Meiotic Behavior of Carica papaya L.: Spontaneous Chromosome Instability and Elimination in Important cvs. in North Indian Conditions

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Summary  Cytogenetical analysis was done in tropical species of papaya that is widely cultivated for its fruits and papain production. The proper and orderly chromosome behavior of the species is a perquisite to ensure high fertility and seed set in the crop. A study on pre-meiotic and post-meiotic stages was done on commercially important cultivars of India and few exotic cultivars. The meiosis in the flower buds was highly disturbed and many meiotic irregularities were recorded from the material fixed in summar. These ranged from chromosome stickiness (44%), chromatin bridges (21.1%), laggards (4.8), chromosome diminution/degeneration (5.2), and micronucleus (5.9%) formation, in the commercially important varieties. Pollen stickiness accounting for persistent pollen adherence in groups of 3–10 was observed in all the varieties ranging from 85.95% in cv. CO2 to 11.64% in CO5. Callose deposition on tetrads accounting for adhesion of pollen grains (permanent tetrads) was also observed. These were found to have a profound effect on the pollen fertility of the cultivars, wherein most of the commercial papaya cultivars had less than 50% of fertile pollen. Thus the role of meiotic irregularities in poor seed setting and seed production capacities under northern Indian conditions, specifically during the summer season has been established. Genetic or abiotic stress factors could be responsible for inducing such kind of anomalies, which are manifested in the form of pollen sterility and poor seed setting. Crop regulation strategies need to be evolved, so that the maximum seed set and fruit production occurs after this period to offset the restrictions imposed due to irregular meiosis.

Key words  Carica papaya, Pollen fertility, Laggards, Micronucleus, Chromosome stickiness, Pollen stickiness, Chromosome disintegration.

Papaya (Carica papaya L.) is a widely cultivated, arborescent, herbaceous plant yielding luscious fruits. Acclimatized in the tropics and subtropics, the family Caricaceae, possesses 5 other genera besides monotypic genus Carica. Earlier Carica L. comprised of 21 species, but recent classifications have placed all species (Other than edible papaya) in Vasconcellea (Droogenbroeck et al. 2004), based on crossability studies and molecular evidences. Among these C. papaya (paw-paw or papaya) is undisputedly most important, and it is heterogenous, outcrossing species the chromosome number being 2n=18; Darlington and Ammal (1945) reported all the members of Caricaceae to be diploids 2n=2x=18. It is polygamous, with three primary sex types: female, male and bisexual. Despite being an important crop of tropics and subtropics, the cytogenetics of the species is minimal, when compared to other species of economic interest. The causes are i) small morphologically indistinguishably chromosomes, ii) lack of development of advanced cytogenical techniques generally used in other species. The economic potential of the crop, for fresh fruit production, processing, latex and in industrial applications, demands continuing efforts for breeding of superior varieties. The unexploited crops (highland papayas) deserve special consideration for their role in possessing...
traits of use in hybridization strategies (National Research Council 1989). In recent years, low seed setting and summer sterility have been reported in papaya from various sources (Giaccenetti 1987). In fact, this is one of the major reasons for poor seed production resulting in limited availability of planting material in papaya in Northern parts of India. Recent endeavors at C.I.S.H. Lucknow, aim towards selecting and developing superior gynodioecious varieties suited to Northern Indian conditions. An understanding of structural and behavioral pattern of chromosomes during meiosis is an essential step towards development of superior varieties by evolving breeding programmes. The cytogenetical analysis is essential for studying stable integration of the genomes in the hybrid progeny and normal progression of genomes during reduction division.

Materials and methods

Meiosis and pollen fertility evaluation was done for 10 papaya varieties from field germplasm collection maintained at Central Institute for Subtropical Horticulture, Rehmankhera farm, Lucknow. Land preparation, cultivar selection and seedling production were done as per production technology given by Singh et al. (2005) in which cultural practice requirements and guidelines for planting, fertilizing, analysis, weed control, trimming, harvesting and disease and pest management are mentioned. Six of them have been developed at TNAU, Coimbatore, and 4 have been evolved at Pusa, Samastipur, Bihar. Flower buds were collected from 3 plants of each, for meiotic analysis and were fixed in FAA (ethanol : formaldehyde : acetic acid 2 : 1 : 1 v/v) for 24 h, after which they were transferred to 70% alcohol and stored at 4°C. Pollen mothers cells (PMC’s) were prepared by the squash technique and stained with 1% acetic carmine. At least 100–250 PMC’s in different phases of meiosis were evaluated for each plant and any abnormalities seen were recorded. Chiasma frequency was calculated by counting the chromosome configurations. The same procedures and stain used for meiotic analysis were employed with open flowers to test pollen sterility. More than 100 pollen grains/plants were analyzed. The data were analyzed statistically in Randomized design (Panse and Sukhatme 1978).

