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In-vivo and *in-vitro* mutagenesis in marigold (*Tagetes erecta*) using ^{60}Co gamma rays

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ABSTRACT

This study was conducted to induce novelty in marigold cultivar Pusa Narangi Gainda through gamma irradiation via *in vivo* and *in vitro* approaches. The seedlings grown under *in vivo* and the proliferated cultures under *in vitro*, were exposed to different doses of ^{60}Co gamma rays (5, 10, 15, 20, 25, 30, 35, 40 Gy). 20 Gy and 15 Gy dose was identified as LD_{50} value for *in vivo* and *in vitro* conditions (53.24 % and 52.55 % plant survival), respectively. There were morphological variations in between irradiated and non irradiated plants. Treatment with 20 Gy induced early flower bud (49, 18 days) compared to control (55.56 days) under *in vivo* study. Under *in vitro* condition, the flower color changed to yellow (yellow 10 YR) compared to the control (orange 5 YR) at 15 Gy. In M_1 generation, two mutants in flower form (m1 and m3), one in colour (m4) and another one in flower earliness (m2) were isolated under *in vivo* mutation, whereas, five mutants in flower colour (vm1, vm2, vm5, vm6 and vm7) and two in flower form (vm3 and vm4) were isolated in irradiated plants which were quite distinct compared to non-irradiated. Variability caused by induced mutations need not to be essentially different from variability caused by spontaneous mutation during evolution, therefore, it is necessary to carry on the M_2 generation. The mutants which were selected from the M_1 generation were selfed to raise the M_2 generation. The study revealed six putative mutants (pm1, pm2, pm3, pm4, pm5 and pm6), which could successfully maintain their distinct traits. Among them, pm[3] produced early flowering in 47.89 days and pm4 gave light orange coloured flower (orange 10 YR).

Key words: Gamma rays, *In vivo*, *In vitro*, Marigold, Mutation

Genetic variability is an important parameter for plant breeders in any conventional crop improvement program (Maluszynski 1995). Therefore, plant breeders successfully recombined the characters from cultivated crop germplasm and related wild species by sexual hybridization; however, it takes several years to develop a new genotype (Arunyanart and Soontronyatara 2002). Hence, it is always appreciated to look for new technologies, which could be combined with conventional breeding method (Yamaguchi *et al.* 2008). Mutation-assisted breeding has played a vital role in inducing novelties in the number of economic traits and an effective method to induce variability within a short span of time, especially in vegetatively propagated crops like rose, gladiolus, bougainvillea etc. (Arunyanart and Soontronyatara 2002).

Marigold (*Tagetes erecta* L.), family Asteraceae, is a native of South and Central America, It can be propagated by seeds as well as cutting, therefore, mutation-assisted breeding can be exploited in this crop as the traits could be fixed easily like any other vegetatively propagated crops. There is a great scope for using mutagenesis in marigold for induction of mutants with novel traits in popular varieties. The variations created through induced mutations could be exploited efficiently under *in vivo* as well as *in vitro* conditions. Moreover, mutation breeding combined with tissue culture has proved more efficient over conventional mutation breeding due to several merits i.e. controlled environment which provides ideal conditions for survival of mutated cells or tissues, higher recovery of induced mutants and isolation of chimeral branches which consumes more time and is risky when multiplied under *in vivo* condition. Selection of appropriate mutagen and isolation of the mutants are difficult, but ^{60}Co gamma ray has proved to be an important in mutagen in many floricultural crops (Bhatt 1990). After mutagenesis, most of mutations are found in M_1 generation may be recessive or chimeric. Hence, it is important to produce M_2 generation for phenotypic screening purposes (Maple and Moller 2007). In the M_2 or later generations the populations get genetically stabilized. Besides, in the M_1 generation, the mutants will be heterozygous and only the dominant traits

