Cytomixis in pollen mother cells of an exotic variety of
Trigonella foenum-graecum L.

N LAKSHMI and P VEERA RAGHAVAIAH
Department of Botany, Cytogenetics Laboratory, Nagarjuna University,
Nagarjunanagar 522 510, India

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Abstract. The phenomenon of cytomixis as well as loss of chromosomes in the
meiotic cells of Trigonella foenum-graecum L. have been described. Because of cyto-
mixis, at diakinesis and metaphase I, 18% of pollen mother cells (PMCs) showed
chromosome numbers ranging from 3-28. Chromatin migration occurred after
pairing through cytoplasmic bands connecting adjacent PMCs. In some PMCs
the chromatin is transformed into thin whip-like structures and then migrated, while
in others it breaks up into variously-sized bits before migration. It is suggested
that this is a natural phenomenon and is under genetic control. The plant is partially
fertile, hence the phenomenon may be of considerable evolutionary significance.

Keywords. Cytomixis; chromatin migration; Trigonella; cytoplasmic bands.

1. Introduction

Cytomixis, the transfusion of nuclear substance into the cytoplasm of adjacent
cells has been described in a large number of plants belonging to diverse families
of angiosperms which include normal species, hybrids, apospots etc. and occurs
in pollen mother cells (PMCs), meristematic and somatic tissues (Digby 1909;
Gates 1911; Korschke 1901; Percival 1930; Katterman 1933; Jacob 1941;
Iyyengar 1943; Sarvek 1958; Bell 1964; Omara 1976; Habib and Chennaveeriah

The present paper records cytomixis for the first time in the genus Trigonella
and an attempt has been made to explain the meiotic behaviour, cytogenetic impli-
cations and evolutionary significance of the phenomenon.

2. Materials and methods

In an attempt to conduct cytogeographical studies on the genus Trigonella, several
germsplasms of Trigonella foenum-graecum L. were obtained from different agro-
climatic regions. In a Finland variety thus raised, one plant was found to be
cytomictic. Suitable flower buds from the aforesaid mutant plant were fixed in freshly prepared acetic-alcohol (1:3) between 9-30 a.m. and 1 p.m. local time, and were changed to 70% alcohol after 24 hrs of fixation. Acetocarmine squash preparations were employed throughout the study. For assessing pollen fertility 4% iodine-potassium iodide solution was used. Observations and photomicrographs were made when the preparations were afresh.

3. Observations

3.1. Morphology

Remarkable phenotypic alteration has been observed in the cytomictic *Trigonella*. It differs from the normal plants in being dwarf, bushy and less fertile with smaller pods and seeds (table 1). Some of the pods were observed to be half-filled leading to reduction in the number of seeds per plant.

3.2. Cytology

Cytological examination revealed certain deviations from the normal meiosis. Evidence of chromatin extrusion and cytomixis was clearly seen in 37.61% of PMCs. The earliest signs of chromatin extrusion were seen at pachytene. Here, the chromatin material could be seen as protruding out of the main chromatin mass at one or more places (figure 6). Protrusions ranging in number from one to eight or more could be easily seen in various PMCs. At various stages of prophase I the movement of chromatin material from the nucleus of one PMC into cytoplasm of an adjacent PMC was noted (figures 1, 2 and 4). In some cases rows of cells were seen connected by cytoplasmic bands like a string of beads (figure 1). The nuclear material may presumably migrate from one cell to the other through these cytoplasmic bands (figures 1, 2 and 4). During migration the chromatin was in the form of thin whip-like structures or pseudopodia-like projections (figure 1). In quite a few PMCs the nuclear mass was found to break up into variously-sized bits of chromatin which migrated into the neighbouring cells (figure 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control plant</th>
<th>Cytomictic plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of the plant (cm)</td>
<td>14.6</td>
<td>9.2</td>
</tr>
<tr>
<td>No. of pods per plant</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>No. of seeds per plant</td>
<td>106</td>
<td>27</td>
</tr>
<tr>
<td>No. of seeds per pod</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Length of pod (cm)</td>
<td>11.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Breadth of pod (cm)</td>
<td>0.34</td>
<td>0.2</td>
</tr>
<tr>
<td>Pollen fertility (%)</td>
<td>92</td>
<td>71</td>
</tr>
</tbody>
</table>

