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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 ^{60}Co source) and EMS (0.25, 0.50 and 1.00%, 5h durations) and mutagenic responsiveness of the species, types (M_2 : 14 macromutant types, 5 non-viable; M_3 : 6 macromutants with combination traints) 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[1], often grown in Indian gardens for its pretty flowers and feathery foliage[2]. The plant is a cut flower species and is also used as pot plant and therefore possesses immense importance in floriculture[3], 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[4-8]. 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 form Royal Botanic Garden, Kew, London (accession no. 0016287), were treated with different doses of gamma-rays (^{60}Cobalt 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 were treated in each lot. Fifty seeds from each treatment along with control were sown immediately in the field to raise M_1 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*[9]. Mitotic index and mitotic abnormalities were noted in control and in treated materials as was proposed earlier by Datta and Biswas[10]. Seed yield (seed sterility was determined as per cent of control) was recorded from M_1 plants. Both viable and non-viable mutant plants (including chlorophyll mutations) were scored from M_2 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 M_1 plants (3-5 randomly selected plants in each dose of treatments) and in macromutants (data of M_2 and M_3 macromutants pooled) in relation to controls (assessed at M_1-Table 1; M_2 and M_3-Table 3) from flower buds fixed in 1 :3 (v/v) propionoalcohol and preserved in 70% alcohol. PMCs and pollens 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 M_2 mutants sown at M_3. the segregation of mutant trait at M_3 into normal and mutant was assessed and χ^2-square test analysis was performed to predict the inheritance pattern.

Results and Discussion

1. *M_1 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%). LD_{50} 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_1 attributes) in *N. damascena.*

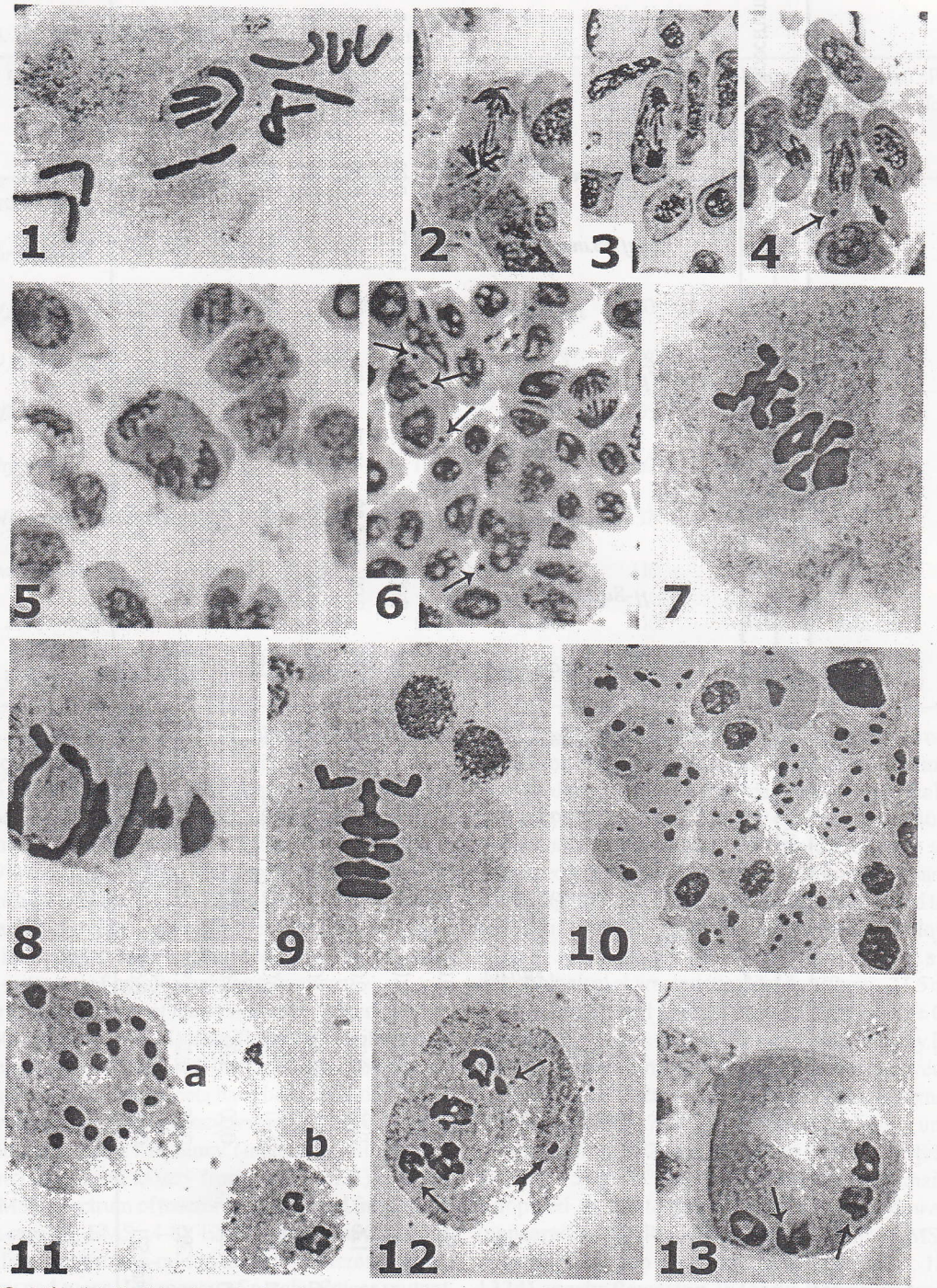
Attributes	Control	Gamma-rays (kR)			EMS (5h)		
		5	10	15	0.25%	0.50%	1.00%
Lethality (%)	-	8.0	10.0	66.0	44.0	48.0	56.0
Injury (%)	-	67.5	84.1	95.3	83.1	83.8	84.4
Mitotic index (%)	10.2 (2510)	9.6 (2214)	9.3 (2758)	5.4*** (1889)	9.7 (1123)	8.3* (1866)	6.8** (1392)
Abnormally dividing cells (%)	5.5 (256)	21.1 (213)	32.7 (257)	64.7 (102)	44.0 (109)	45.2 (155)	40.0 (95)
Spectrum of mitotic anomalies	4	10	10	8	5	5	3
Mean chiasma / cell	8.14	8.10	7.82	-	7.69	7.82	8.01
Mean univalents / cell	0.36	0.47	0.42	-	0.42	0.36	0.36
Mean bivalents / cell	5.82 (50)	5.54 (547)	5.53 (162)	-	5.79 (135)	5.82 (84)	5.82 (85)
Mean quadrivalents/cell	-	0.11	0.15	-	-	-	-
Equal AI separation	96.83 (63)	77.60 (509)	87.29 (115)	-	92.39 (92)	93.55 (62)	100.00 (49)
Pollen sterility (%)	16.07 (112)	22.15 (2280)	19.76 (1225)	-	17.61 (159)	19.35 (1240)	18.48 (552)
Flower sterility (%)	37.3	53.1	46.4	-	28.4	37.7	40.4
Capsule sterility (%)	64.1	78.2	65.6	-	52.1	65.7	73.5
Seed sterility (%)	0.0	46.1	58.5	-	4.1	28.5	78.0

