Cytomixis and Associated Meiotic Abnormalities in Pollen Mother Cells of *Chlorophytum tuberosum* (Roxb.) Baker

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Summary  Cytomixis associated with chromosomal anomalies was recorded in the meiotic division of pollen mother cells of the wild genotypes of *Chlorophytum tuberosum* (Roxb.) Baker for the first time. The migration of chromosomal substances was more prevalent in Meiosis-I than Meiosis-II. Cytomixis has been found both in juxtaposed cells by direct contact as well as between remote cells through cytoplasmic strands. During cytomixis, various chromosomal abnormalities, such as chromosomal stickiness, laggards, chromosomal bridges, unequal separation of chromosome, micronuclei formation and loss of chromosome, were recorded. A significant number of empty pollen formations leading to pollen sterility was recorded which is assumed to be a direct result of cytomixis.

Key words  Chromosomal abnormality, *Chlorophytum tuberosum*, Cytomixis, Meiosis, Pollen Mother Cells.

The herbaceous species *Chlorophytum tuberosum* (Roxb.) Baker belongs to the family Agavaceae and is commonly known in India as ‘Safed musli’. Nearly 235 species of the genus are distributed throughout the tropical and sub tropical zones of the world. Out of the 15 species reported in India, the natural occurrence only of *C. tuberosum* has been reported from West Bengal (Mandal and Nandi 2012a, b). The species is widely known due to its importance as a source of aphrodisiac medicine and propagates mostly through vegetative reproduction and with a very low amount of seed production.

Pollen meiosis for this species was studied by Kumar and Rao (1958), who claimed the chromosome number of *n*=8 and also reported the presence of inversion bridges. Spontaneous chromosome inversion has also been reported in another congeneric species, *C. elatum* (Sheriff 1957). The mixing of cytoplasm of two cells – cytomixis has been reported in *C. comosum* (Thumb.) Jacq. by Lattoo et al. (2006) and chromosomal abnormalities in pollen meiosis has also been reported by others (Pagliarini et al. 1993, Gudadhe et al. 2012). Cytomixis, a phenomenon of mixing nuclear as well as cytoplasmic materials of two or more cells lying in close proximity, was reported first by Körnicke (1901). Although this phenomenon has been reported, in various plants, by earlier researchers (*e.g.* Gottschalk 1970, Cheng et al. 1975, Omara 1976, Premachandran et al. 1988, Peng et al. 2003, Zhouhui 2003, Sheidai and Attaei 2005, Sidorchuk et al. 2007), its occurrence in *C. tuberosum* has been recorded for the first time in this paper.

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Materials and methods

Individual plants of *Chlorophytum tuberosum* (Roxb.) Baker were collected from five different wild locations of the southern lateritic zone of West Bengal and the collected germplasms were maintained in the garden of Department of Botany and Forestry, Vidyasagar University. The collections made at five different locations were all Accession No. RNB177 (Ranibandh Block) from the Bankura district and four others, namely Accession No. NYG102 (Nayagram), Accession No. SLB 155 (Salbani), Accession No. AML122 (Amlachati) and Accession No. KLB130 (Kalabani), were collected from different natural forest areas of the Paschim Medinipur district, West Bengal, India. Standard nursery practices were adopted to maintain all the populations in the departmental garden.

For the study of meiotic division in pollen mother cells (PMCs), young fresh buds were collected from five different in situ populations and also from the plants growing in the university garden. They were primarily preserved in modified Carnoy’s fixative (6:3:1) for 24 h and then transferred to 70% ethanol and kept at 18°C in refrigerator for long-term preservation. Both fresh and preserved buds were stained with 1% aceto-carmin solution and observed under the higher magnifications of a light microscope (*Leica* DM1000). Ten plants from each ecotype were collected and maintained in the institute garden. Flower buds were collected from five plants of each ecotype and meiotic slides were prepared separately for them. From each slide, 10 plates were scored and their mean data were deduced with respective standard deviations.

