INDUCED MUTAGENESIS IN NIGELLA DAMASCENA L.

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For induction of desirable 'plant type' mutations in the ornamental Nigella damascena L. var. Miss jekyll blue (love-in-a-mist, family : Ranunculaceae), dry seeds (moisture content 8.67%) were treated with gamma-rays (5, 10 and 15kR from 60Co source) and EMS (0.25, 0.50 and 1.00%, 5h durations) and mutagenic responsiveness of the species, types (M₁ : 14 macromutant types, 5 non-viable; M₂ : 6 macromutants with combination traits) and frequency of induced macromutants and their cytogenetical behaviour have been ascertained and discussed.

Keywords: EMS; Floriculture; Gamma-rays; Induced mutagenesis; Macromutants; Nigella damascena.

Introduction
Nigella damascena L. (love-in-a-mist) is an erect annual herb (family : Ranunculaceae) commonly cultivated in temperate gardens throughout the world, often grown in Indian gardens for its pretty flowers and feathery foliage. The plant is a cut flower species and is also used as a pot plant and therefore possesses immense importance in floriculture, although new novel 'plant types' are lacking in the species to enhance its marketing potentiality. The methodology of induced mutation has been adopted in different ornamentals for developing new varieties of interest. With a view to it, present authors have undertaken a comprehensive research programme on induced mutagenesis in N. damascena and this communication describes the responsiveness of the species to gamma-rays and EMS (ethyl methane sulfonate), frequency and types of macromutants induced and their cytogenetical behaviour.

Materials and Methods
Seed samples (moisture content 8.67%) of Nigella damascena L. (cultivated variety Miss jekyll blue), the mother stock of which was obtained from Royal Botanic Garden, Kew, London (accession no. 0016287), were treated with different doses of gamma-rays (60Co source) and EMS (solution prepared in phosphate buffer 0.2M, pH 6.8, temperature 18°C ± 1°C) as cited in Table 1. Hundred seeds from each treatment along with control were sown immediately in the field to raise M₁ generation, while the rests were grown in petriplates (lined with moist filter papers) to assess germination and seedling growth (7 days from treatments). Biological damages like injury (seedling growth) and lethality (germination frequency) were estimated under uniform environmental conditions (18°C ± 1°C) as suggested by Konzak et al. Mitotic index and mitotic abnormalities were noted in control and in treated materials as was proposed earlier by Datta and Biswas. Seed yield (seed sterility was determined as per cent of control) was recorded from M₁ plants. Both viable and non-viable mutant plants (including chlorophyll mutations) were scored from M₁, mutagenized plant-population from germination to maturity. Colour of leaves, flowers and fruits of normal and mutant plants (of identical maturity) were confirmed from Horticultural Colour Chart I and II (1968).

Meiosis was performed in M₁ plants (3-5 randomly selected plants in each dose of treatments) and in macromutants (data of M₂ and M₁ macromutants pooled) in relation to controls (assessed at M₁, Table 1; M₂ and M₁, Table 3) from flower buds fixed in 1:3 (v/v) propionoalcohol and preserved in 70% alcohol. PMCs and pollen were stained in 1% propionocarmine and fully stained pollen grains were considered fertile. Photomicrographs were taken from temporary squash preparations.

Inheritance patterns of the mutant traits of the macromutants were only studied from selfed seeds of M₂ mutants sown at M₁, the segregation of mutant trait at M₁ into normal and mutant was assessed and χ²-square test analysis was performed to predict the inheritance pattern.