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% capule (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 (specturm 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-Figs. 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 M_1 plants (Figs. 7-9). Mean chromosome association per cell at MI in control has been 5.82II + 0.36I and it varied from 0.11IV + 5.54II + 0.47I to 0.15IV + 5.53II + 0.42I in irradiated materials and 5.79II +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 M_2.

Doses		No. of plants scored	Macromutant plant types (%)														Total	Spectrum
			Non-viable						Viable									
			Chloroxantha-I	*Chloroxantha-II*	*Viridis*	*Small flower-I*	*Small flower-II*	*Lax branching*	*Early flowering-I*	*Early flowering-II*	*Late flowering*	*Patchy flower*	*Deeply pigmented flower*	*Round fruit*	*Dark coloured fruit*	*Dark coloured round fruit*		
Gamma - rays	5kR	69	0.00	0.00	0.00	1.45	0.00	1.45	0.00	0.00	1.45	8.70	2.90	0.00	5.80	0.00	23.19	6
	10kR	57	1.75	0.00	0.00	0.00	0.00	1.75	1.75	0.00	1.75	12.28	0.00	0.00	10.53	0.00	31.58	6
	Total	126	0.79	0.00	0.00	0.79	0.00	2.38	0.79	0.00	2.38	10.32	1.59	0.00	7.94	0.00	26.98	8
EMS	0.25%, 5h	299	0.00	0.33	1.00	0.00	0.67	1.00	0.00	0.00	0.00	13.71	0.00	2.01	3.34	0.67	22.74	8
	0.50%, 5h	71	1.41	0.00	0.00	0.00	0.00	7.04	4.23	4.23	0.00	7.04	0.00	0.00	2.82	0.00	26.76	6
	1.00%, 5h	25	0.00	0.00	0.00	4.00	0.00	0.00	0.00	0.00	4.00	16.00	0.00	0.00	16.00	0.00	40.00	4
	Total	395	0.25	0.25	0.76	0.25	0.51	2.03	0.78	0.78	0.25	12.66	0.00	1.52	4.05	0.51	24.56	13
	Grand Total	521	0.38	0.19	0.58	0.38	0.38	2.11	0.77	0.58	0.77	12.09	0.38	1.15	4.99	0.38	25.14	14

Table 3. Meiosis in control and in viable macromutants.

