



## Effect of different doses of gamma radiation on growth parameters of Mulberry (Morus) variety Kosen

H. L. Ramesh<sup>1</sup>, V. N. Yogananda Murthy<sup>2\*</sup> and Munirajappa<sup>3</sup>

<sup>1</sup>Department of Sericulture, V. V. Pura College of Science, Bangalore - 560 004 (Karnataka), INDIA

<sup>2</sup>Department of Life Sciences, Ganga Kaveri Institute of Science and Management, Bangalore-560 021(Karnataka), INDIA

<sup>3</sup>Department of Sericulture/Life Sciences, Jnana Bharathi Campus, Bangalore University, Bangalore-560 056 (Karnataka), INDIA

\*Corresponding author. E-mail: yoga16@rediffmail.com

**Abstract:** The present study was carried out to evaluate the effect of different doses of gamma radiations (1kR-10kR from  $Co^{60}$ ) on different growth parameters of mulberry variety Kosen. Overall results revealed that a declined trend in all the growth parameters i.e. sprouting (83.66%-18.66%), rooting (77.96%-19.59%), height of the plant (105.00 cm-58.03 cm), number of the branches (7.73-4.23), internodal distance (3.76 cm-3.94 cm), petiole length (2.89 cm-2.59 cm) and pollen fertility (77.93%-40.66%) were recorded and showed a deleterious effect. But the response of growth parameters against different doses showed variant behaviour in case of rooting as constant declined trend was not observed. Similarly fluctuating state has been found in other growth parameters also.

**Keywords:** Gamma, Irradiation, Kosen, Mulberry, Rooting, Sprouting, Survivability

### INTRODUCTION

Sericulture is a multidisciplinary, export orient, rural based cottage industry. Several indigenous and exotic varieties available in the country. But all the mulberry plants/ varieties are not utilized to the maximum extent due to lack of one or the other morpho-economic traits. India being a vast country with complex agro climatic zones, there is wide scope for having a number of elite mulberry varieties for cultivation in different areas. Mutation breeding has been widely employed (Tikader *et al.*, 1996; Deshpande *et al.*, 2010) for improving vegetatively propagated crop plants. Gamma ray has been proved to be highly potent in inducing variability in mulberry plant. Kosen is a temperate mulberry variety and has been introduced in India. It is a poor rooter and poses problem of acclimatization in tropical agro-climatic conditions and leaf production to the extent of 28-32 MT/hectare/year. The present investigation aims at improving the morpho-agro botanical traits of already existing mulberry cultivar Kosen.

### MATERIALS AND METHODS

Kosen mulberry variety plants were used as test plant material in the present investigation. The mulberry plants of Kosen were selected from the mulberry germplasm bank maintained at Jnana Bharathi campus, Bangalore University, Bangalore-560 056. Juvenile twigs of the Kosen mulberry variety were used for cuttings preparation and only the middle parts of the twigs were

taken. Each cutting measured about 15cms in length having 2-3 active vegetative buds, which were free from pathogen and pests. Care was taken to avoid damage to the buds and cut ends while preparing the cuttings. The juvenile cuttings were irradiated with different doses of gamma rays radiations (1kR-10kR) from  $Co^{60}$  gamma unit installed at the Indian Institute of Horticulture Research [IIHR], Hesaraghatta, Bangalore-560 088. Irradiation was conducted during April-May months and ten replications were maintained for the calculation of mean values of all the parameters encountered.

Irradiated cutting were planted in earthen pots which were filled with a mixture of well dried pulverized garden soil, fine sand and well decomposed farmyard manure in the proportion of 1:1:1. Each replication having 10 cuttings, each were maintained for six months before transplanting them in to the main field. The pots were arranged in rows giving a spacing of ½ feet between the pots and 1 foot between the rows. The transplanted saplings were maintained in randomized block design (RBD) with 90cmx90cm spacing. Necessary cultural operations such as timely irrigation, weeding, intercultivation, manuring, protection against desiccation, diseases and pests were ensured. Suitable controls were also maintained in similar conditions for comparative studies. At  $M_1$  and  $M_2$  generations, data related to growth responses such as sprouting, rooting, survivability, internodal distance, branching pattern, leaf area and pollen fertility were recorded (Dandin and Jolly, 1986;

Shamachary and Jolly, 1988; Dandin and Kumar, 1989; Sanjappa, 1989). The data collected on various parameters in the present investigation were analysed statistically according to the design of the experiment (Sundarraaj *et al.*, 1972; Singh and Choudhary, 1979).