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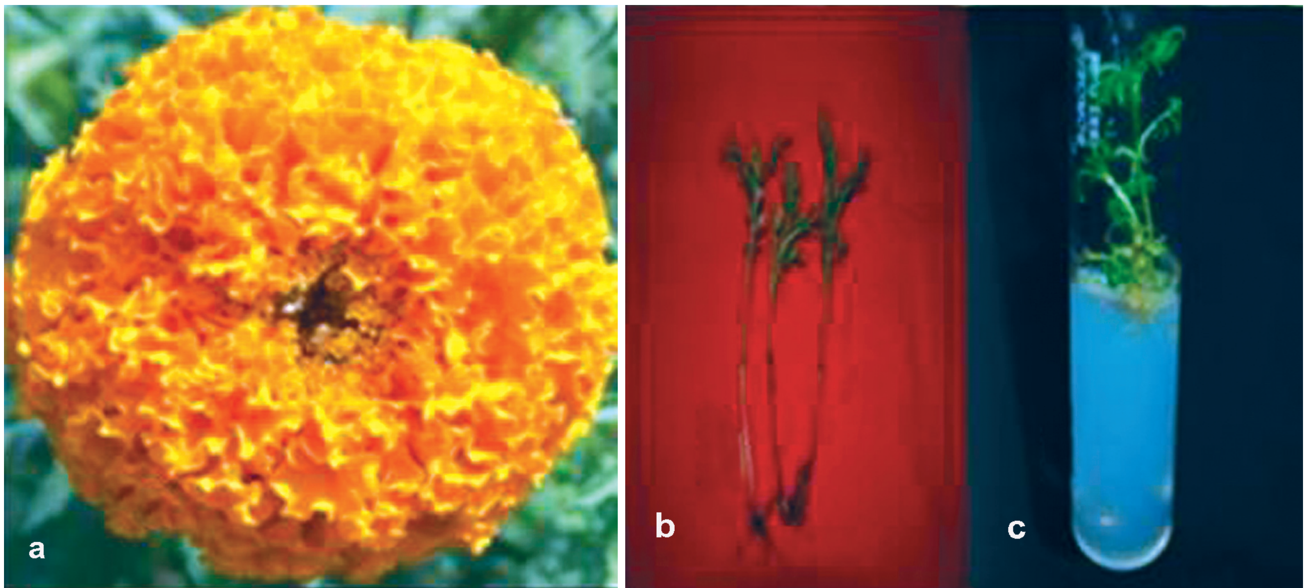

Fig 1 Materials for mutation breeding a) Parent cultivar Pusa Narangi Gainda, b) *in vivo* raised seedlings, c) *in vitro* raised proliferated culture.

