Cytomixis during Microsporogenesis in Some Populations of Croton bonplandianum of North India

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Summary Present investigations were carried out to study the microsporogenesis in populations of Croton bonplandianum Baill. from 5 cities of north India. The chromosome count of n=10 was uniform in all the populations. Among the analysed populations 32.50–40.96% of PMCs were recorded to be interconnected through cytoplasmic connections, with many of them showing actual transfer of genetic material between the cells. Cytomixis was observed to involve 2–8 cells at a time. Presence of laggards and resultant micronuclei has been observed in all the populations. As a result of cytomixis and its associated abnormalities heterogeneous pollen size and variation in pollen fertility were also recorded.

Key words Cytomixis, Croton bonplandianum, Pollen size, Pollen fertility.

Cytomixis is a phenomenon that involves the migration of chromatin/chromosomes between the meiocytes through cytoplasmic channels. Since its first report by Koernicke (1901) in the pollen mother cells of Crocus sativus, the phenomenon has been reported during microsporogenesis in a wide range of flowering plants (Heslop-Harrison 1966, Gottschalk 1970, Cheng et al. 1975, Omara 1976, Singhal and Gill 1985, Guochang et al. 1987, Bedi 1990, Bione et al. 2000, Bellucci et al. 2003, Datta et al. 2005, Ghaffari 2006, Latino et al. 2006, Singhal et al. 2007). Cytomixis is also known to occur in somatic cells of leaves (Tarkowska 1960), tapetum (Cooper 1952), ovary (Koul 1990), root meristem (Sarvella 1958) and shoot apex (Guzicka and Wozny 2005). The process of chromatin transfer, which has a profound effect on the meiotic process and its end-products, is of considerable evolutionary significance (Falistocco et al. 1995, Morikawa and Leggett 1996, Ghanima and Talaat 2003).

Croton bonplandianum Baill. (vernacular kala bhangra), a native of America but now naturalised in India, is an important medicinal plant that occurs widely along roadsides, railway tracks, abandoned fields, wide-open ravines, etc. The plants are erect, herbaceous, branched, rough (whitish satellite) with latex. Leaves are alternate, simple and lanceolate with toothed margin. The inflorescence is a terminal protogynous raceme with female flowers at the base and male at the top. Flowering and fruiting is from April to October. Bark and roots are alternative and cholangic, seeds are purgative and the latex of plants has a healing effect on cuts and wounds (Chandel et al. 1996).

The present investigations were carried out to evaluate the cytological diversity within this introduced species, if any. The plants of Croton bonplandianum were collected from 5 different locations in north India (Table 1). Flower buds of suitable size were fixed in Carnoy's fixative and stored in 90% alcohol at 4°C till further use. Meiotic studies were conducted from temporary mounts of young anthers squashedin 2% acetocarmine. Careful observations were made on a number of slides to confirm the chromosome count and occurrence of various abnormalities was recorded. Pollen fertility was determined by mounting the pollens from mature anthers in glycerol–acetocarmine mixture (1:1) and heating at 60°C for 5 min and observing after 2–4 h. Well

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filled and uniformly stained pollens were scored as fertile. Photographs of preparations depicting various observations were taken using Nikon 80i digital microscope.

Meiotic studies of the plants of all 5 populations of the species *viz.* Patiala, Chandigarh, Ludhiana, Panipat and Ropar revealed the haploid chromosome number of $n=10$ (Fig. 1a, b). The present count of haploid chromosome number is in line with earlier records for the species by Patil (1958), Gajapathy (1962), Datta (1967), Choda and Mehra (1972), Hans (1973), Koul et al. (1976), Bir and Sidhu (1979, 1980), Sidhu and Bir (1983), Soontorchainak and Chaiyasut (1999).