## RESULTS AND DISCUSSION

The present study observed that, sprouting percentage showing decreasing trend with the increase in dosages of gamma rays. It was highest at 1kR (83.68 %) and least at 10kR (18.66%) compared to control (85.56 %) Table 1. In control plants sprouting was noticed on 8<sup>th</sup> day of planting and delayed sprouting was observed when the cuttings were irradiated at 1kR to 5kR gamma rays and taken 13-15 days to sprout. The results are in conformity with the findings of Rao *et al.* (1984); Jayaramaiah and Munirajappa (1987); Tikader *et al.* (1996). Sprouting is the inherent capacity of the plant material to unfold the buds and produce new flush of shoots. It is an established fact that, the role of agro-climatic conditions and moisture is a binding factors influencing sprouting (Dandin and Kumar, 1989). The sprouting is adversely affected by higher doses of gamma rays. RaoEswar *et al.* (2004) and Deahpande *et al.* (2010) reported that the gamma rays are more potent and are highly penetrating in nature might have developed cells which were undergoing meiotic division in the bud region. Katagiri (1970) reported that the decrease in sprouting percentage with the increase in gamma rays dosage is due to partial cell death. Decrease in sprouting percentage has been attributed to destruction of auxin (Skoog, 1935) or due to inhibition of auxin synthesis (Gordon, 1957). It may also be due to variation in temperature, water content and oxygen tension at the time of treatment (Nybom *et al.*, 1952). Similar reduction in sprouting and survival percentage of vegetatively propagated crops was reported by several workers (Banerji and Datta, 1991; Hemalatha, 1998). According to Kaicker (1992) reduction in sprouting may be due to the toxic effect of higher core of gamma rays whereas the same at lower levels hastened the metabolic activity. Progressive reduction in growth parameters can be interpreted on cytological, physiological and anatomical view points. These include interference in normal mitosis and frequent occurrence of mitotic aberrations, inhibition of rate of assimilation and consequent change in the nutrient of plants (Cesarett, 1968).

Among the irradiated cuttings, highest rooting was noticed at 2kR (78.06%) and least at 9kR (13.76%) and control cuttings showed 81.70% rooting. The reduction in rooting was found to be significant with the increase in the dosage of gamma rays. Higher the dosage of gamma rays lesser is the rooting rate and root proliferation (Table 1). Rooting behavior of a variety is

purely a genetic character (Hartman and Kester, 1976). Present findings are in line with the observations of Fujitha and Wada (1982) who reported that relatively decreased percentage of rooting (50%) with the increase in the gamma rays doses in mutant strains of Ichinose over its control (78%). They have also reported that, the spontaneous mutants of mulberry variety KNG produced low percentage of rooting (16%-30%) where as normal KNG mulberry variety showed 96% of rooting.

Survivability of plant cuttings varied depending upon the dosage of gamma rays administered. Saplings recovered from cuttings irradiated with higher doses of gamma rays (10kR) though sprouted and grew initially, they fail to develop further after 45-50 days. Survivability percentage was maximum at 1kR (78.70%) and minimum at 9kR (21.30%) and control plants show 86.90% of survivability (Table 1). The decrease in survival percentage after radiation treatment was attributed by Skoog (1935) and Smith and Kersten (1942) to the destruction of auxin. Gray (1990) observed that the decrease in the survivability of irradiated plant material to the series of events occurring at the cellular level which affect the vital macromolecules and result in physiological imbalance. Survivability indicates the capacity of treated population to withstand even the severe dosage of gamma rays. Survival percentage studied in all the irradiated mulberry varieties showed a continuously decreasing trend. Sensitivity of the plant material depends on the genetic constitution, dose employed, DNA amount, its replication time at initial stages, moisture content, stage of development and genotype (Deshpande *et al.*, 2010). Jayaramaiah and Munirajappa (1987) and Nakajima (1972) have concluded that low doses of irradiation could be used as safe and effective methods in mulberry. Sastry *et al.* (1974) reported that survivability in mulberry varieties S<sub>30</sub> and K<sub>2</sub> showed that the injury was directly proportional to the concentration of mutagen. The authors also opined that survivability depending on the disturbances caused at the physicochemical level in cells or acute chromosomal damage or due to the combined effect of both.