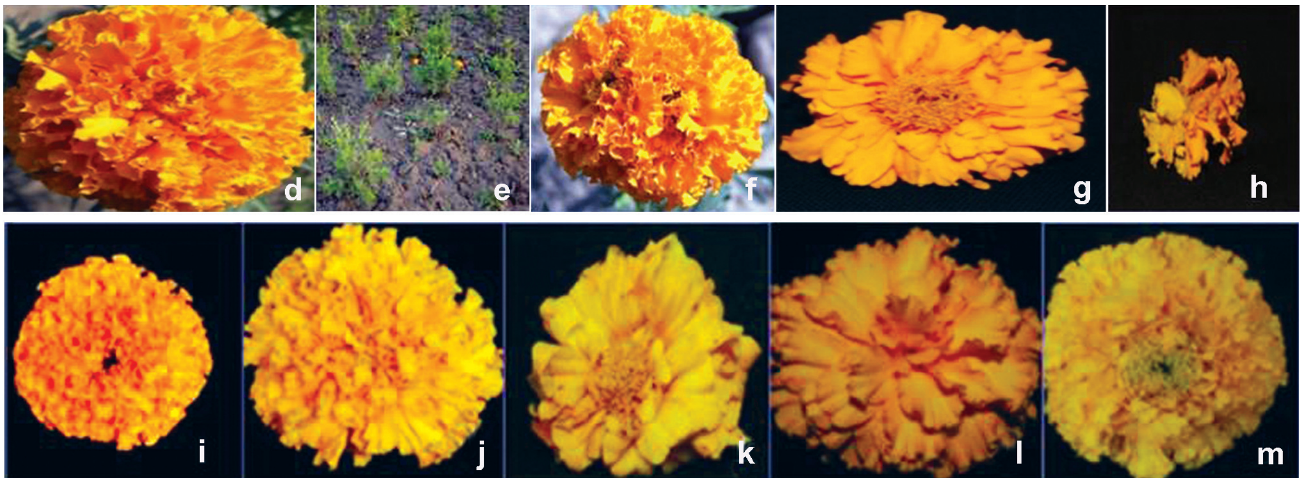

Fig 2 Variants (*in vivo* and *in vitro*) derived in M_1 generation d) m1,e) m2, f) m3, g) m4, h) vm1, i) vm3, j) vm4, k) vm5, l) vm6, m) vm7.

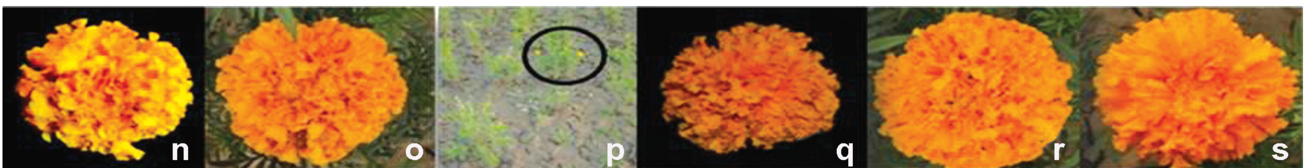

Fig 3 Putative mutants derived in M_2: n) pm1, o) pm2, p) pm3, q) pm4, r) pm5, s) pm6.

with respect to the original genotype or wild type would be expressed (Leyser 2000).

Keeping above aspects in view, the present investigation is focused on induced variability in marigold under *in vitro* and *in vivo* approach using gamma rays as mutagen during 2008-11 at IARI, New Delhi.

MATERIALS AND METHODS

The study was conducted consecutively for 3 years (2008-09 to 2010-11) at farm and Central Tissue culture Laboratory, NRCPB, IARI, New Delhi. long healthy 12-15 cm seedlings (Fig 1b) from nursery and proliferated cultures (Fig 1c) on MS Medium were subjected to an acute exposure of 0, 5, 10, 15, 25, 30, 35 and 40 Gy of ^{60}Co gamma ray doses at NRL, IARI, New Delhi. The treated seedlings were directly transplanted at 30×30 cm distance in the field with three replications. For *in-vitro* study, after irradiation plantlets were sub-cultured twice in the proliferation medium supplemented with BAP (3.0 mg/l) and NAA (0.2 mg/l). Then elongated shoots were transferred individually in culture vessels containing half-strength of MS medium fortified with NAA (0.5 mg/l) and IBA (0.5 mg/l) for

Table 1 Effect of gamma irradiation on vegetative and floral characteristics in *in vivo* and *in vitro* (after acclimatization) raised plants in M_1

Treatment (Gy)	Plant survival (%)		No. of primary branches/explant		No. of secondary branches/explant		Abnormalities in vegetative parts		Abnormality (%)		Days to bud initiation		Days to flowering		No. of flowers		Flower diameter (cm)	
	In vivo	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*
Control (Not irradiated	86.54 (68.44)	68.69 (55.92)	8.03	8.33	8.45	10.24	Normal	Normal	0.00 (0.00)	0.00 (0.00)	55.40	58.45	70.45	74.92	53.43	40.56	5.55	5.45
5.0	73.64 (59.08)	63.24 (52.65)	8.60	8.78	8.09	8.56	Normal	Normal	0.00 (0.00)	0.00 (0.00)	55.45	61.67	72.45	75.23	50.23	35.18	5.45	5.32
10.0	70.95 (57.35)	61.76 (51.77)	7.10	5.15	7.85	6.78	Normal	Smaller leaves, bushy plant	0.00 (0.00)	15.34 (23.03)	59.65	62.32	78.94	77.34	35.24	18.83	5.15	4.09
15.0	67.45 (55.12)	52.12 (46.20)	6.06	3.50	4.35	3.67	Leaves small, a few variegated leaves	Leaves small, a shorter inter-node	3.15 (22.96)	19.24 (25.99)	63.34	63.33	80.56	78.34	19.45	10.67	4.35	3.16
20.0	53.24 (46.78)	28.90 (32.52)	1.93	1.35	2.67	1.67	Leaves thinner, shorter internode, joining of buds	Dwarfing of plants, getting purple leaves and most of them died	15.04 (25.89)	25.67 (30.40)	59.18	64.45	79.30	79.47	6.45	4.59	3.37	3.08
25.0	34.38 (34.79)		1.56		2.34		Leaves thinner shorter internode		18.90 (26.49)		57.56		78.87		4.34		3.02	
CD (P=0.05)	9.50	6.85	0.91	0.84	1.04	1.11			2.27	3.18	2.35	3.56	4.64	NS	2.19	1.94	0.54	0.65