Results

All wild populations showed cytomixis alongside several types of chromosomal abnormality, with a preponderance of stickiness and chromosomal bridges. Other types observed were unequal separation of chromatids, laggard chromosomes, late movement of chromatids in Anaphase-I. PMCs with greater as well as lesser amounts of genomic content were also witnessed. Transfer of chromatin material from one PMC to another was noticed in all cases of cytomixis. Based on the forms of cytomixis observed, it may be classified into two types. In some cases, the direct fusion of conjugant PMCs was recorded and in others, cytomixis occurred by the formation of cytoplasmic strands. These strands were noted in some cases to involve more than two cells, even up to eight cells (Fig. 1b). Some of the populations showed a higher frequency of cytoplasmic strand formation (Table 1 and Fig. 1b) and a few PMCs showed direct fusion amongst many contiguous cells in a chain (Fig. 1a). In many populations, the formation of cytoplasmic strands was noted to form even between two remote cells. In direct fusion of cells, nuclear material (Fig. 1a) was found to congregate on the margins of the cell and to intersperse through the cell wall in the form of tiny beads of chromatin material (Fig. 1b) or as large lumps (Figs. 1a, b). Careful observation confirmed the prevalence of cytomixis in Meiosis-I (63.50%) in contrast with Meiosis-II (3.82%). Transfer of chromatin was either partial, involving a small segment of a chromosome, or complete. As a consequence of the transfer of chromosomal parts, many cells showed the formation of micronuclei (Figs. 1c, d) and an excess amount of chromatin matter as separate lump(s) (Figs. 1c, d). A few PMCs contained single isolated bivalents at metaphase-I (Fig. 1g). The diverse nature of the abnormalities in meiosis was encountered in all five populations. The highest and lowest percentages of chromosomal abnormality were shown by the SLB155 and NYG110 populations respectively. Amongst all types of abnormalities observed, stickiness of chromatin was more frequent in all populations and mainly occurred at Metaphase-I. In most of cases, chromosomes lost their individuality completely due to stickiness (Figs. 1c, d, h). Some PMCs showed lagging of chromatin material, isolated from the main lumps of chromatin (Fig. 1c), chromatin bridges at Anaphase-I (Fig. 1e) and late movement of bivalents at Anaphase-I (Fig. 1f).
A study of the later stage of meiosis showed many empty pollens and petite pollens of a sterile nature (Fig. 2b). The present work registered the occurrence of empty pollen to vary within a range between 3.97% and 10.09% among plants of different populations (Table 1 and Fig. 1j).

**Discussion**

Cytomixis in *Chlorophytum tuberosum* has been noted in the present study to occur in the different stages of meiotic division of PMCs. Such occurrence in PMCs, meristematic cells, tapetal cells and ovary cells of a wide range of flowering plants has been reported by many earlier researchers (Heslop-Harrison 1966, Bellucci *et al.* 2003, Bobak and Herich 1978, Koul 1990).

![Fig. 1.](image)

Table 1. Cytological events associated with cytomixis in *C. tuberosum.*

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>% of cytoplasmic strand formation</th>
<th>% of direct cell fusion</th>
<th>% of abnormal cells</th>
<th>% of cytomixis</th>
<th>% of empty pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML122</td>
<td>2.75±0.07</td>
<td>2.69±0.09</td>
<td>20.67±11.83</td>
<td>5.44±5.36</td>
<td>93.49±3.99</td>
</tr>
<tr>
<td>KLB130</td>
<td>4.30±3.06</td>
<td>2.39±3.04</td>
<td>21.12±13.07</td>
<td>6.69±9.16</td>
<td>94.01±7.83</td>
</tr>
<tr>
<td>NYG102</td>
<td>2.65±5.32</td>
<td>2.71±8.01</td>
<td>18.32±15.16</td>
<td>5.36±7.42</td>
<td>96.03±3.83</td>
</tr>
<tr>
<td>SLB155</td>
<td>3.41±3.07</td>
<td>3.52±6.11</td>
<td>22.21±13.39</td>
<td>6.93±7.31</td>
<td>89.91±3.36</td>
</tr>
<tr>
<td>RNB177</td>
<td>4.13±7.76</td>
<td>2.55±5.79</td>
<td>21.53±12.87</td>
<td>6.68±7.49</td>
<td>90.93±3.70</td>
</tr>
</tbody>
</table>

Mean (±S.D.)