Plant height was found to be highest at 1kR (105.00 cm) and least at 9kR (52.13 cm). Height of the irradiated population of Kosen was significantly reduced with the increase in gamma rays administered. Mulberry is basically a polygenic plant and plant height is a quantitative trait which is predominantly controlled by polygene. Each gene contributes small effects, which is called genetic additive effect (Thohirah Lee Abdullah *et al.*, 2009). It has been observed that, plant height was increased at 8 kR (63.16 cm) compared 7 kR (52.20 cm). Several authors (Anon, 1977; Kearsy and Pooni, 1996) were of the view that, in some cases mutation are not stable, they will undergo recombination during meiosis.

**Table 1.** Effect of gamma irradiation on propagation and growth attributes of Kosen mulberry variety at M<sub>1</sub> generation.

Treatment	Sprouting %	Rooting %	Survival %	Height of the plant (cm)	Number of branches (no.)	Internodal distance (cm)	Leaf area (cm <sup>2</sup> )	Petiole length (cm)	Number of flowers/ inflorescence	Inflorescence Length (cm)	Pollen fertility (%)
Control	85.66	81.70	86.90	123.83	7.60	4.35	79.36	3.28	25.00	3.28	85.20
1kR	83.66	77.96	78.70	105.00	7.73	3.76	86.63	2.89	22.00	3.11	77.93
2kR	82.33	78.06	74.90	96.29	5.86	4.17	70.80	3.07	27.66	3.16	67.66
3kR	65.66	69.96	72.30	88.00	5.10	4.06	72.33	3.31	27.33	2.81	68.36
4kR	70.33	51.36	69.50	77.56	5.13	2.94	56.63	3.42	20.33	3.07	51.90
5kR	56.66	59.60	51.70	95.90	5.33	4.13	72.03	2.70	20.00	3.50	63.56
6kR	50.33	28.10	32.70	68.03	4.60	4.73	66.46	2.85	23.33	3.14	49.93
7kR	46.00	34.90	37.80	52.20	3.60	4.74	45.00	2.55	27.66	2.53	63.86
8kR	46.66	19.83	26.20	63.16	4.00	4.16	61.80	2.66	12.66	2.99	43.20
9kR	22.33	13.76	21.30	52.13	3.80	3.98	53.46	2.78	10.00	3.09	53.56
10kR	18.66	19.59	24.50	58.03	4.23	3.94	45.53	2.59	11.33	2.95	40.66
SEM	--	--	--	--	0.23	0.07	1.07	0.04	1.28	0.04	1.58
<b>CD at 5%</b>	NS	NS	NS	NS	1.28	1.82	2.21	1.57	2.38	1.09	NS

NS: Non significant



**Fig.1.** Mutant of Kosen mulberry variety recovered at 8kR gamma irradiation showing chlorophyll deficient leaves.



**Fig.2.** Hard wood cuttings of Kosen mulberry variety irradiated at 10kR gamma rays showing withering of inflorescence before maturity.



**Fig.3.** Leaf mutant of Kosen mulberry variety recovered at 8kR gamma irradiation showing biforked leaves.



**Fig.4.** Mutants of Kosen mulberry variety recovered at 8kR gamma irradiation showing chlorophyll deficient leaves.

Multicellular organisms have the ability to recover from sub lethal doses of ionizing radiations. Even with in a cell, non damaged molecule may be able to take over metabolic process and exerts a gradual recovery to normal levels. The efficiency of selecting the desired mutant is controlled by single gene (Brunner, 1995).