(*Arc Sin √% transformed data).

rhyzogenesis. *In vitro* rooted plantlets were further carefully removed from flasks, washed thoroughly, dipped in carbendazim® (0.1%) (BASF) for few seconds and acclimatized in glass jars with polypropyline lids (Kasablanka, Mumbai) filled with peat, vermiculite and agropeat (2:1:1) supplemented with one quarter strength of MS medium. The plantlets were kept in culture room before transferring to greenhouse for acclimatization at 25±1^{0}C under fluorescent white light (47 μmol/m^2/s) at a photoperiod of 16/8 hr light and dark cycles. The plants were transferred under full sunlight and a comparative study was made on *in vitro* and *in vivo* grown irradiated plants in M_1.

Selfed seeds of four variants derived by *in vivo* mutation (m1, m2, m3 and m4) and seven variant of *in vitro* mutation (vm1, vm2, vm3, vm4, vm5, vm6 and vm7) with their respective control were harvested separately. The seeds were then grown in nursery and finally transplanted in main field at 30×30 cm distance in three replications separately for raising M_2 generation.

The observations were taken at weekly intervals and finally data were analyzed employing completely randomized design (CRD). The treatment means were separated using Fisher's Critical Difference (α = 0.05). The percentage data were subjected to arc sin √% transformation before ANOVA.

RESULTS AND DISCUSSION

Survival (%)

The sprouting and survival of plant were found to be linearly decreased with increase in gamma ray doses. The highest explant survival (86. 54 and 68.69 %) was observed in control for *in vivo* and *in vitro,* respectivly, followed by 5 Gy (73.64 and 63.24 %) (Table 1). The decrease in survival of explant can be attributed to the drop in the level of auxin concentration and chromosomal aberrations caused by gamma irradiations (Kumar 2002, Yamaguchi *et al*. 2008). The LD_{50} dose for bud sprouting and survival of irradiated axillary bud explants for cv. Pusa Narangi Gainda was estimated at 20 Gy with 53.24% survival for *in vivo* condition. *In vitro* proliferated cultures are very delicate and thus lower doses are shown to give more mortality (Kuksova *et al*. 1997).

Vegetative growth

The maximum plant height (75.89 and 55.67 cm) was found in control, i.e. without gamma rays treatment in both the conditions *in vivo* and *in vitro* followed by 5 Gy (69.45 and 45.56 cm), while, the minimum height (20.84 and 18.85 cm) was recorded at 25 and 20 Gy in *in vivo* and *in vitro* condition respectively (Table 1). Inhibitory effect of gamma irradiation was seen by Lee *et al.* (1997).

Maximum number of primary branches (8.60 and 8.78) were observed at 5 Gy dose followed by control (8.03 and 8.33), in both conditions *in vivo* and *in vitro,* respectively. The maximum number of secondary shoots were seen in control (8.45 and 10.24) followed by 5 Gy (8.09 and 8.56) in *in vivo* and *in vitro* conditions, respectively (Table 1). Inhibitory effect of gamma irradiation was observed by many workers (Kasumi *et al*. 2001).