Results and Discussion
1. M₁ attributes : Estimation of different parameters are represented in Table 1.
   i. Lethality, injury and sterilities : Lethality, injury and seed sterility demonstrated dose dependent enhancement mostly (excepting : seed yield increased over control in 0.25%, 5h EMS). Injury was considerably higher among the employed doses (gamma-rays : 67.5% to 95.3%; EMS : 83.1% to 84.4%); while, lethality and seed sterility were maximum in 1%, 5h EMS. High sterility was also manifested in doses of gamma-rays (46.1% to 58.5%). LD₅₀ was ascertained (gamma-rays : between 10 and 15kR; EMS : between 0.5% and 1.0%) from the employed doses. Germinated seedlings in 15kR attaining a few millimeter of...
Table 1. Mutagenic responsiveness (M, attributes) in N. damascena.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Control</th>
<th>Gamma-rays (kR)</th>
<th>EMS (5h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Lethality (%)</td>
<td>-</td>
<td>8.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Injury (%)</td>
<td>-</td>
<td>67.5</td>
<td>84.1</td>
</tr>
<tr>
<td>Mitotic index (%)</td>
<td>10.2</td>
<td>(2510)</td>
<td>(2214)</td>
</tr>
<tr>
<td>Abnormally dividing cells</td>
<td>5.5</td>
<td>(256)</td>
<td>(213)</td>
</tr>
<tr>
<td>Spectrum of mitotic anomalies</td>
<td>4</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean chiasma / cell</td>
<td>8.14</td>
<td>8.10</td>
<td>7.82</td>
</tr>
<tr>
<td>Mean univalents / cell</td>
<td>0.36</td>
<td>0.47</td>
<td>0.42</td>
</tr>
<tr>
<td>Mean bivalents / cell</td>
<td>5.82</td>
<td>(50)</td>
<td>(547)</td>
</tr>
<tr>
<td>Mean quadrivalents / cell</td>
<td>-</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>Equal AI separation</td>
<td>96.83</td>
<td>(63)</td>
<td>(509)</td>
</tr>
<tr>
<td>Pollen sterility (%)</td>
<td>16.07</td>
<td>(112)</td>
<td>(2280)</td>
</tr>
<tr>
<td>Flower sterility (%)</td>
<td>37.3</td>
<td>53.1</td>
<td>46.4</td>
</tr>
<tr>
<td>Capsule sterility (%)</td>
<td>64.1</td>
<td>78.2</td>
<td>65.6</td>
</tr>
<tr>
<td>Seed sterility (%)</td>
<td>0.0</td>
<td>46.1</td>
<td>58.5</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate total number of cells / pollens scored; ** and *** significant at 0.05, 0.01 and 0.001 probability level.

growth turned brownish. No plants could be raised in field at 15kR. Control plants possessed 37.3% flower (flowers not transformed into capsule) and 64.1% capsule (abortive) sterilities and those sterilities enhanced in treatments (excepting: 0.25%EMS).

ii. Mitotic consequences : Mitotic index in control was 10.2 and it decreased in treatments, although the reduction was significant only in a few doses (15kR, 0.50% and 1.0 EMS). Control samples (2n=12 - Fig. 1) had clumped chromosomes, diplochromosomes, sticky bridges and laggards in 5.5% dividing cells (spectrum 4), the frequency of which enhanced in treatments (spectrum 3 to 10; Gamma-rays: 8 to 10; EMS: 3 to 5). EMS induced predominantly clumping and sticky behaviour of chromosomes and diplochromosomes as compared to breakages following gamma irradiations (rings, fragments - Fig. 4, bridges-Fig. 2-3 and micronuclei - Fig. 6). Spindle abnormalities like tripolar organization of chromosomes (Fig. 5) and formation of laggards (Fig.3) were also encountered in treated materials. Study of mitotic consequences in mutagen treated materials is an important aspect to assess responsiveness of the species to mutagens and forms an integral part of mutagen experiment.

iii. Meiosis : PMC squashes revealed 2n=12 chromosomes uniformly in M1 plants (Figs. 7-9). Mean chromosome association per cell at MI in control has been 5.82I + 0.36I and it varied from 0.11I + 5.54II + 0.47I to 0.15IV + 5.53III + 0.42I in irradiated materials and 5.79I + 0.42I to 5.82II + 0.36I in EMS treatments. Control had 2.28 ± 0.18 rings and 3.52 ± 0.16 rods per cell at MI and the frequency of ring bivalents decreased in treatments reflecting the effect in the number of chiasmata per nucleus as was evident from correlation values between the attributes (r = 0.82 at 5 DF; p<0.05).
Figs. 1-13. Mitotic (1-6) and meiotic (7-13) chromosomes in Nigella damascena. 1. Metaphase showing 2n=12 chromosomes. 2. Double bridge formation at anaphase. 3. Anaphase with bridge and laggards. 4. Fragment (→) at late metaphase. 5. Tripolarity at anaphase. 6. Resting cells showing micronuclei formation (→). 7-9. 6II at MI with ring and rod bivalents. 10. Group of meiocytes showing chromatin bodies of variable number and sizes. 11. Two variable sized meiocytes with differential chromatin content (a-18 chromatin bodies; b-2 chromatin masses). 12-13. Chromatin bodies forming chromosome like structures - 8II(1+3+3+1)+2I(→)+1 fragment (↔)- Fig. 12; 6II (two overlapped→)- Fig. 13.
### Table 2. Frequency (%) of macromutants at M₉.