The course of meiosis is quite abnormal with a good number (32.50–40.96%) of PMCs involved in cytomixis i.e., PMCs interconnected with the help of cytoplasmic channels showing transfer of chromatin as well (Table 2). The PMCs at the diakinesis, metaphase-I and II and tetrad stages were observed to be involved in cytomixis (Fig. 1d–i). The migration of chromatin material from one cell to another is a well established phenomenon reported in large number of plants (Rieger *et al.* 1976, Saggoo and Bir 1983, Zheng 1983, Consolaro and Pagliarini 1995, Bellucci *et al.* 2003, Lattoo *et al.* 2006, Singhal and Kumar 2008, Kumar *et al.* 2010). The phenomenon has been credited with a role in evolution (Cheng *et al.* 1980), an additional means for the conservation of genetic heterozygosity of gametes (Villeux 1985) and the production of aneuploids and polyploids (Falistocco *et al.* 1995). On the other hand, some researchers like Heslop-Harrison (1996) and Haroun (1995), consider cytomixis as an artifact created during fixation. Other cited causes of cytomixis are pathological infections (Bobak and Herich 1978, Morisset 1978), physiological changes (Bell 1964, Bahl and Tyagi 1988), pollution (Haroun *et al.* 2004), pesticides (Bobak and Herich 1978), temperature (Narain 1976), and genes (De and Sharma 1983, Ghanima and Talaat 2003). According to Zheng *et al.* (1999), actin and its associated proteins are involved in the intercellular nuclear migration during cytomixis.

Cytomixis has been observed in PMCs of *Croton bonplandianum* in all the 5 north Indian populations.

### Table 1. Sampled populations of *Croton bonplandianum* Baill with geographical location

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Locality</th>
<th>Accession number (PUN)*</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chandigarh</td>
<td>50882</td>
<td>30°44'14 N</td>
<td>76°47'14 E</td>
<td>320</td>
</tr>
<tr>
<td>2</td>
<td>Ludhiana</td>
<td>50883</td>
<td>30°53'60 N</td>
<td>75°50'60 E</td>
<td>242</td>
</tr>
<tr>
<td>3</td>
<td>Panipat</td>
<td>50884</td>
<td>29°25' N</td>
<td>77°02' E</td>
<td>218</td>
</tr>
<tr>
<td>4</td>
<td>Patiala</td>
<td>50885</td>
<td>30°23' N</td>
<td>76°26' E</td>
<td>249</td>
</tr>
<tr>
<td>5</td>
<td>Ropar</td>
<td>50886</td>
<td>31°57'59 N</td>
<td>76°31'59 E</td>
<td>259</td>
</tr>
</tbody>
</table>

* Herbarium code of Botany Department, Punjabi University, Patiala (Inadi) as per “Index Herbariorum” by Holmgren and Keuken (1974).

### Table 2. Cytomixis, meiotic course and pollen fertility in studied populations of *Croton bonplandianum*

<table>
<thead>
<tr>
<th>Population</th>
<th>PMCs involved (%)</th>
<th>No. of PMCs involved</th>
<th>Meiotic stages</th>
<th>PMCs with laggards (%)</th>
<th>Pollen fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chandigarh</td>
<td>36.41</td>
<td>2–5</td>
<td>P-I, M-I, A-I</td>
<td>24.13</td>
<td>92.21</td>
</tr>
<tr>
<td>Ludhiana</td>
<td>35.07</td>
<td>2–8</td>
<td>M-I, M-II, T</td>
<td>14.28</td>
<td>90.78</td>
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<tr>
<td>Panipat</td>
<td>36.17</td>
<td>2–4</td>
<td>P-I, M-I, M-II, A-II</td>
<td>10.52</td>
<td>94.35</td>
</tr>
<tr>
<td>Patiala</td>
<td>32.50</td>
<td>2–6</td>
<td>D, M-I, M-II, A-I, A-II, T</td>
<td>30.43</td>
<td>93.86</td>
</tr>
<tr>
<td>Ropar</td>
<td>40.96</td>
<td>3–5</td>
<td>P-I, M-I, T</td>
<td>20.83</td>
<td>90.01</td>
</tr>
</tbody>
</table>

