Cytogenetical and agronomical aspects of radiation induced marker trait mutants in sesame (Sesamum indicum L.)

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Abstract: Morphological mutants in sesame (Sesamum indicum L.; Family : Pedaliaceae) with distinctive marker traits namely leaf of narrow type, elongated, thick, ovate, ternate with long petiole and with white and pigmented flowers induced following X-ray and gamma-ray irradiations showed monogenic recessive inheritance. Control and mutants had 2n = 26 chromosomes always and the chromosomes formed bivalent (control: 12.93/cell, mutant: 12.26/cell; control 0.14/ cell; mutant 0.14/ cell to 1.48/ cell) which tended to form variable groups (3 to 10) in 46.81% to 65.20% meioocytes (8 group class being frequent). Univalent frequency/cell was relatively higher in long petiole (1.48/cell). Predominant chromosomal association noted among the plant types was 13 II (control: 92.96%; mutants: 50.00 % to 95.88%). Anaphase I segregation of chromosome was mostly equal (13/13) in the plant types (control: 100.0%, mutant: 98.80% - 100.00%). Pollen fertility was 83.50% in control and varied from 38.90% to 80.20% in the mutants. Pollen fertility and A1 chromosome separation was non-correlated. Analysis of quantitative parameters at M4 (true breeding plants were assessed by RBBD with three replications each) revealed that most of the mutants ( thick leaf, narrow leaf, elongated leaf) were beneficial for their direct selection, while the others may be exploited in cross breeding programme.

Keywords: Sesame, mutants, marker traits, cytogenetics and agronomical features.

Introduction
Improvement of crop plants through induction of mutation has been emphasized (Gaul, 1964; Brock, 1977; Datta & Biswas, 1985; Kharkwal, 2000; Datta & Rang, 2001; Mukherjee & Datta, 2006; Iqbal & Datta, 2007). A research programme on radiation induced mutagenesis has been initiated to induce genetic variations and to screen desirable ‘plant type’ mutations for efficient plant breeding in Sesame (Sesamum indicum L.; Family: Pedaliaceae), an oil yielding plant of commerce and 20 viable morphological mutants are screened (Chowdhury & Datta, 2008). This communication reports on the cytogenetical and agronomical aspects of some morphological marker mutants (related to leaf and pollen) induced following different doses of x-ray and gamma-ray irradiations. Marker traits are always useful in genetics and breeding as they are easily scorable and selectable in field condition.

Materials and Methods
Dry and filled seeds (moisture content: 9.56%) of Sesamum indicum L. var. B-67(obtained from Pulses and Oil Seed Research station, Berhampur, West Bengal;) highly adaptive to plains of West Bengal (Sengupta & Chatterjee, 1982) were treated with gamma-rays (doses : 50, 100, 200 and 300 Gy; source Co at the rate of 1.3 Gy per minute, irradiation at Saha Institute of Nuclear Physics, Salt Lake, Kolkata, West Bengal) and X - rays (doses: 50, 100, 200 and 300 Gy; source to distance 10 cm; at the rate of 20.16 Gy per minute; irradiation at CRIJAF, Nilganj, West Bengal). Control and treated seeds were sown (50 seeds in each lot) in the experimental field of Kalyani University (spacing of 15 cm between plants and 40 cm between lines) to raise M1 and subsequently M2 generation (plant to row) during rain fed seasons of 2005 and 2006 respectively. Macromutants were scored at M2. Selfed M2 plant types were used to raise M3 and subsequently M4 generations.

Several leaf and flower mutants recovered at M2 were compared with their respective control and ‘t’ test was performed whenever needed. Crossings were made between normal ( as female parent) and mutants (narrow leaf, elongated leaf, thick leaf, ovate leaf, ternate and long petiole; male parent) and F1 and F2 plants were raised. The F2 were used for estimating the segregating ratio of different leaf traits using the Chi-square (X²) test segregation patterns of white flower and pigmented flower mutants (colours confirmed by Horticultural Colour Chart I and II) were studied from selfed M2 mutants grown in M3 generation and the segregating ratios were confirmed by X²-test.

Meiotic analysis (PMC and pollen grains were stained in 2% aceto - carmine solution) was performed in control and mutant plant types (flower buds fixed from 2 to 3 plants of each category in 1:3 (v/v) acetic alcohol and preserved in 70% alcohol) from M2 and M3 plants. Photomicrographs were taken from temporary squash preparations.

Control and mutant types were compared for 10 different agronomic parameters (Table 1) by growing them in randomized block design at M4 both at irrigated (January to April) and rain fed kharif (May to August) seasons 2008 with three replications. Plot size was 3m X 1.5 m with 4 rows in each plot and each row was 250 cm long. Spacing was 30 cm between rows and 10 cm between plants. Five plants were randomly taken from each replication and a total of 15 plants were assessed for each plant type and the data obtained were statistically analyzed.

Seed protein was extracted following Osborne (1962) and estimated as per Lowry et. al. (1951). Oil was extracted in petroleum ether (60 - 80°C b. p. for 5 hours) by soxhlet apparatus from seeds dried in the sun (2 consecutive days, 5 hours).

