Impact of genome doubling on cytomorphological characters of *Sesamum indicum* L. (Pedaliaceae)

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**ABSTRACT.** Traditionally, tetraploids have been generated asexually using a genome-doubling agent, such as colchicine. Seeds and apical buds of seedlings of sesame (*Sesamum indicum* L., 2n=26) were treated with colchicine solutions of different concentrations for different durations. No polyploidy could be obtained in the seed treatment process, while seedling treatment resulted in five autotetraploid plants at 0.5% concentration for 3 consecutive day’s treatment along with the recovery of 12hrs each day. The morphology (plant height, number of branches, number of capsules, days to flowering, days to maturity, seed weight etc), cytology, pollen fertility and seed set of induced autotetraploid plants (4n=52) and diploids (2n=26) have been analyzed. Pollen fertility and stomatal frequency registered a decrease over diploids while size of pollen grain and stomata increased in the autotetraploids. Meiotic studies of the autotetraploids revealed a number of uni-, bi-, and multivalents. The present study was undertaken to induce genome doubling in sesame under greenhouse conditions, so as to develop a suitable protocol for the maximum recovery of autotetraploids and an economic use of colchicine.

**KEYWORDS:** Apical buds, Autotetraploids, Colchicines, Seedlings, Sesame

Polyplody is recognized as the most important feature of evolution in plants. One of the most spectacular advances of the genomics era has been a renewed appreciation of the pervasiveness and importance of genome doubling in plant evolution. Although the prevalence of polyplody in plants has classically been recognized from comparative analyses of chromosome numbers (Stebbins 1950; Grant 1981) and other biosystematics approaches (e.g., Masterson 1994). Polyploids often result in better adaptability, wider ecological niches and successful colonization of a greater range of different habitats (Samuel *et al* 1990). Enough variability created by the induction of polyplody and selection from segregating generation would yield better genotype for crop improvement (Singh 1991). Since the discovery of chromosome doubling property of colchicine, numerous reports have been published on colchicine induced polyplody.

The doubling of one genome requires a series of genetic and genomic adjustments that govern proper centromere recognition, chromosome pairing, and balanced assortment of chromosomes during meiosis. Genome doubling may further be complicated by other factors that may collectively be referred to as ‘genomic shock’ (McClintock 1984).

Sesame (*Sesamum indicum* L.) is one of the most important ancient oil seed crops (Bedigian and Harlan 1986). It is grown in tropical and subtropical areas (Ashri 1998) of the world. Sesame contains about 50-60% seed oil (Uzun *et al.* 2002; Arslan *et al.* 2007), which is of superior quality, nearly matching olive oil. Sesame oil is highly stable as compared to other edible oils, mainly due to the presence of antioxidants like sesamin, sesaminol, sesamolinol and squalene (Mohamed and Awatif 1998). Sesame oil also contains a high level of polyunsaturated fatty acids (Wood 1999). It has a reducing effect on plasma cholesterol and it also lowers the blood pressure (Sankar *et al.* 2005). Potential benefits of sesame on human health have recently renewed the interest in this ancient crop (Laurentin and Karlovsky 2006). Sudan, India, Myanmar and China are the most important sesame producers with 68% of the total world production (Laurentin and Karlovsky 2006). Despite the nutritional value and oil quality, the research on sesame has been scarce (Bedigian 2003). Average productivity of this important oil seed crop in India is 453 kg of seed/ha, which is far below the average productivity in China (1127 kg/ha) and Egypt (1211 kg/ha) (Banerjee and Kole 2009).

There is an ample scope for improving the productivity of this important oil seed crop through varietal improvement and hybrid cultivar development. The aim of this study was to bring results that could help sesame breeders to select suitable parental material for crossing and to increase the efficiency of selection in conjugation with other available information on morphological and molecular diversity.

**MATERIALS AND METHODS**

Seeds of sesame were obtained from Akola (Regional station of National Baeuro of Plant Genetic Resources, New Delhi), Maharastra. In Order to induce Polyploidy, treatments with aqueous solutions of colchicine were given on the seeds and seedlings as follows:

**Seed treatment** Seeds were presoaked in water for 12 h, and after removing the surface moisture with blotting paper, dipped in 0.1%, 0.3%, 0.5% colchicine solution for 5 h. Then, the treated seeds after washing well in running water, were allowed to germinate in petridishes on moist...
Seedling (bud) treatment Healthy seeds were sown and seedlings were treated after 2-3 days of their germination and before the emergence of third leaf. Cotton plugs were kept on the apical vegetative bud and 0.5% colchicine solutions were added to it by dropper. The seedlings were then covered with earthen pots as a measure to check evaporation. Colchicine solution was added on the plugs from time to time to avoid drying out. Treatments were given for different durations (12, 24 and 36h) for 1, 2 and 3 consecutive day’s alongwith recovery of 12 h. After specific period of treatments, cotton plugs were removed and shoot tips was washed well with water.

Cytological studies Buds of suitable sizes were fixed in acetic acid alcohol solution (1:3). Slides were prepared using anther squash technique with 2% aceto-carmine. Pollen fertility was evaluated by acetocarmine stainability test where sterile pollen grains remain unstained whereas the fertile pollen grains stained red. Photographs in transmitted light were made using an Aksioskop 2 plus microscope with an MC 80 camera and objective 40 xs. Data on different morphological characters of the plants were also recorded.