Results

The study of meiosis in different varieties of papaya was done and sequentially pre-meiotic, meiotic and post-meiotic stages were studied. The PMC’s undergoing pre-meiosis were small and chromosomes highly entangled mass until zygotene stage of meiosis. Hence, the pachytene analysis could not be done.

Normally, meiotic division proceeds from pairing of chromosome (9 pairs of bivalents), to tetrad formation in a coordinated and sequential manner. However, surprisingly large-scale meiotic abnormalities were recorded in the flower buds (fixed in summer season) leading to disturbances in chromosome movement to poles (Fig. 1). The meiotic abnormalities observed among the varieties included various forms of instability viz. chromosome stickiness, precocious migration of univalents, secondary associations, laggards, bridges and micronuclei formation. The meiotic phases generally most affected by these abnormalities were metaphase I and anaphase I.

Sticky chromosomes were the most commonly encountered anomaly in the studied sample. This phenomenon of sticky metaphase chromosomes involving few to entire genome complement was found in all the studied varieties (Table 1). It ranged from 21.6% in CO₃ to 44.0% in CO₂. There was wide variation of expression ranging from PMC’s with slight chromosome stickiness to more intense chromatin clusters, resulting in clumpy mass, impaired chromosome segregation unsuited to meiotic progression towards pollen grains (Fig. 1e and 1f).

Precocious univalent migration was found to occur in varieties CO₃, CO₄, CO₆ and CO₇. This abnormality nevertheless was observed in relatively lower frequency and the variational response
was nonsignificant. Desynapsis or no crossing over and univalent formation was found in large numbers (Fig. 1a).

Secondary association of bivalents involving 8–10 chromosomes was also observed in some varieties (Fig. 1g). Irregular chromosome segregation like Laggard chromosomes in Anaphase I; bridges and laggards in Anaphase II were other common abnormalities. Spindle disturbances along with stickiness in genome resulted in formation of restitution or micronuclei in range of 5.9–1.2% (Table 2). Chromosome disintegration due to stretching of chromosomes was also recorded (5.3–0.4%). This resulted in few chromosomes participating in the meiosis (up to 4 bivalents were...
found) (Fig. 1b). The abnormalities due to aberrant behavior of chromosomes in meiosis resulted in large variations during tetrad formation. This was seen by presence of triad and pentad.

The role of meiotic abnormalities present in such high frequencies was bound to have consequences and hence pollen fertility of the cultivars was examined from the selected plants. The 10 varieties of papaya, had high frequency of pollen sterility accounting for poor pollen fertility ranging from 51.2% in cv. Pusa Nanha to nil in CO₂ and CO₆ varieties (Fig. 2a). Pollen stickiness accounting for persistent pollen adherence in groups of 3–10 (Fig. 2b) was observed in all the varieties ranging from 85.95% to 11.64% (Table 2). Callose deposition on tetrads accounting for adhesion of pollen grains (permanent tetrads) was also observed (Fig. 2c). Analysis of variance revealed significant differences ($p<0.05$) in the pollen fertility behavior of the studied varieties (Table 2).

### Discussion

Pollen fertility is the most important criterion for genetic superiority of a variety, because the ability of a variety to reproduce and to survive depends on it. The pollen fertility directly or indirectly is dependent upon the efficiency of the meiotic process. Studies in different plant species have revealed the role of meiotic irregularities for declining seed setting and production capabilities (Dewald and Jalal 1974, Smith and Murphy 1986, Consolaro et al. 1996, Pagliarini and Pereira 1992).

