed that only 0.1% concentration of EMS (rather than 0.3% and 0.5%) induces this type of breakage and reunion of segments of chromosomes. A logical interpretation of this phenomenon may be that, although higher doses of EMS should directly relate with the segmental breakage only but lower doses of EMS might be involved in the segmental interchange. Such breakpoints of non-homologous chromosomes form rings and chains. A gene or a set of genes responsible for pairing might have been affected due to translocation and have given rise to univalents (Sinha and Acharia 1974).

During metaphase I, non co-orientation of the chromosomes of a translocation heterozygote can give rise to duplicate-deficient gametes following 2:2 segregation or aneuploid gametes following a 3:1 segregation (Hagberg 1954). Laggards and bridges at anaphase I may also be the after effects of translocation as the chromosomes could not separate easily in the mean time of early anaphase. Adjacent orientations seem to be more responsible for the formation of laggards where there is always a possibility of unequal and delayed separation (Kumar and Singh 2003).

Heterozygote has immense value in inbreeding practices as it has the potential for preserving genetic heterozygosity under conditions of inbreeding. We have reported heterozygosity in T. ammi for the first time; however, it will be interesting to observe whether heterozygosity will pass to the subsequent generations or not. Further studies are required to support these results and to explore our understanding related with the involvement of chromosomes in translocations for their

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**Table 3. Types of separation at anaphase I of the translocation heterozygote.**

<table>
<thead>
<tr>
<th>Separations</th>
<th>No. of PMCs</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 : 9</td>
<td>39</td>
<td>30.23</td>
</tr>
<tr>
<td>8 : 10</td>
<td>48</td>
<td>37.21</td>
</tr>
<tr>
<td>Laggards</td>
<td>25</td>
<td>19.38</td>
</tr>
<tr>
<td>Bridges</td>
<td>17</td>
<td>13.17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>129</strong></td>
<td></td>
</tr>
</tbody>
</table>
resourceful exploitation in linkage studies in the present crop.

Acknowledgements

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References


