Cytological Studies in *Psidium freidrichsthalianum* Niedenzu

H. C. Srivastava

Fruit Breeding Laboratory, Department of Horticulture,
Haryana Agricultural University, Hissar, India

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*Psidium freidrichsthalianum*, Niedenzu is the native of South Mexico and Central America (Bailey 1919). It is a small tree with slender branches and smooth dark brown bark. Leaves are 3 to 3.5 cm long and 1 to 1.5 cm wide, sparsely pellicid punctate, almost glossy above and puberulent below. Blooms during spring and monsoon. Ripe fruits are small, very sweet and display moderate amount of seeds on cutting. It is in cultivation in Costa Rica, Guatemala and parts of Central America. The fruits are considered valuable for jelly making in those countries. Study of the literature shows that cytologists have not given their attention toward this species. A preliminary cytological observation has been reported by the author in 1972. This communication presents a detailed cytological studies of the species.

Materials and methods

*Psidium freidrichsthalianum*, Niedenzu growing in the Central Circle Botanical Survey of India, Allahabad was used for the studies.

Root tips were treated in .002 M solution of 8-hydroxyquinoline for 3 hours at 14°C. Afterwards the tissue was transferred in fixing cum staining mixture of 2% aceto orcein and N-hydrochloric acid in the ratio of 8:2 respectively. The root tips were squashed in 1% aceto orcein.

For the study of meiosis floral buds were fixed in Carnoy’s fixative. Addition of a pinch of ferric acetate in the fixative was very useful. Anthers were squashed in 1% propiono carmine.

Photomicrographs were taken from the fresh preparations at a magnification of 2325 x. Drawings were prepared by bleaching of photomicrograph.

Observations

Cytological observations of somatic tissue have revealed *P. freidrichsthalianum*, Niedenzu to be 2n=22 (Fig. 1). The basic chromosome number of *Psidium* is n=11 (Atkinson 1947) hence, this species is a diploid. The chromosomes were mostly rod shaped, however, few chromosomes appeared to be J shaped. Due to the small size of the chromosomes their morphology could not be studied in detail.

At diakinesis in majority of the cases there was regular bivalent formation. Eleven bivalents were commonly observed (Fig. 2). In a few PMC varying proportions of bivalents and univalents have been seen. Number of univalents have been found to be 2. These presumably have resulted due to the failure of pairing.
Occasionally a bivalent was observed to be attached with the nucleolus (Fig. 2).

Behaviour of the chromosomes at metaphase I was studied from a large number of pollen mother cells. An analysis of 105 clear cells is presented in Table 1.

### Table 1.

<table>
<thead>
<tr>
<th>Configuration</th>
<th>No. of pollen mother cells</th>
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<tr>
<td>11</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
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<tr>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>105</strong></td>
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</table>

**Mean number of quadrivalent per cell** = 0.066  
**Percentage of cells enclosing quadrivalent** = 6.66%

**Mean number of trivalent per cell** = 0.057  
**Percentage of cells enclosing trivalent** = 3.80%

**Mean number of bivalent per cell** = 10.713  
**Percentage of cells enclosing bivalent** = 100.00%

**Mean number of univalent per cell** = 0.133  
**Percentage of cells enclosing univalent** = 3.80%

At metaphase I generally 11 bivalents have been observed (Fig. 3). In few cases a quadrivalent (Fig. 4) or a trivalent with three univalents (Fig. 5) have been noted. This is the first record of association of more than two chromosomes in a diploid species of *Psidium*. Bivalents oriented regularly at the equator. In certain cases it has been observed that ten bivalents were lying at the equator and two univalents were opposite to each other away from the equator. Their orientation opposite to each other leads to the conclusion that they have arisen due to precocious separation. In another case ten bivalents with one univalent have been found at the equator and one univalent was away from it. It appears that no pairing has occurred in this bivalent (Fig. 6). Occasionally two univalents were oriented in midst of bivalents within the equator. In 6.66% PMCs single quadrivalent was seen. Maximum number of quadrivalent was only one. Similarly one trivalent has been observed in 3.8% PMCs.

Anaphase I was usually regular. Eleven chromosomes were observed moving towards respective poles (Fig. 7). However, about 20% PMCs revealed abnormal anaphase I. In 8% cases a distribution of 12 and 10 chromosomes was seen (Fig. 8). About 10% cases revealed one to three chromosomes to behave as laggards. A PMC having three laggards and a distribution of 9 and 10 chromosomes is observable in Fig. 9. Occasionally a chromosome was observed to be lying away from the spindle region. Besides these irregularities twelve per cent pollen mother cells revealed two dicentric bridges accompanied by fragment (Fig. 10).

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Figs. 1–14. *Psidium freidrichsthalianum*, Niedenzu. 1, 2n=22. 2, 11 bivalents at diakinesis. 3, 11 bivalents at M I. 4, one quadrivalent at M I. 5, Y shape trivalent at M I. 6, 10 II+2I at M I. 7, 11:11 distribution at A I. 8, 12:10 distribution. 9, three laggards at A I. 10, two dicentric bridges. 11, a chromosome lying in between spindles at M II. 12, normal A II. 13, collapse of a spindle. 14, two laggards and a chromosome away from the spindle at A II.
various types of distribution at anaphase I

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</thead>
<tbody>
<tr>
<td>No. of PMC</td>
<td>50</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Percentage</td>
<td>80.32</td>
<td>1.64</td>
<td>4.91</td>
<td>8.03</td>
<td>3.28</td>
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During telophase I some of the laggards which were unable to access the poles have been observed lying in the cytoplasm. Some of the stray chromosomes occasionally lead to formation of micronuclei.