All the measures are averages of 7 measures except plant height of cytomictic mutant.
Figures 1–6. Different meiotic stages of the cytomic mutant in *Trigonella*. 1. Whip-like streaks of chromatin migrating from one meiocyte to another, × 1875. 2. Migration of chromatin between adjacent meiocytes after dividing into bits, × 1950. 3. Hypoploid meiocyte, showing only three chromosomes, × 4500. 4. Formation of cytoplasmic bridges between adjacent PMCs, × 2387. 5. Anaphase I cell with 17 chromosomes showing unequal segregation of 10:7, × 3750. 6. Chromatin extrusion at pachytene, × 3254.
At diakinesis, metaphase I and anaphase I, in addition to the normal cells which were present in maximum frequency (57.98%), 42.02% PMCs with varying chromosome numbers ranging from 3-28 were also observed (figures 3 and 5, table 2). About 8% of cells showed more than 16 chromosomes and 28% of cells displayed lower chromosome number. The PMCs with higher chromosome number were found to be large in size which is attributable to the migration of both cytoplasmic and chromatin material. In some preparations a few cells were found to be enucleate. Multivalent formation was absent, thereby indicating that cytomixis is operating only after bivalent formation.

At anaphase I, 24.02% of cells showed variable segregations with one to six laggards (table 2). Anaphase abnormalities include laggards, division of univalents and non-disjunction of chromosomes (table 2). Likewise, a few lagging chromosomes, scattering of chromosomes, variable number of chromosomal patches were also observed at anaphase II. At sporad stage, in addition to 5-6 micronuclei, dyads and triads were noted in 30% of cells.

Notwithstanding the meiotic abnormalities 71% of the pollen grains were stainable. The plant is semisterile and a few healthy seeds were secured for progeny studies.

4. Discussion

Cytological abnormalities observed in the present study such as chromatin extrusion, chromatin agglutination, chromatin transfer, formation of cytoplasmic bridges, deviation of chromosome numbers from the normal one, presence of laggards, irregular segregation of chromosomes, formation of micronuclei and

<table>
<thead>
<tr>
<th>No. of cells</th>
<th>No. of chromosomes</th>
<th>Segregational pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>18</td>
<td>8 + 7 + 3L</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>4 + 5 + 2L</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>5 + 5 + 3L</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3 + 0</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>5 + 6 + 2L</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>6 + 5 + 1L</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>16 + 4 + 4L</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3 + 4</td>
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<tr>
<td>1</td>
<td>28</td>
<td>17 + 8 + 3L</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>7 + 10</td>
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<tr>
<td>3</td>
<td>8</td>
<td>2 + 6</td>
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<td>3 + 1</td>
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<tr>
<td>2</td>
<td>6</td>
<td>2 + 2 + 2L</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>4 + 1</td>
</tr>
</tbody>
</table>
presence of polyploidy were perhaps as a consequence of cytomixis and some may be due to the operation of other phenomena.

The movement of chromatin material in *Trigonella* was not uniform and the contents of one cell could be seen in two or three neighbouring cells. A similar trend has been recorded by Katterman (1933) in *Oenothera*. Since there is no multivalent formation, it is inferred that migration of chromatin is taking place after the synopsis of the homologous chromosomes. Cytomixis has been observed in the present investigation at and after pachytene. The same has been recorded by Mendez and Rijo (1951), Sadasivaiah and Magoon (1965), Kamra (1960), Habib and Chennaveeraiah (1976) and Lakshmi and Rao (1977). However, in *Hemerocallis*, cytomixis was recorded by Prakash Narain (1979) as late as second telophase. From this it is evident that cytomixis can occur at any stage in the division cycle of meiocytes.

Although, there are views that several factors like action of fixatives, mechanical pressure, temperature anomalies, pathological conditions, disturbance in the hydrostatical state of sporogenous tissues, application of herbicides may cause cytomixis, in the present case, cytomixis appears to be a natural phenomenon and is under the control of genes, since all the plants were raised under uniform environmental conditions. Further it is not an artifact due to fixation and handling, since the phenomenon is present both in fixed and unfixed materials and in squashes and smears. In *Trigonella*, the phenomenon appears to be playing a significant role in bringing about evolutionary divergence since variable chromosome numbers leading to the production of aneuploid gametes were present in 42·03% cells. Further, some of these gametes seem to be viable as the plant was partially fertile and produced viable seeds. However, this fact has to be confirmed by further progeny studies. This opinion gains support from the studies of Selassie (1970) who assumed that production of aneuploid PMCs and unreduced gametes could be an important factor in creating spontaneous variation in *privus*.

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* Originals not seen