Per cent vegetative leaf abnormalities with respect to abnormal leaf shape, leaf lamina, fused leaves, albinism or leaves with less chlorophyll, variegated leaves with stunted growth etc. were noticed at higher doses of gamma rays. Such types of abnormalities were detected in the early stages of growth after irradiation treatment, which were usually recoverable later on. Foliar abnormalities may be due to inhibitory effect of mutagen on cell division and cell proliferation. Formation of small leaf was the most common deformity in the genotype (Roy *et al.* 2004) through mutation breeding.

Floral development

Under *in vivo* condition, i.e. increasing the dose from 5 (57.45 days), 10 (59.65 days) and 15 Gy (63.34 days) over the control (55.40 days) constantly increased the days to flower bud initiation. Stimulatory effects of gamma irradiation on bud sprouting was recorded in this experiment which corroborates the earlier findings (Benetka 1985) whereas deleterious effect of gamma rays at higher doses was observed by Singh *et al.* (1999). In *in vitro* condition

Table 2 Isolation of *in vivo* and *in vitro* raised variants in M_1 generation

In vivo derived variants		
Variants	Derived from	Characters
m1	10 Gy	Larger flower diameter (8.2 cm) than the parent cultivar Pusa Narangi Gainda (5.5 cm)
m2	20 Gy	Earlier flower bud appearance by 7 days (on 48 days after transplanting) as compared to control (55.40 days days after transplanting)
m3	20 Gy	Larger sized, compact and joined bloom (9.3 cm dia.) than control.
m4	20 Gy	Yellow flower (yellow 5Y) compared control (orange 5 YR) *in vitro* derived variants
vm1	10 Gy	Half the sphere of the capitulum with orange coloured (orange 5 YR) and half of it with yellow petals (yellow 2.5 Y)
vm2	10 Gy	Orange petal (orange 10 R) than control (orange 5 YR)
vm3	15 Gy	Larger bloom (7.5 cm) with broader petals width (18.0 mm) than control (5.45 cm and 12.0 mm)
vm4	15 Gy	Yellow flower colour (yellow 10 YR) compared to control (orange 5 YR)
vm5	15 Gy	Yellow flower (yellow 10 YR) compared to the control (orange 5 YR)
vm6	15 Gy	Yellow flower (yellow 5 Y) with smaller bloom (3 cm diameter)
vm7	20 Gy	Light yellow flower (yellow 5 Y) with dwarf stature plant(20cm height)

Table 3 Morphological analysis of variants in M_2

Name	Days to seed germination	Days to anthesis	No. of flowers/plant	Flower diameter (cm)	Flower colour	Width of petal (mm)	Harvest index (%)	100 seed wt.(mg)
Parent	5.67	70.80	53.24	5.71	orange	11.24	48.33(43.86)	240.93
m1	5.67	71.89	30.65	5.37	orange	12.04	44.33(41.55)	241.24
m2	5.54	67.89	21.34	5.59	orange	12.25	45.34(42.30)	239.45
m3	5.65	70.23	25.67	5.45	orange	12.47	38.67(38.35)	238.78
m4	5.45	70.45	40.78	5.71	orange	12.33	45.45(42.30)	240.13
vm1	5.52	70.16	30.56	5.67	orange	11.45	35.78(36.69)	235.67
vm2	5.55	69.9	26.67	5.33	orange	11.19	32.56(34.76)	236.45
vm3	5.80	71.45	31.78	5.34	orange	12.45	34.87(35.85)	239.56
vm4	5.65	70.12	27.45	5.50	orange	12.55	32.12(34.51)	240.56
vm5	5.38	71.45	29.04	5.65	orange	12.45	34.12(35.73)	238.94
vm6	5.45	69.23	24.56	5.35	orange	12.45	31.45(34.08)	241.35
vm7	5.23	70.11	25.67	5.47	orange	12.34	31.34(34.02)	238.78
CD (P=0.05)	1.19	9.92	7.85	0.27	-	0.48	1.85	11.23

it is an evident in Table 1 that lowest days required to bud initiation was in control (58.45), whereas the highest was in 20 Gy (64.45).