Results and discussion
Morphology: Narrow leaf (narrow, oblong to lanceolate, entire margin; length: mutant - 15.5 cm ± 0.41, control - 13.7 cm ± 0.19, t = 1.68, df=28, p> 0.05; breadth: mutant -
Table 1. Records on quantitative traits in different plant types at M₂ of S. indicum in kharif and irrigated seasons

<table>
<thead>
<tr>
<th>Plant types</th>
<th>Attributes</th>
<th>Control</th>
<th>Narrow leaf</th>
<th>Elongated leaf</th>
<th>Thick leaf</th>
<th>Ovate leaf</th>
<th>Ternate</th>
<th>Long petiole</th>
<th>White flower</th>
<th>Pigmented flower</th>
<th>CB at 5% level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>K 113.4±1.3</td>
<td>K 60.0±1.3</td>
<td>K 83.2±0.3</td>
<td>K 185.7±2.7</td>
<td>K 67.2±1.5</td>
<td>K 74.3±1.0</td>
<td>K 85.5±0.8</td>
<td>K 67.4±0.6</td>
<td>K 98.3±2.7</td>
<td>K 3.1±1.7</td>
</tr>
<tr>
<td></td>
<td>No of primary brancher s/ plant</td>
<td>5.9±0.3</td>
<td>2.1±0.1</td>
<td>2.3±0.2</td>
<td>6.4±0.2</td>
<td>2.4±0.1</td>
<td>2.9±0.1</td>
<td>2.9±0.1</td>
<td>3.4±0.4</td>
<td>4.0±0.1</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td></td>
<td>Total brancher s/ plant</td>
<td>9.1±0.1</td>
<td>3.3±0.1</td>
<td>5.0±0.1</td>
<td>12.8±0.4</td>
<td>5.1±0.1</td>
<td>4.1±0.1</td>
<td>4.6±0.2</td>
<td>7.5±0.3</td>
<td>6.0±0.2</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td></td>
<td>Distance from base to 1 branch (cm)</td>
<td>25.5±2.7</td>
<td>21.3±0.7</td>
<td>25.1±1.5</td>
<td>69.1±1.3</td>
<td>20.6±0.3</td>
<td>21.7±0.2</td>
<td>26.8±0.9</td>
<td>18.6±0.3</td>
<td>34.1±3.8</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td></td>
<td>Capsule on the main axis</td>
<td>22.2±1.2</td>
<td>23.9±2.8</td>
<td>21.9±0.6</td>
<td>41.1±0.6</td>
<td>13.9±0.3</td>
<td>24.0±0.6</td>
<td>19.9±0.4</td>
<td>19.5±0.4</td>
<td>23.6±2.7</td>
<td>3.0±0.5</td>
</tr>
<tr>
<td></td>
<td>Total capsule/ plant</td>
<td>62.7±1.5</td>
<td>51.7±1.1</td>
<td>48.1±6.2</td>
<td>82.6±1.9</td>
<td>29.9±0.2</td>
<td>35.8±1.9</td>
<td>39.9±1.0</td>
<td>46.8±0.3</td>
<td>44.2±3.2</td>
<td>5.3±0.9</td>
</tr>
<tr>
<td></td>
<td>Capsule length (cm)</td>
<td>3.2±0.0</td>
<td>2.1±0.1</td>
<td>3.3±0.1</td>
<td>3.0±0.2</td>
<td>3.6±0.1</td>
<td>2.1±0.1</td>
<td>2.0±0.1</td>
<td>2.3±0.5</td>
<td>2.5±0.0</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td></td>
<td>Seed yield/ plant (gm)</td>
<td>6.6±0.03</td>
<td>7.2±0.2</td>
<td>6.8±0.6</td>
<td>7.9±0.1</td>
<td>5.3±0.1</td>
<td>6.6±1.0</td>
<td>4.0±1.0</td>
<td>3.6±1.0</td>
<td>6.0±0.5</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td></td>
<td>Seed protein content (%)</td>
<td>12.8±0.2</td>
<td>7.2±0.2</td>
<td>16.0±3.3</td>
<td>21.4±0.6</td>
<td>15.4±0.6</td>
<td>13.4±0.4</td>
<td>14.9±0.2</td>
<td>15.6±0.4</td>
<td>15.0±0.6</td>
<td>1.5±0.7</td>
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<tr>
<td></td>
<td>Seed fatty oil content (%)</td>
<td>34.4±0.8</td>
<td>30.5±0.3</td>
<td>33.4±1.7</td>
<td>27.6±0.4</td>
<td>32.9±0.8</td>
<td>32.4±0.3</td>
<td>33.4±0.5</td>
<td>37.4±0.4</td>
<td>37.6±1.3</td>
<td>1.87</td>
</tr>
</tbody>
</table>