Maximum number of branches were noticed at 1kR (7.73) and minimum at 7kR (3.60) compared to control (7.60). Higher dosages of gamma rays such as 9kR (3.80) and 10kR (4.23) drastically reduced the number of branches in the irradiated Kosen mulberry variety. Increased dosage of gamma ray irradiation lowered the formation of new shoots. If the doses are too high, too many plants will be killed because mutagens can have direct negative effect on plant tissue and many mutations can be lethal. This is due to the fact that primary injuries are retardation or inhibition of cell division, cell death affects the growth habit and changes in plant morphology. If the dose is too low, there will not be enough mutation because of low mutation frequency and results in small mutated sector (Nazir *et al.*, 1998). Branching pattern showed variations in number of branches and it was observed that, at 8kR

(4.00), 9kR (3.80) and 10kR (4.23) respectively. Mulberry is polygenic in nature and branching pattern is controlled by polygenes. In some cases mutation are not stable and vary with the mutagenic sensitivity (Anon, 1977). Hazama (1968); Katagiri (1976) and Rao *et al.* (1984) have reported that radio sensitivity and bud mutations of mulberry in gamma irradiated populations showed deformations, inhibition of height and branching pattern. Internodal distance was found to be less in most of the treated plants compared to control. However, in plants irradiated with other dosages, the internodal distance did not vary much from the control. There was a marked reduction in the petiole length at 7kR (2.55 cm) and leaf-petioles of cuttings irradiated at 2kR were short and stout (Table 1). The internodal length was found to be affected by cell number and cell length or both in Barley (Blonstein and Gale, 1984). Similar observations in Mysore local mulberry variety treated by gamma irradiation have been reported by Jayaramaiah and Munirajappa (1987).

Leaf area was considerably decreased due to gamma irradiation (Table 1). Plants irradiated with 1kR gamma rays showed slightly bigger leaves (86.63cm<sup>2</sup>) compared



to control and miniature leaves were observed at 10kR (45.53 cm<sup>2</sup>). It was also observed that some irradiated populations at 8kR, 9kR and 10kR exhibited small, crumpled, biforked and chlorophyll deficient leaves (Figs. 1-4). Mulberry is a cross pollinated and heterozygous plant, induced mutations creates a new genetic variations in leaf area at different doses were due to genetic mutation instead of somatic mutation. Somatic mutation occurs when the mutant cell continues to divide, the individual cell contain a patch of tissue with genotype different from cells of the rest of the body (Brar and Jain, 1998). These include karyotype changes, point mutations, somatic crossing over, somatic gene arrangement, changes in DNA amplification and segregation of pre-existing chimera tissue (Kearsey and Pooni, 1996). The abnormalities observed in leaves is due to various causes such as disturbances in phytochromes, chromosomal aberrations, mitotic inhibition, disrupted auxin synthesis, disturbance in DNA synthesis etc. (Matsumura and Fujii, 1957; Abraham and Ninan, 1968; Mikaelsen *et al.*, 1968). Irradiated population showed lowered pollen fertility which gradually decreased with the increased rate of gamma ray dosage. Only 48.20% and 40.66% of pollen fertility were observed at 8kR and 10kR respectively. Radiation induced pollen sterility has been reported by different workers in some of the crop plants (Yamaguchi, 1963; Singh and Roy, 1991).

### Conclusion

Mulberry variety Kosen has been subjected to different doses of gamma irradiation (1kR-10kR) and various growth parameters were recorded. It is commonly observed in the variety that, with increase in gamma ray dosage, there was linear decrease in the performance of various growth parameters viz., sprouting, rooting, survivability, plant height, leaf area and pollen fertility. Gamma ray is a potent physical mutagen which could induce variability in mulberry variety Kosen. There was no remarked variation observed in the treated population in the first generation. Leaves showed abnormalities such as biforked and chlorophyll deficient leaves. Height of the plants was drastically reduced at higher doses (7kR-10kR).