The highest number of flowers/plant was recorded in control (53.43 and 40.56) followed by 5 Gy (50.23 and 35.18) and 10 Gy (35.24 and 18.83) of gamma ray doses (Table 1). Similar effect of gamma rays on number of flowers was also reported by previous workers (Raghava *et al.* 1995).

The maximum flower diameter under *in vivo* (5.55 cm) was obtained in non-irradiated plants followed by 5 Gy (5.45 cm) and 10 Gy (5.15 cm). In *in vitro,* lower dose, i.e. 5 Gy gave quite similar diameter (5.32 cm) to that of control (5.45 cm). In *in vitro* condition there was increase in flower diameter at lower dose of gamma irradiation that can be explained as plant vigour in terms of biomass was caused to increase by stimulation of certain amino acid synthesis, namely lysine, phenylalanine (Antonov *et al.* 1989).

Flower colour variations

Gamma irradiation resulted in induction of changes in flower colour and form. The treated explant resulted in the flower colour variations at 20 Gy *in vivo* condition however, at 10 and 15 Gy of gamma ray doses gave change in flower colour in *in vitro* approach. The radiations induced flower colour changes may be due to chromosomal aberrations, change in chromosome number, gene mutation, rearrangement of different histogenic layers and mutations occurring in the biochemical pathways leading to an increase or decrease or both in the concentration of one or more existing pigments or may be due to blockage in synthesis of pigments or origin of new pigments (Dhaduk 1992)

Table 4 Isolation of putative mutants in M_2 generation

Variants	Derived from	Specification
pm1	m4	Light orange (orange 10 YR) but not exactly yellow (5 Y) like m4.
pm2	m3	Larger flower (8.5 cm dia.) than the control (5.5 cm dia.).
pm3	m2	early flowering (47.89 days) than control (55.67 days) after transplanting.
pm4	vm6	Flowers were light orange (orange 10 YR) although in vm6 it was yellow (yellow 5 Y).
pm5	vm3	large bloom (7.5 cm dia.) with wider petals width (14.00 mm) than control (5.5 cm dia. and 12.00 mm petal width).
pm6	vm4	Larger flower (7.5 cm dia.) compared control (5.5 cm dia.).

Morphological characterization in M_2 generation

The maximum days for seed germination were taken in vm3 (5.8 days), followed by Parent 1 and m1 (5.67 days) (Table 3). In cross pollinated crops, selfing is required to maintain the purity of mutant. It might be attributed to the occurrence of seeds with completely developed embryos which could not be affected by the gamma irradiation (Omar *et al.* 2008). It was evident from Table 3 that the maximum number of days required for the flower bud initiation as well as anthesis was noted in the m1 (56.71 and 71.89 days) followed by the vm3 (56.23 and 71.45). The number of flowers/plant was recorded the maximum in both the parents, i.e. Parent 1 (53.24) and Parent 2 (55.56) followed by m4 (40.78), m4 exhibited non-significantly maximum average flower diameter (5.71 cm) with equivalence to Parent 1 (5.71 cm) while, minimum in vm2 (5. 33 cm). Marigold is a heterozygous crop, so there may be presence of widely diverse genes, the primary DNA sequence could be altered through irradiation but could not transferred in all the mutant population in second generation (M_2) the similar type results was obtained by Lefort *et al.* (1999). The flower color remained orange (similar to the parent) in all the mutants generated in M_2 along with their parent with a little alteration. Mutation can result in several different types of change in DNA sequences; these can have no effect, alter the product of a gene, or prevent the gene

from functioning. May be these are the reasons of deriving colour mutant (*m*4, vm1, vm2, vm5, vm6 and vm7) in M_1. However, to the damaging effects that mutations can have on cells, organisms have evolved mechanisms such as DNA repair to remove mutations. That would be the primary reason of not getting a true colour putative mutant, the conclusion lend the support from the finding of Bandyopadhyay *et al*. (1997) and Reiter *et al*. (1992).