Metaphase II has been observed to be regular in most of the cases. Occasionally number of chromosomes at the two spindle deviated from the normal. It was due to unequal distribution during first division which gave rise to two nuclei having different chromosome numbers. Occasionally one chromosome was oriented between the two metaphase plates (Fig. 11).

Anaphase II was regular in most of the cases (Fig. 12), however, 4% cases revealed a chromosome lying outside the spindle region. Sometimes 1–3 chromosomes behaved as laggards. A PMC displaying two laggards and a chromosome lying off the spindle may be seen in Fig. 14. Spindle functioned regularly but 2.56% cases revealed collapse of one of the spindles (Fig. 13). The observations from 78 well prepared pollen mother cells at anaphase II are enclosed in Table 3.

<table>
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<tr>
<th>Particulars</th>
<th>No. of cells</th>
<th>Percentage</th>
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<tr>
<td>One laggard</td>
<td>12</td>
<td>15.37</td>
</tr>
<tr>
<td>Two laggards</td>
<td>8</td>
<td>10.37</td>
</tr>
<tr>
<td>Three laggards</td>
<td>6</td>
<td>7.69</td>
</tr>
<tr>
<td>Normal disjunction</td>
<td>50</td>
<td>64.11</td>
</tr>
<tr>
<td>Spindle fusion</td>
<td>2</td>
<td>2.56</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>78</strong></td>
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Telephase II was regular in several cases. Some chromosomes which were not included in the telophasic nuclei appeared to remain in the cytoplasm. They behaved as stray chromosomes. Besides stray chromosome micronuclei have also been observed.

Discussion

*Psidium freidrichsthalianum*, Niedenzu a diploid species reveals association of more than two chromosomes resulting in the formation of ring and a Y shaped configuration. This presumably is the first record of multivalent formation in an diploid species of *Psidium*. The highest association observed is a quadrivalent and trivalent. Both these configurations have not been observed in the same pollen mother cell. Raghuvanshi (1958) reported in diploid *Citrus assamensis* 18 chromosomes, forming one quadrivalent, six bivalents and two univalent. He explained
this condition as having resulted by pairing of non homologous chromosomes, McClintock (1933) and Burnham (1934) observed intimate association of non-homologous chromosomes in prophase of Zea mays but due to lack of chiasma formation in this region this association could not persist upto metaphase.

In P. freidrichsthalianum, in addition to trivalent and quadrivalent bridges have been observed. This is of great significance from evolutionary point of view because it clearly indicates the role played by both translocations and inversions in speciation. Here is an instance where both the structural changes translocation and inversions of major evolutionary importance in plant species occur in the same plant.

The inversion heterozygosity appears to be more frequent than translocation. The explanation of this is found when we consider as to how these structural changes are produced. There are two distinct stages in the production of structural changes chromosome breakage and reunion of fragments. A fracture of chromosomes produce two "injured" surfaces which are for certain time capable of reunion with other surface any where in the nucleus (Muller 1940 and Helfer 1941). In case many reunions are possible, the fragments, whose ends happen to be geometrically closest, have the greatest chances of effecting a junction.

In this species of Psidium appearance of univalents at meiosis is fairly common. The univalents arise in three ways. It is either a chromosome which has altogether failed to pair at zygotene or it is one which paired to form a bivalent but whose two component chromosome separated at diplotene because no chiasma was formed between them or due to precocious disjunction of bivalent. In the present material zygotene and pachytene stages could not be studied. Consequently it was not possible to determine whether exactly the occurrence of univalent at diakinesis and metaphase I is due to entire absence of synopsis or due to the subsequent disassociation following synopsis.

Appearance of univalents is influenced by translocation, inversion and hybridization. Dicentric bridge configuration has been seen in P. freidrichsthalianum in one or occasionally two bivalents. Presence of inversions in this species obstructed in regular pairing. It appears that inversion as well as translocation affects univalent formation in Psidium freidrichsthalianum, Niedenzu.

Formation of univalent in the present material may have also been effected by environmental factors. Environmental factors like temperature etc. have been demonstrated by several workers to effect the behaviour of chromosomes (Heilborn 1930, Katayama 1931 and Sax 1931). Working on Ribes Meurman (1928) reported a marked variation in conjugation of chromosomes in different cells of the same anthers which he explained as due to differential nutritional condition of the cell. However in the present investigation special care was taken to collect the buds. The collection was performed when the environmental conditions were quite normal. The plants were well irrigated and healthy. Under such circumstances the effect of these various environmental factors were reduced to minimum. No doubt the fact that these various factors might have also caused univalency to certain extent in Psidium freidrichsthalianum can not be ignored completely.

Pollen sterility in the present material has been found to be 50 per cent. This may be mainly due to production of gametes with duplications and deficiencies for
certain chromosome section. Since the duplication and deficiencies are generally lethal these unbalanced types are destroyed and the reproductive potentialities of the plant are significantly reduced.

Summary

*Psidium freidrichsthalianum* Niedenzu is a diploid species having a chromosome number 2n = 22. Quadrivalent, trivalent and univalent have been observed concomitant with bivalents. In addition to this dicentric bridges accompanied by fragments have been seen. It is presumably the consequence of inversion crossing over at the preceding diplotene. Beside these, other abnormalities such as precocious disjunction, unequal separation, lagging chromosomes, spindle fusion and stray chromosomes have been recorded. Pollen fertility is 50 per cent.

References