<table>
<thead>
<tr>
<th>Doses</th>
<th>No. of plants scored</th>
<th>Non-viable</th>
<th>Macromutant plant types (%)</th>
<th>Viable</th>
<th>Total</th>
<th>Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Choloroxantha-I</td>
<td>Chloroxantha-II</td>
<td>Viridis</td>
<td>Small flower-I</td>
<td>Small flower-II</td>
</tr>
<tr>
<td>5kR</td>
<td>69</td>
<td>0.00</td>
<td>0.00</td>
<td>1.45</td>
<td>0.00</td>
<td>1.45</td>
</tr>
<tr>
<td>10kR</td>
<td>57</td>
<td>1.75</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>0.79</td>
<td>0.00</td>
<td>0.00</td>
<td>0.79</td>
<td>0.00</td>
</tr>
<tr>
<td>0.25%, 5h</td>
<td>299</td>
<td>0.00</td>
<td>0.33</td>
<td>1.00</td>
<td>0.00</td>
<td>0.67</td>
</tr>
<tr>
<td>0.50%, 5h</td>
<td>71</td>
<td>1.41</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1.00%, 5h</td>
<td>25</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>395</td>
<td>0.25</td>
<td>0.25</td>
<td>0.76</td>
<td>0.25</td>
<td>0.51</td>
</tr>
<tr>
<td>Grand Total</td>
<td>521</td>
<td>0.38</td>
<td>0.19</td>
<td>0.58</td>
<td>0.38</td>
<td>0.38</td>
</tr>
</tbody>
</table>
Table 3. Meiosis in control and in viable macromutants.

<table>
<thead>
<tr>
<th>Plant types</th>
<th>No. of PMCs assessed at MI</th>
<th>Mean / cell</th>
<th>Mean chiasma per cell±SE</th>
<th>No. of cells scored at AI</th>
<th>Balanced (6/6) A1 separation (%)</th>
<th>Total no. of pollen scored</th>
<th>Pollen fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120</td>
<td>5.88</td>
<td>0.25</td>
<td>8.2±0.2</td>
<td>74</td>
<td>91.9</td>
<td>498</td>
</tr>
<tr>
<td>Lax branching</td>
<td>48</td>
<td>5.96</td>
<td>0.08</td>
<td>8.3±0.2</td>
<td>54</td>
<td>81.5</td>
<td>359</td>
</tr>
<tr>
<td>Early flowering I</td>
<td>38</td>
<td>5.76</td>
<td>0.47</td>
<td>7.2±0.3</td>
<td>40</td>
<td>87.5</td>
<td>294</td>
</tr>
<tr>
<td>Early flowering II</td>
<td>38</td>
<td>5.53</td>
<td>0.95</td>
<td>8.2±0.3</td>
<td>28</td>
<td>71.4</td>
<td>486</td>
</tr>
<tr>
<td>Late flowering</td>
<td>99</td>
<td>5.88</td>
<td>0.24</td>
<td>7.6±0.1</td>
<td>36</td>
<td>83.3</td>
<td>480</td>
</tr>
<tr>
<td>Patchy flower</td>
<td>67</td>
<td>5.67</td>
<td>0.66</td>
<td>7.9±0.2</td>
<td>69</td>
<td>85.5</td>
<td>512</td>
</tr>
<tr>
<td>Deeply pigmented flower</td>
<td>71</td>
<td>5.86</td>
<td>0.28</td>
<td>7.7±0.1</td>
<td>57</td>
<td>89.5</td>
<td>350</td>
</tr>
<tr>
<td>Round fruit</td>
<td>123</td>
<td>5.59</td>
<td>0.83</td>
<td>7.6±0.1</td>
<td>110</td>
<td>72.7</td>
<td>356</td>
</tr>
<tr>
<td>Dark coloured fruit</td>
<td>53</td>
<td>5.79</td>
<td>0.42</td>
<td>7.8±0.1</td>
<td>110</td>
<td>84.0</td>
<td>712</td>
</tr>
<tr>
<td>Dark coloured round fruit</td>
<td>127</td>
<td>5.85</td>
<td>0.30</td>
<td>8.2±0.3</td>
<td>108</td>
<td>88.6</td>
<td>125</td>
</tr>
</tbody>
</table>

About 96.8% A1 cells had balanced (6/6) separation of chromosomes and it varied from 77.6% to 100.0% in treatments. Unequal separation (5/7) of chromosomes, laggards (1-2) and bridges were the abnormalities formed at AI. Pollen fertility in control plants was 83.9% and it ranged from 77.9% to 82.4% in treatments. Cytologically balanced A1 cells and pollen fertility were significantly correlated between them (r=0.82 at 5 DF; p<0.05) indicating that the variations noted in pollen fertility was the outcome of cytological consequences.