A study of the later stage of meiosis showed many empty pollens and petite pollens of a sterile nature (Fig. 2b). The present work registered the occurrence of empty pollen to vary within a range between 3.97% and 10.09% among plants of different populations (Table 1 and Fig. 1j).
Kwiatkowska et al. (2003) and Ventela et al. (2003) claimed cytomixis to happen in lower plants as well as during the spermatogenesis of animals. Cytomixis by cytoplasmic strands and direct contact of cells in the species, that under study here, is supported by the observations of similar occurrences in different plants made by other researchers (De and Sharma 1983, Sapre and Deshpande 1987, Bahl and Tyagi 1988, Haroun 1995, Singhal and Kumar 2008, Zhen-Qiao and Xing-Feng 2009). The higher rate of chromatin transfer in the first meiotic division, in comparison to the second division, observed here for all the individuals, is also in accordance with the findings of earlier reports (De and Sharma 1983, Consolaro and Pagliarini 1995, Bellucci et al. 2003, Lattoo et al. 2006).

A host of anomalies, such as spindle disturbance, unequal separation of chromosomes, chromosomal bridges, lagging and early or late separation of chromosomes, stickiness of chromosomes, excess chromatin and formation of micronuclei, all observed for *C. tuberosum* in this study, comply with the findings of previous researchers (Sheriff 1957, Kumar and Rao 1958, Pagliarini et al. 1993, Haroun 1995, Lattoo et al. 2006, Singhal and Kumar 2008, Zhen-Qiao and Xing-Feng 2009) in this species as well as in many other plant species. The formation of micronuclei (Figs. 1c, d) resulted with the chromatin content separate from the main mass; this has also been reported in other studies (Bhat et al. 2006). The occurrence of cells with a reduced number of chromosomes was also noted in Metaphase-I (Fig. 1i) of this species. However, the meiotic chromosome number (*n*=8) found in this study (Fig. 1k) goes in accordance with the previous record of Kumar and Rao (1958) in *C. tuberosum*. Although there are no earlier reports on cytomixis in *C. tuberosum*, the work of Kumar and Rao (1958) depicting some unusual features in pollen meiosis in the species, holds much significance and extends support to the present work. Reports on the occurrence of inversion bridges in this species by Kumar and Rao (1958) and John et al. (1989) has also been adduced with the similar cases (Fig. 1h) observed here, and the presence of laggard chromosomes by the side of the bridges in the present study might suggest its origins may be found in inversion. The occurrence of inversion bridges has also been confirmed in another congeneric species, *C. elatum*, by Sheriff (1957). As pointed out by Kumar and Rao (1958), the significance of inversion, more specifically, of pericentric inversion accompanied with crossing over therein, in playing a role in the evolution of congeneric species of the genus appears to be plausible.

Individuals showing cytomixis also had a greater frequency of empty pollen (89.91–96.03%) in the studied species, a feature quite similar to earlier experiences in other species (Bahl and Tyagi 1988, Yen et al. 1993, Lattoo et al. 2006, Singhal and Kumar 2008, Zhen-Qiao and Xing-Feng 2009). Empty pollen is most likely the result of cells being emptied of cytoplasm in the course of cytomixis. Unequal genomic content in the meiotic products of PMCs is also expected to cause aneuploidy, in effect, a fact claimed in earlier reports, too (Sarvella 1958, Sapre and Deshpande 1987, Datta et al. 2005), in addition to developing some perishable void products.

Notwithstanding the enigmatic nature of cytomixis, some researchers have claimed it to be a regular and genetically controlled event, which is also influenced by some physiological and environmental factors (De and Sharma 1983, Zheng et al. 1987, Bellucci et al. 2003, Boldrinia et al. 2006, Lattoo et al. 2006). However, no difference in the frequency or types of cytomixis was observed in this study among the five populations of *Chlorophytum tuberosum* collected from different environments and edaphic conditions. The unusual proximity, adherence of cytomictic cells and also the appearance of isolated chromatin lumps migrated towards the margins of cells, clearly occurring prior to the initiation of the cell division process, as revealed in this study, appear to be axiomatic for the involvement of a chemical signaling in commencing as well as progressing the meiotic event.

There have been many attempts to surmise the nature of origin of cytomixis, its mechanism, fate and significance, as well as its role in evolution, but as yet these attempts have been to no avail due to lack of sound evidence to support them. Despite this, it seems quite apparent that cytomixis is instrumental in checking the success rate of sexual reproduction and also in introducing a change
in chromosome number in the process of natural evolution. The check in reproductive success might have an ecological significance too, which probably has been implicated in its vegetative propagation.

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References