### REFERENCES

- Abraham, A. and Ninan, C. A. (1968). Genetic improvement of the coconut palm : some problems and possibilities. *Indian J. Genet. and Plant Breeding*, 3 : 97 – 103.
- Anon, (1977). Manual on mutation breeding. Technical report series No.119, IAEA, Vienna, 1974, pp.169 – 192.
- Banerji, B. K. and Datta, S. K. (1991). Induction of somatic mutation in chrysanthemum cultivar 'Anupam'. *Journal of Nuclear Agriculture Biology*, 19 : 252 – 256.
- Blonstein, A. D. and Gale, M. D. (1984). Cell size and cell number in dwarf mutants of barley in semi dwarf cereal mutants and their use in cross breeding II (Teidsc 407), *FAO/IAEA, Vienna*, 19 – 29.
- Brar, D. S. and Jain, S. M. (1998). Somaclonal variation: Mechanism and applications in crop improvement. In: Somaclonal variation and induced mutations in crop improvement, Jain, S. M., D. S. Brar and B. S. Ahloowalia (Ed.) *Kluwer Academic Publisher*, ISBN: 0792348621, pp.15.
- Brunner, H. (1995). Methods of induction of mutations. Plant breeding Unit, Joint FAO/IAEA Programme, IAEA Laboratories, Seibessdorf, Austria.
- Cesarett, A. P. (1968). Effect of radiation on higher plants and plants communities. *Radiation Biology*, United State Atomic Energy Commission, Washington DC, 264 – 309.
- Dandin, S.B. and Jolly, M.S. (1986). Mulberry descriptor. *Sericologia*, 26(4):465 – 475.
- Dandin, S.B. and Kumar, R. (1989). Evaluation of mulberry genotypes for different growth and yield parameters. In : Genetic resources of mulberry and utilization. Ed. By Sengupta and Dandin, S. B. CSR&TI, Mysore, 143 – 151.
- Deshpande, K. N., Mehete, S. S. and Pingle, S. D. (2010). Effect of different mutagens for induction of mutations in mulberry. *Asian J. Exp. Biol. Sci. Spl.*, 104 – 108.
- Fujitha, H. and Wada, M. (1982). Studies on mutation breeding in mulberry (*Morus* spp.). In: Induced mutation in vegetatively propagated plants. *IAEA, Vienna*, 249 – 279.
- Gray, E. (1990). Evidence of phenotypic plasticity in mulberry (*Morus L.*). *Castanea*, 55 (4) : 278 – 281.
- Gordon, S. A. (1957). The effect of ionizing radiation on plants, bio-chemical and physiological aspects. *Quant. Rev. Biol.*, 32: 3 – 14.
- Hartman, H. T. and Kester, D. E. (1976). Plant propagation – Principles and Practices. Prentice Hall of India.
- Hazama, K. (1968). Breeding of mulberry tree. *J.A.R.Q.*, 15 – 19.
- Hemalatha, K. (1998). Induction of mutation in carnation (*Dianthus caryophyllus L.*) through gamma rays and EMS. *Ph.D. Thesis*, University of Agricultural Sciences, Bangalore.
- Jayaramaiah, V. C. and Munirajappa. (1987). Induction of mutations in mulberry variety 'Mysore Local' by gamma irradiation. *Sericologia*, 27 (2) : 199 – 204.
- Katagiri, K. (1970). Varietal differences of mutation rate and mutation spectrum after acute gamma ray irradiation in mulberry. *J. Sericult. Sci. Japan*, 39 (3) : 194 – 200.
- Katagiri, K. (1976). Radiation damage and induced polyploids in mulberry *Morus alba L.* *Environ. Exp. B A.*, 16 (2/3) : 119 – 130.
- Kaicker, U. S. (1992). Rose breeding in India and Cytology of induced mutation of H.T.Cv, 'Folklore'. *Acta Horticulture*, 320 : 105 – 112.
- Kearsey, M. J. and Pooni, H. S. (1996). The genetic analysis of quantitative traits. Plant genetic school of Biological Science, University of Birmingham, U. K. Chapman and Hall, pp 381.
- Matsumura, S. and Fuji (1957). Induction of bud sprouts by x-rays and gamma – rays. *Ann. rep. Nat. Inst. Genet.*, Japan, 8: 94 - 95.
- Mickaelsen, K., Ahnstrom, G. and Li, W. C. (1968). Genetic effects of alkylating agents in Barley. Influence of pest storage, metabolic state and pH of mutagen solution. *Heredities*, 59 : 353 - 374.
- Nazir, M. B., O. Mohamad., A. A. Affida and A. Sakinah