Clumping of chromosomes forming pycnotic nuclei that soon degenerate and coalescing of nuclei has been reported in Gramineae (Mendes-Bonato et al. 2001). Chromosome stickiness characterized by chromosome clustering during different stages of cell cycle, is caused by genetic or environmental factors, and genetically controlled stickiness has been reported in maize (Beadle 1932a, b), millet (Rao et al. 1990) and wheat (Zanella et al. 1991). Apart from genetic reasons various abiotic factors such as temperature and herbicides also trigger this phenomenon (Ericksson 1968, Caetano-Pereira et al. 1995). Defective functioning of one or more types of non-histone proteins involved in chromosome organization is important for chromatid separation and segregation. In certain species plant genotype and environment interactions have been found to cause chromosome stickiness resulting in partial or complete failure of pollen germinability (Gaulden 1987).

The anomaly in chromosome segregation is widely reported in maize (Pagliarini et al. 1989), rubber (Pagliarini et al. 1992), and Brassica sp. (Souza et al. 1997). The causes attributed are low chiasmata, or presence of asynaptic genes in prophase I (Gottschalk and Kaul, 1980a, b). Correlation between this character with impaired pollen fertility and low seed setting has been observed in all the studied species. Absence of chiasmata, which are responsible for maintenance of bivalents permitting normal chromosome segregation is one of the reasons that can be ascribed for the precocious

### Table 2. Pollen fertility and pollen clumping in papaya varieties during summer season

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Varieties</th>
<th>Number of pollen grains analyzed</th>
<th>Pollen stickiness (range) (% value)</th>
<th>Pollen fertility (% value)</th>
<th>Pollen sterility (value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CO₂</td>
<td>760</td>
<td>90–100</td>
<td>83.95</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>CO₃</td>
<td>742</td>
<td>44–94.7</td>
<td>65.67</td>
<td>50.8</td>
</tr>
<tr>
<td>3</td>
<td>CO₄</td>
<td>461</td>
<td>22–100</td>
<td>50.61</td>
<td>34.69</td>
</tr>
<tr>
<td>4</td>
<td>CO₅</td>
<td>831</td>
<td>43–100</td>
<td>11.64</td>
<td>24.64</td>
</tr>
<tr>
<td>5</td>
<td>CO₆</td>
<td>520</td>
<td>95–100</td>
<td>24.17</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td>CO₇</td>
<td>882</td>
<td>16–77</td>
<td>48.02</td>
<td>44.07</td>
</tr>
<tr>
<td>7</td>
<td>Pusa delicious</td>
<td>802</td>
<td>40–62</td>
<td>24.8</td>
<td>44.5</td>
</tr>
<tr>
<td>8</td>
<td>Pusa nanha</td>
<td>900</td>
<td>40–80</td>
<td>32.0</td>
<td>51.2</td>
</tr>
<tr>
<td>9</td>
<td>Pusa majesty</td>
<td>742</td>
<td>20–80</td>
<td>21.2</td>
<td>45.8</td>
</tr>
<tr>
<td>10</td>
<td>Red fleshed</td>
<td>623</td>
<td>21–100</td>
<td>21.36</td>
<td>44.8</td>
</tr>
</tbody>
</table>
migration of univalents. Such disturbances in meiosis in highly heterogeneous and out crossing species perhaps indicate the hybrid nature of genomes, resulting in cell cycle variations, meiotic disturbances, chromosome bridges. The behavior of these and laggards, which also imply an abnormal spindle, leads to micronucleus formation and loss of chromatin.

Secondary association in groups of 2–3 observed in some varieties, implies homeology within genomes, suggesting for hybrid nature of these varieties as papaya is cross-pollinated species. The high amount of aberrations in meiotic cycle culminated in abnormal tetrads, sticky pollen grains and shriveled pollens. The sterility was also very high. Rao et al. (1990) and Golubouskaya (1989) observed that pollen grains resulting due to chromosome stickiness are inviable for fertilization. In our study too, chromosomes stickiness in all the varieties was on the major contributing factors towards impaired pollen fertility.

Conclusion

The meiosis in the flower buds of most of the important papaya cultivars was highly disturbed and many meiotic irregularities were found an in the summer fixed material. This leads to poor pollen fertility ultimately rendering the seed production capability of the varieties highly dysfunction-al. Hence seed production programme during this season in papaya is rendered abortive under typical subtropical conditions of Lucknow. Therefore papaya cultivation requires crop regulation strategies, so that the maximum seed set and fruit production occurs after summer season.

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References