Plant types	No. of PMCs assessed at MI	Mean / cell		Mean chiasma per cell±SE	No. of cells scored at AI	Balanced (6/6) AI separation (%)	Total no. of pollens scored	Pollen fertility (%)
		II	I					
Control	120	5.88	0.25	8.2±0.2	74	91.9	498	89.8
Lax branching	48	5.96	0.08	8.3±0.2	54	81.5	359	53.5
Early floweing I	38	5.76	0.47	7.2±0.3	40	87.5	294	95.6
Early flowering II	38	5.53	0.95	8.2±0.3	28	71.4	456	62.9
Late flowering	99	5.88	0.24	7.6±0.1	36	83.3	480	73.6
Patchy flower	67	5.67	0.66	7.9±0.2	69	85.5	512	80.0
Deeply pigmented flower	71	5.86	0.28	7.7±0.1	57	89.5	350	61.4
Round fruit	123	5.59	0.83	7.6±0.1	110	72.7	356	76.4
Dark coloured fruit	53	5.79	0.42	7.8±0.1	110	84.0	712	82.6
Dark coloured round fruit	127	5.85	0.30	8.2±0.3	105	88.6	125	69.6

About 96.8% AI 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 AI 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 M_2 (verified from M_3 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* $60/_2$, 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 colyledonary 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 (SF 1 and SF2 had similar morphological characteristics like dwarfness - 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/cell - 2n = 12 with balanced AI chromosome separaton mostly- 79.1% as compared to highly unstable male meiosis in SF2), *lax branching* (lax natured branches forming 75° to 85° angle of divergence with main axis compared to 40° to 45° 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* $_7 39/_1$ colour in petalloid sepals), *deeply pigmented flower (Moorish Blue* $_7 39/_1$ colour compared to *French Blue* $43/_1$ colour in control flowers; mutant trait

was in concomitant association with late floweriing and dark coloured fruit traits), *round fruit* (round shaped fruits compared to globular-oblong fruits in controls), *dark coloured fruit* (colour uniform throughout the fruits - *Pansy Violet* colour $_0$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 M_2 mutant seeds sown at M_3 segregated into normal and mutant plants (1 DF) to a close fit of 1:1 (*lax branchng :* normal-28, mutant-26, χ^2-0.074, p value 0.7-0.8; *early floweing* I : normal-22, mutant-20, χ^2-0.095, p value 0.7-0.8; *late flowering :* normal-11, mutant-10, χ^2- 0.048, p value 0.7-0.8; *patchy flower* : normal-11, mutant-10, χ^2-0.48, p value 0.7-0.8; *round fruit* : normal -31, mutant-29, χ^2-0.067, p value 0.7-0.8; *dark coloured fruit* : normal-21, mutant-19, χ^2-0.10, p value 0.7-0.8) and 9 : 7 *(early flowering* II : normal-19, mutant -12, χ^2-0.319, p value 0.5-0.6 and *dark coloured round fruit* : normal-10, mutant-8, χ^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 M_3 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 53.5-95.6% 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 M_3* : Progenies of M_2 lines (M_2 seeds were bulked from each treatment other than the macromutants and 100 seeds from each lot were sown in line at M_3) gave rise to some unusual mutants showing combination of M_2 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 M_4 with same combination of traits but in very low frequencies (2.78% to 6.52%). The mutants had normal (2n=12) chomosome behaviour.

The *crumpled fruit* mutant had disturbed meiosis 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). AI and AII 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 / Advertising Sales Kit © 1999-2000 Britannica.com Inc.
2. Wealth of India 1966, A dictionary of Indian raw materilas and industrial products. CSIR Publications, New Delhi. **7(N-P)** 63-65.
3. Swarup V 1967, Garden Flowers. National Book Trust. 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.
6. Banerji B K and Datta S K 1986, Induction of single flower mutant in *Hibiscus* cv. 'Alipur Beauty'. *J. Nuclear Agric. Biol.* **15(4)** 237-240.
7. Datta S K and Banerji B K 1994, "Mahara Variegata" - A new mutant of bougainvillea. *J. Nuclear Agric. 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.
9. Konzak C F, Nilan A, Wagner J and Foster R J 1965, Efficient chemical mutagenesis. In : The use of mutations in plant breeding. *Rad. Bot.* **5** (Suppl) 49-70.
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* **4** 1-2