2. Types and frequency of macromutants: Macromutant types (total-14; non-viable-5) identified at M₁ (verified from M₂ segregating population) with their estimated frequencies in different mutagen treatments have been shown in Table 2. Maximum mutation frequency was noted at 1.0% EMS. Non-viable mutation frequency (including chlorophyll mutations) was found to vary from 1.45% (5KR) to 4.0% (1.0% EMS). Spectrum of macromutants ranged from 4 to 8 (gamma-rays: 6, EMS: 4-8). EMS seems to have higher potentiality than gamma-rays in inducing macromutation frequency and types. Frequency of patchy flower mutant was maximum (12.09%) when assessed over the mutagen treated population.

The types of macromutants are chloroxantha I (colour- Agathia Green 60/₁, had thick leaves and died within 20-32 days from emergence), chloroxantha II (Pea Green 61/₁, dissected thin pinnae, total chlorophyll content - 0.692 mg/gm of tissue as compared to 1.51 mg/gm of tissue in control, dried up at flowering stage), viridis (plants died at cotyledonal leaf stage-7-10 days from emergence), small flower I (semi-dwarf-27.5 to 32.0 cm compared to 40.3cm ± 1.4 in controls; flower size : 2.9 cm ± 0.1 x 2.9 cm ± 0.1 as compared to 3.6 cm ± 0.2 x 3.5 cm ± 0.2 in control plants, late flowering - 160d to 170d from sowing as compared to 121d to 132d in controls, mutant plant types dried up at flowering stage), small flower II (SF1 and SF2 had similar morphological characteristics like dwariness - 23.5 cm and 27.0 cm; small sized flowers - 3.0 cm ± 0.1 x 3.0 cm ± 0.1, 2.7 cm ± 0.1 x 2.7 cm ± 0.1 but differed conspicuously in their meiotic chromosome behaviour - SF1 had an average of 5.7II + 0.7I/II - 2n = 12 with balanced A1 chromosome separation mostly- 79.1% as compared to highly unstable male meiosis in SF2), lax branching (lax natured branches forming 75o to 85o angle of divergence with main axis compared to 40o to 45o in controls), early flowering I (appeared in 10KR gamma-rays and 0.5% EMS only, flowering- 110d to 120d from sowing; control : 121d to 132d), early flowering II (early flowering trait was concomitantly associated with lax branching, patchy flower and dark coloured fruits), late flowering (flowering- 149d to 152d from sowing), patchy flower (irregular patches of Moorish Blue 39/₁, colour in petaloid sepalas), deeply pigmented flower (Moorish Blue 39/₁, colour compared to French Blue 43/₁, colour in control flowers; mutant trait
was in concomitant association with late flowering and
dark coloured fruit traits), round fruit (round shaped fruits
compared to globular-oblond fruits in controls), dark
coloured fruit (colour uniform throughout the fruits - Pansy
Violet colour 33 along the sutures only in control fruits) and
dark coloured round fruit (spotted only in 0.25%, 5h EMS).

3. Cytogenetic analysis of macromutants

i. Inheritance patterns: Selfed M2 mutant seeds sown at
M1 segregated into normal and mutant plants (1DF) to a
close fit of 1:1 (lax branching : normal-28, mutant-26, \( \chi^2 \)
0.074, p value 0.7-0.8; early flowering I: normal-22, mutant-
20, \( \chi^2 \)-0.095, p value 0.7-0.8; late flowering : normal-11,
mutant-10, \( \chi^2 \)-0.048, p value 0.7-0.8; patchy flower : normal-
11, mutant-10, \( \chi^2 \)-0.48, p value 0.7-0.8; round fruit : normal
-31, mutant-29, \( \chi^2 \)-0.067, p value 0.7-0.8; dark coloured
fruit : normal-21, mutant-19, \( \chi^2 \)-0.10, p value 0.7-0.8) and
7: (early flowering II : normal-19, mutant -12, \( \chi^2 \)-0.319, p
value 0.5-0.6 and dark coloured round fruit : normal-10,
mutant-8, \( \chi^2 \)-0.004, p value 0.95) ratios indicating possible
monogenic (1:1) and digenic (9:7) mode of inheritance
patterns. Thirty selfed seeds from deeply pigmented
flower mutant sown at M1 yielded only 5 plants, of which 4
segregated to normal and 1 mutant.