6.13 cm ± 0.15, control - 11.06 cm ± 0.27, t = 7.62, df=28, p<0.001, area: mutant - 42.67 sq.cm ± 2.74, control - 87.2 sq.cm ± 4.19, t = 13.74, df = 28, p< 0.001), elongated leaf (length - 17.83cm ± 0.14, breadth - 11.1 cm ± 0.48, area - 106.67 sq.cm ± 4.25), thick leaf (leaves were leathery), ovate leaf (serrated margins, hairy on upper surface, short and thick petiole), ternate (three leaves per node and each leaf axil bearing three fruits, altered phyllotaxy opposite decussate to whorled), long petiole (length - 19.0cm to 22.0 cm in mutant compared to 7.0 cm to 9.0 cm in control; petioles more or less right angles to main axis), white flower (corolla tube and flap white [8/2], phlox purple [632 to 632], colour in flaps and white colour corolla tube in control and pigmented flower (mutant corolla tube - phlox purple [632 to 632], flap - philox purple [632 to 632]) mutant plant types were spotted at M2 (Figs. 1-9) in different doses of x-ray and gamma- ray irradiations (Chowdhury & Datta, 2008). Mary and Jayabalanan (1995) induced mutation affecting leaf morphology in sesame at M₂ following EMS treatments to seeds. Sengupta and Datta (2005) identified a narrow leaf mutant in sesame following nitrous acid and hydrogen peroxide treatments in different doses, and the mutant yielded higher number of capsule/plant on the main axis than control.

Inheritance of traits: F₂s raised from different set of crosses were made between normal and mutants were of normal phenotypes. F₂ segregation revealed that narrow leaf (normal 33, mutant 12 , total 45, χ² = 0.067 for 3:1 at df, p>0.75), elongated leaf (normal 44, mutant 12, total 56, χ² = 0.423 for 3:1 at df, p> 0.50), thick leaf (normal 86, mutant 27, total 113, χ² = 0.073 for 3:1 at df, p> 0.75), ovate leaf (normal 35, mutant 11, total 46, χ² = 0.029 for 3:1 at df, p> 0.80), ternate (normal 27, mutant 08, total 35, χ² = 0.085 for 3:1 at df, p> 0.75), long petiole (control 62, mutant 19, total 81, χ² = 0.103 for 3:1 at df, p> 0.70) traits were monogenic recessive to normal trait(s). M3 segregation of white flower (normal 08, mutant 03, total 11, χ² = 0.029 for 3:1 at df, p< 0.90) and pigmented flower (normal 21, mutant 06, total 27, χ² = 0.111 for 3:1 at df, p> 0.70) also suggested possible monogenic recessive inheritance of the flower colour traits. Most mutations in angiosperm are reported to be controlled by single pair of recessive alleles (Brock, 1971; Gaur & Gour, 1999).

Meiosis: Meiotic analysis revealed 2n = 26 chromosomes always and the chromosomes formed bivalents (control: 12.93 /cell; mutant: 12.26/ cell to 12.93/ cell) and univalents (control: 0.14/ cell; mutant 0.14/ cell to 1.48/ cell). Among the mutants, univalent frequency was higher in long petiole (1.48/ cell). Bivalents and univalents tended to form variable groups (3 to 10) in control (57.74% meiocytes) and in mutant plant types (46.81% to 65.20%). Predominant group class noted among the plant types was 8 (control: 28.17% cells, mutant: 17.02 % to 47.62% cells) and it was in conformity to earlier findings of Sengupta and Datta (2003). Predominant chromosomeal association noted among the plant types was 13 II (control: 92.96%; mutants: 50.00 % to 95.88%).

Average chromosome associations per cell at metaphase I of the plant types were recorded to be 12.93 II + 0.14 I in control, 12.89 II + 0.21 I in narrow leaf, 12.93 II + 0.14 I in elongated leaf and thick leaf, 12.90 II + 0.19 I in ovate
leaf, 12.87 II + 0.27 I in ternate, 12.26 II + 1.48 I in long petiole, 12.92 II + 0.16 I in white flower and 12.91 II + 0.18 I in pigmented flower. Compared to 100.00% cells with equal (13/13) anaphase I separation in control, the mutants had 98.80% to 100.00% cells. Pollen fertility studied in control was 83.50% and it varied from 38.90 % (white flower) to 80.20% (ternate). Pollen fertility and AI chromosome separation was non-correlated (r = 0.34, df 8, p>0.05). Analysis of cytogenetical parameters indicated that the mutants were possibly the outcome of genetical causes rather than cytological disturbances.

Agronomic traits: Results indicated (Table 1) that irrespective of seasons thick leaf mutant was most desirable plant type and found to possess superior agronomic traits like plant height, primary and total branches per plant, capsule on main axis, distance from base to first branching, total capsule per plant, seed yield and seed protein content than control. Compared to control, seed protein content also enhanced in narrow leaf, elongated leaf, white flower and pigmented flower mutants; while pigmented flower mutant plants were with increased seed fatty oil content. Capsule length also increased in pigmented flower mutant than control. Thus, these mutants correspond closely to the ideotype being looked for in the species and offer scope of their direct selection; while ovate leaf, ternate and long petiole plant types may be used as parents in crossing programmes for crop improvement effectively utilizing the marker trait(s). Leaf of thick type, ovate, elongated, ternate with long petiole mutants are reported first time for the species.

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