Results and Discussion Survival of treated buds After 30 days of treatment the survival of buds was recorded on the basis of the greenness of the buds. Nongrowing, brown buds were considered to be dead, while green, growing buds were considered to have survived. The survival rates were 96±0.52, 91.5±0.99, and 89.1±1.36 respectively; at 0.1%, 0.3% and 0.5% seed treatments of colchicine, respectively and survival rates in 0.5% colchicine bud treatment were 84±0.64, 78±2.71 and 66±1.23 respectively on 1, 2 and 3 consecutive days as compared to the 100±0.00% survival in the control.

There was an inverse relation between colchicine concentration and survival of buds.

Bud emergence and shoot growth The first visible effect of colchicine treatment was delayed sprouting and growth of the treated buds. In untreated explants, bud emergence and growth initiation were observed within three days of sowing. The growth of treated buds was slower than that of the controls. The concentration of colchicine was observed to have a marked influence on the growth of shoots. The lengths of the shoots varied from 140±1.52 to101±2.08 cm after 60 days of growth using 0.5% concentration of colchicine, with the longest (140±1.52cm) at 12 h of 0.5% colchicine treatment and the shortest (101±2.08cm) at 36 h. Untreated shoots were 138.5±0.28 cm long after 60 days of growth. The retarded growth rate may be due to the reduced rate of cell division that results from the physiological disturbance caused by colchicine (Swanson 1957). Similar observations of initial retardation of growth were also reported from ex vitro studies by earlier workers (Sikder and Jolly 1994).

Leaf characteristics The basal leaves of shoots that developed from colchicine treated buds were distorted while the upper newly formed leaves were small, thick and dark green with stronger venation. Such an increase in thickness could be due to an increase in cell size (Dwivedi et al. 1986). The length and breadth of the leaves were reduced in colchicine-induced tetraploid plants as compared to that of untreated plants (Fig. 2). The number of leaves that emerged after 60 days of growth also varied using the three different concentrations of colchicine.

Screening of autotetraploids The initial screening for autotetraploids was based on leaf, stomatal and pollen morphology. Further confirmation was by chromosome counts. No polyploids could be obtained in the seed treatment process, while Seedling treatment resulted in 1 autotetraploid plant at 0.5% concentration for two days treatment and 4 at three days of colchicine treatment. The

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of colchicine (%)</th>
<th>Duration (hours)</th>
<th>Survival of buds</th>
<th>Autotetraploid observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00</td>
<td>5</td>
<td>100±0.00</td>
<td>–</td>
</tr>
<tr>
<td>Seed treatment</td>
<td>0.1</td>
<td>5</td>
<td>96±0.52</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>5</td>
<td>91.5±0.99</td>
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<tr>
<td></td>
<td>0.5</td>
<td>5</td>
<td>89.1±1.36</td>
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<tr>
<td>Bud treatment</td>
<td>0.5</td>
<td>12</td>
<td>84±0.64</td>
<td>–</td>
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<tr>
<td></td>
<td>0.5</td>
<td>24</td>
<td>78±2.71</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>36</td>
<td>66±1.23</td>
<td>4</td>
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<thead>
<tr>
<th>Characteristics</th>
<th>Diploid plant (2n=26)</th>
<th>Autotetraploid plant (2n=52)</th>
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<tbody>
<tr>
<td>Plant height (cm)</td>
<td>140 ± 1.52</td>
<td>101± 2.08</td>
</tr>
<tr>
<td>No. of capsule/Plant</td>
<td>41.66±3.75</td>
<td>8.64±1.76</td>
</tr>
<tr>
<td>Size of pollen grains (μm)</td>
<td>38.00±1.48</td>
<td>51.2±0.86</td>
</tr>
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pollen grain size was $48.00 \pm 1.48 \mu m$ in diploids and $56 \pm 0.86 \mu m$ in autotetraploids (Table 2). In tetraploids the size of stomata was almost double as compared to those observed in diploids; while the frequency of stomata was lower. Sikder et al. (1986) used this technique to screen tetraploids after colchicine treatment of the apical bud of
Fig. 2. Sesame plants studied. A. Normal diploid (2n=26) and autotetraploids (4n=52) set of sesame. B. Leaf of normal diploid (2n) and autotetraploids (4n) plant. C. Normal fertile flower of diploid plant. D. Arrow showing sterile flower of autotetraploids plant.
Morus alba L. under ex vitro conditions. In the present study tetraploids also had a higher number of stomatal chloroplasts (almost double) than diploids. Cytological studies further confirmed the tetraploid status of induced autotetraploids. The meiotic chromosome number of diploid plants was, n=13 and that of tetraploid plants was, 4n=52 (Fig. 1). Regular distribution of 52 chromosomes at the metaphase plate of each plant derived from 0.5% colchicine concentration at 24 h and 36 h of treatments also revealed some mixoploids among the autotetraploids. Mixoploidy is a major problem in the recovery of autotetraploids.

**CONCLUSIONS**

Chromosome doubling induces an increase in cell size, a reduction in the rates of mitosis and meiosis, and may affect enzymatic activity per unit protein (although not necessarily in the same direction for all genes). These effects are generally species and often genotype-dependent. The ploidy manipulation approach could be immediately exploited for commercial cultivation of species for the production of high-value edible oil with enhanced productive efficiency, as well as to realize qualitative improvement per se. The present investigation revealed that treatment of the apical bud with 0.5% colchicine for three consecutive days was more effective than the other concentrations tested for the induction of autotetraploidy in sesame. The present study, therefore, represents a novel method for the large-scale induction of autotetraploids in sesame.

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**LITERATURE CITED**