ii. Meiosis: Meiosis in macromutants in relation to control
revealed 2n=12 chromosomes always and the chromosomes
formed bivalents and univalents at MI. Univalent formation
has been found to be relatively higher in round fruit, early
flowering II and patchy flower mutants. Mean chiasma per
cell was 8.2±0.2 in control and it varied from 7.2±0.3 to 8.3
±0.2 in mutant plants. About 91.9% AI cells in control had
balanced segregation of chromosomes (rests formed 5-1-6
separation only) with an average pollen fertility of 89.8%.
In mutants, frequency of balanced AI cells and pollen
fertility varied from 71.4-89.5% and 53.5-96.6% respectively.
Laggard(s) (1-4) and unequal separation of chromosomes
(5/7 and 5-1-6) were uniformly found in the mutants
(Table 3).

4. Macromutants spotted at M1: Progenies of M2 lines (M2
seeds were bulked from each treatment other than the
macromutants and 100 seeds from each lot were sown in
line at M1) gave rise to some unusual mutants showing
combination of M1 macromutant traits mostly, like dark
coloured fruit with lax branching and round fruit (10kR-
1 plant), round fruit with patchy flower and dark coloured
fruit (0.25% EMS - 1 plant), late flowering with round fruit
(0.25% - 1 plant) and with solitary flower (0.25% - 1 plant)
and dark coloured fruit with Patchy flower (0.50% - 1
plant) and with crumpled fruit (0.50% - 1 plant). Excepting
crumpled fruit mutant, all the other mutants were viable
and appeared at M1 with same combination of traits but in
very low frequencies (2.78% to 6.52%). The mutants had
normal (2n=12) chromosome behaviour.

The crumpled fruit mutant had disturbed meiosis and
and fromed unequal sized irregularly shaped chromatin
masses of differential condensation varying from 1 to 22
per cell (Figs. 10-11). The chromatin masses were
agglutinated and showed fuzzy and distorted appearances.
Unequal sized meiocytes with differential chromatin
contents were also evidenced (Fig. 11). Chromatin bodies
forming bivalent like structures (Figs. 12-13) were observed
in a few meiocytes (6II like structure in 6 cells, 5II + 2I like
structure in 4 cells and 5II + 2I+1 fragment found in 2 cells).
All cells were distorted in appearances and rarely
observed.

Present investigation reveals that the ‘plant type’
raised in N. damascena are genic rather than cytological.
Patchy flower, deeply pigmented flower and mutants with
combination of floral trait(s) may be promising for
ornamental values. Further, fruit mutants may add
decorative values to the species. The early flowering, late
flowering, solitary flower and lax branching mutants may
serve as germplasm resources in developing desirable
recombinant genotypes.

References
1. Encyclopedia Britannica - About Britannica.com /
Comments & Questions / Company Information /
2. Wealth of India 1966, A dictionary of Indian raw
materials and industrial products. CSIR Publications,
New Delhi. 7(N-P) 63-65.
110-111.
4. Abraham V and Desai B M 1977, Radiation induced
variegation mutants in Bougainville. Curr. Sci. 46(10)
351-352.
5. Broertjes C 1969, Mutation breeding of Streptocarpus.
Euphytica. 18:33-339.
flower mutant in Hibiscus cv. ‘Alipur Beauty’. J.
Biol. 23(2) 114-116.
8. Datta S K 1996, Effect of gamma irradiation on mutant
genotypes : Chrysanthemum cultivar ‘D-5’ and its
mutants. J. Indian Bot. Soc. 75(1-2) 133-134.
Efficient chemical mutagenesis. In : The use of
10. Datta A K and Biswas A K 1981, EMS induced mitotic
aberrations in Nigella sativa L. and N. damascena L.
Cell Chro. News Letter 41-2