- (1998). Research highlights on the use of induced mutations for plant improvement in Malaysia. Malaysian Institute for Nuclear Technology Research (MINT), Bangi.
- Nakajima, K. (1972). Induction of useful mutation in mulberry by gamma irradiation. *JARQ*, 6 (4) : 195 – 198.
- Nybom, N., Gustaffsson, A. and Ehrenberg, L. (1952). On the injurious action of ionizing radiation in plants. *Bot. Notice.*, 105 : 343 – 365.
- RaoEswar, M. S., Dandin, S. B., Mallikarjunappa, R. S., Venkateshaiah, H. V. and Bongale, U. D. (2004). Evaluation of induced tetraploid and evolved triploid mulberry genotypes for propagation, growth and yield parameters. *Indian J. Seric.*, 43 (1) : 88 – 90.
- Rao, P., Rao, J. M. M and Sarojini, N. L. (1984). Mutation breeding in mulberry *Morus indica* L. *Indian J. BA.*, 7 (1): 106 – 111.
- Sanjappa, M. (1989). Geographic distribution and exploration of the genus *Morus* L. (*Moraceae*). In: Genetic resources of mulberry and utilization. Ed. By Sengupta, K. and Dandin, S. B. Central Sericultural Research & Training Institute, Mysore, 4 – 7.
- Sastry, C. R., Venkataramu, C. V., Azeez Khan and Krishna Rao, J. V. (1974). Chemical mutagenesis for productive breeding in mulberry. *Paper presented at the seminar organised in commemoration of silver jubilee of Central Silk Board, India.*
- Shamachary and Jolly, M. S. (1988). A simple device for quick determination of mulberry leaf area in the field. *Indian J. Seric.*, 27 (1) : 51 – 54.
- Singh, R. K. and Choudhury, B. D. (1979). *Bio-metrical methods in quantitative genetic analysis*. Kalyani Publishers, New Delhi.
- Singh, A. and Roy, R. P. (1991). X-ray irradiation studies on *Trigonella foenum – graecum* L. *J. Genet. Iber.*, 23: 49 – 66.
- Skoog, F. (1935). The effect of radiation on Auxin and plant growth. *J. Cell. Physiology*, 7 : 227 – 270.
- Smith, G. F. and Kersten, H. (1942). Auxins in seedlings from x-rayed seeds. *Amer. J. Bot.*, 29 : 785 – 819.
- Sundararaj, G. L., Nagaraju, M. N., Venkataramu and Jaganath. (1972). *Design and Analysis of field experiments*. U.A.S., Misc., Series No. 22, Bangalore, India: 424 – 440.
- Thohirah Lee Abdullah, Johari Endan and B. MohdNazir. (2009). Changes in flower development, chlorophyll mutation and alteration in plant morphology of *curcuma alismatifolia* by gamma irradiation. *American J. Applied Sciences*, 6 (7) : 1436 – 1439.
- Tikader, A., Vijayan, K., Roy, B. N. and Pavankumar, T. (1996). Studies on propagation efficiency of mulberry (*Morus* spp.) at ploidy level. *Sericologia*, 36 (2): 345 – 349.
- Yamaguchi, H. (1963). Genetic effects of Pile radiation in rice. Biological effects of neutron and proton irradiation. *IAEA, Vienna*, 371 – 382.