The result obtained in this present study indicates that there are ultimate six putative (pm1 to pm6) mutants were found out in M_2 generation with specifications mentioned in Table 4. The morphological changes obtained are stable in M_2 and they can be further studied in the next generations.

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REFERENCES

Arunyanart S and Soontronyatara S. 2002. Mutation induction by gamma ray and X-ray irradiation in tissue cultured lotus. *Plant Cell Tissue Organ Culture* **70**: 119–20.

Bandyopadhyay P, Das D K and Chattopadhyay T K. 1997. Correlation and path analysis in seed production of marigold as affected by the micronutrient application. *Horticulture Journal* **10**: 73–8.

Benetka V. 1985. Some experience of methodology with the isolation of somatic mutation in rose cultivar 'Sonia'. *Acta Pruhoniciana* **50**: 9–25.

Bhatt M S. 1990. Studies on *in vivo* and *in vitro* propagation techniques in rose (*Rosa hybrida* L.). Ph D thesis, PG. School, Indian Agricultural Research Institute, New Delhi.

Dhaduk B K. 1992. Induction of mutation in garden gladiolus (*Gladiolus* L.) by gamma rays. Ph D thesis, Indian Agricultural Research Institute, New Delhi.

Kasumi M, Takatsu Y, Manabe T and Hayashi M. 2001. The effects of irradiating gladiolus cormels with gamma rays on callus formation, somatic embryogenesis and flower colour variation in the regenerated plants. *Journal of Japanese Society of Horticulture Science* **70**: 126–8

Kuksova V B, Piven N M. and Gleba Y Y. 1997. Somaclonal variation and *in vitro* induced mutagenesis in grapevine. *Plant Cell Tissue Organ Culture* **49**: 17–27.

Kumar S. 2002. Studies on *in vitro* mutagenesis in rose (*Rosa hybrida*). Ph D thesis, CCS University, Meerut.

Lee Y I, Lee I S and Lim Y P. 2002. Variation in sweet potato regenerates from gamma ray irradiated embryogenic callus. *Journal of Plant Biotechechnology* **4**: 163–70.

Lefort F and Douglas G C. 1999. An efficient micro-method of DNA isolation from mature leaves of four hard-wood tree species. A*nnals of Forest Science* **56**: 259–63.

Leyser, M 2000. Genetics and Analysis of Quantitative Traits, 980p. Sinaure Assocs. Inc., Sunderland, Massachusetts, USA.

Maluszynski M, Nichterlein K, van Zanten L and Ahloowalia B S. 2005. Officially released mutant varieties—the FAO/IAEA database. *Mutation Breeding Review* **12**: 1–84.

Maple A J and Moller J P. 2007. Cytogenetics of mutants in triticale. *Plant Breeding Review* **5**: 41–93.

Omar C, Alikarnanoglub S, Acik L and Canbolatb Y. 2008. Plant breeding program aided by radiation treatment. *California Aggriculture* **14**(7): 4.

Raghava S P S. 1995. Genetic improvement of African marigold. (*In*) *Proceeding of National Seminar on Ornamental Horticulture and Environment*, Calcutta, 6-8 October 1995,pp 111–9.

Reiter R S, Williams J G K, Feldman K A, Rafalski A, Tingey S V and Scolnik P A. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. *Proceeding of National Academy of Science* USA. **89**: 1477–81.

Roy P K, Mamun A N K and Ahmed G. 2004. *In vitro* plantlets regeneration of rose. *Plant Cell Tissue Organ Culture* **14**(4): 149–54.

Singh K P, Singh B, Raghava S P S and Kalia C S. 1999. *In vitro* induction of mutation in carnation through in vitro application of chemical mutagen. *Indian Journal of Genetics* **60**(4): 535–9.

Yamaguchi H, Shimizu A, Degi K and Morishita T. 2008. Effects of dose rate of gamma ray irradiation on mutation induced and nuclear DNA content in chrysanthemum. *Breeding Science* **58**: 331–5.