PMC: pollen mother cell; D: diakinesis; P-I: prophase-I; M-I: metaphase-I; M-II: metaphase-II, A-I: anaphase-I; A-II: anaphase-II; T: tetrad.
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Fig. 1. Meiotic chromosome number, and meiotic abnormalities in *Croton bonplandianum* (a–i). a: A PMC at diakinesis showing 10 bivalents. b: A PMC at metaphase I showing 10 bivalents. c: A PMC at telophase I showing laggards (arrows). d: Three PMCs showing multiple cytomictic channels (arrows). e: PMCs showing transfer of chromatin through cytomictic channel (arrow). f: PMCs showing chromatin transfer (arrow) and cytomictic channels of varying breadth. g: PMCs at diakinesis showing chromatin transfer through cytomictic channels (arrows). h: Enucleate PMC (arrow) and another PMC showing double chromosome complement. i: Presence of cytomictic channels at tetrad stage (arrows).
populations where meiotic analysis was carried out. The process was observed to involve up to 8 PMCs at a time and occurred in the form of simple cytoplasmic connections to direct fusion of the PMCs, first type being more common in all the populations. Sometimes adjoining PMCs were observed to be connected by 2 or more channels of varying breadth (Fig. 1d, f). Actual chromosome/chromatid transfer was greater in PMCs at the first meiotic division and here too at metaphase-1 than the PMCs at the second meiotic division. More frequent occurrence of cytomixis

**Fig. 2.** Meiotic abnormalities in *Croton bonplandianum* (a–f). a: A PMC at telophase II showing laggards (arrows). b–d: Tetrads showing micronuclei (arrows). e: Polyad. f: Stained apparently fertile and transparent apparently sterile pollen grains showing heterogeneous size (arrows).
during meiosis-I as compared to meiosis-II has been recorded by earlier researchers also (Cheng et al. 1980, Mantu and Sharma 1983, Consolaro and Pagliarini 1995, Pierozzi and Benatti 1998, Lattoo et al. 2006, Singhal and Kumar 2008, Kumar et al. 2010). The amount of chromatin transfer varied from a small part to the entire complement thus making the donor cell empty (Fig. 1h). In some PMCs in the plants of the Chandigarh and Panipat populations, chromatin was observed being transferred to 2 or more PMCs simultaneously. In the populations of Patiala, Ludhiana and Ropar, cytomixis was observed at the tetrad stage of the PMCs also (Fig. 1i). These observations are in line with the earlier reports of Koul (1990) in *Alopecurus arundinaceous*, Sen and Bhattacharya (1988) in *Vigna glabrescens* and Kumar et al. (2010) in *Clematis orientalis*.

Apart from cytomixis there were other meiotic irregularities in the form of laggards at anaphase and telophase of meiosis I and II (Figs. 1c, 2a) and micronuclei at the tetrad stage (Fig. 2b–d). The number of laggards varied from 1–3 among the meioocytes belonging to different populations of plants. Bhat et al. (2006) have postulated that the formation of micronuclei is entirely due to laggards.

The phenomenon of cytomixis and associated meiotic abnormalities have been considered responsible for pollen infertility and heterogeneity in pollen size. In present investigations the size of the pollen grains was found to be heterogeneous in all the populations (Fig. 2f). Broadly 3 categories of pollen were observed. Medium sized pollen grains (40–42×40–41.6 μm) were frequent in all the populations. The large sized pollen grains varied between 43–54 μm in diameter whereas the small sized were below 22 μm in diameter. The small sized (micropollens) were mainly sterile in nature and contributed to the overall pollen sterility.

The pollen fertility among the 5 populations of *Croton bonplandianum* varied from 90.01 to 94.35%, the minimum being in the Ropar population and the maximum in the Panipat population.

References

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Datta 1967


