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EFFECT OF GAMMA RADIATION ON MORPHOLOGICAL AND GROWTH PARAMETERS OF MULBERRY VARIETY M_5

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ABSTRACT
Mulberry variety M_5 is an open pollinated hybrid (OPH) selected from the seedling population of Mysore local variety. M_5 mulberry variety cuttings were used for irradiation with different doses of gamma rays (1kR to 10kR). During the course of investigation, various propagation parameters viz., sprouting percentage, rooting percentage, survivability, height of the plant, internodal distance, leaf area were recorded. Moderate dosages such as 4kR to 7kR were found to be fruitful in the induction of beneficial variability. Morphological leaf mutants like enlarged leaf (4kR), curvy leaf (8kR), mosaic leaf (8kR) and biforked leaf (9kR) were found in the treated population. In M_2 generation, important features like shortened internodal distance and increased leaf area were observed. Increase in rooting % was observed at 4kR (90.03%) when compared to control (89.20%). LD_{50} of M_5 was found to be good between 6kR and 7kR, while acute doses like 9kR and 10kR proved to be lethal for the taxa studied. Height of the irradiated population significantly reduced with the increase in the dosages of gamma rays. Slightly increased plant height was recorded in the population irradiated at 5kR. Increased tendency in the number of branches was noticed at 4kR. Higher doses of gamma rays i.e., 8kR to 10kR drastically reduced the number of branches. The treated plants are grown for full maturity in order to test their potentiality in breeding programme to produce lines.

KEY WORDS: Gamma irradiation, morphology, sprouting, rooting, survivability, plant height, internodal distance, leaf area.

INTRODUCTION
Mulberry is an outstanding bio-energy food plant of silkworm *Bombyx mori* L. and mulberry variety M_5 is the most popular cultivar in Karnataka. Many mulberry genotypes are available in nature, but all are not utilized for rearing silkworms since they lack in one or the other required beneficial trait. Successful exploitation of various mutagenic agents for inducing aberration has become one of the most important lines of contemporary research. Mutation induction in mulberry started towards the end of 1950's in Japan (Sugiyama and Tojyo, 1962; Tojyo, 1966; Hazama, 1967a; Katagiri, 1970). Mutation induction techniques such as radiation or chemical mutagens are good tools for increasing variability in crop species because spontaneous mutations occur with an extremely low frequency. Mutation techniques have significantly contributed to plant improvement worldwide and have made an outstanding impact on the productivity and economic value of some crops. Mutation breeding has been widely employed in recent times for improving vegetatively propagated crop plants and gamma rays have been proved to be highly potent in inducing variability in mulberry plant (Deshpande *et al.*, 2010, Tikader *et al.*, 1996). Present investigation aims at improving the morpho-economic traits with higher yield and nutritionally superior leaves of the existing mulberry cultivars by using gamma irradiation.

MATERIALS AND METHODS
In the present investigation, mulberry variety M_5 was procured from mulberry germplasm bank maintained at Jnana Bharathi Campus, Bangalore University, Bangalore. Juvenile twigs of M_5 mulberry genotype were used for cuttings preparation and only the middle part of the twigs were taken. The newly prepared juvenile cuttings were irradiated with different doses of gamma rays (1kR to 10kR) from Co^{60} gamma unit installed at the Indian Institute of Horticulture Research (IIHR), Hesaraghatta, Bangalore – 560 088. Irradiation was conducted during summer months and ten replications were maintained for the calculation of mean values of all the parameters encountered.
The irradiated cuttings 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 with three replications having ten cuttings each and the same were maintained for six months before transplanting them in to the main field. The transplanted twigs were planted in the randomized block design with 90cm x 90cm 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; Sanjappa, 1989). The data collected on various parameters in the present investigation were tabulated using "Method of Analysis of Variance" appropriate to the design of the experiment (Sundarraj *et.al*., 1972; Singh and Choudhary, 1979).

RESULT AND DISCUSSION

Sprouting: Sprouting is the inherent capacity of the plant material to unfold the buds and produce new flush of shoots and it is an established fact that, the role of agro – climatic conditions and moisture are binding factors influencing sprouting (Dandin and Kumar, 1989). The gamma irradiation population of M_5 showed linear decrease in sprouting with the increased dosage. Low dosages of gamma rays 1kR (81.30%), 2kR (78.70%) and 3kR (69.20%) did not affect sprouting but in the cuttings irradiated with 4kR to 8kR the sprouting was drastically reduced in all the varieties irradiated. 9kR (29.40%) and 10kR (21.50%) proved to be lethal in M_5 variety. Decrease in sprouting has been attributed to the destruction of auxin (Skoog, 1935) or due to inhibition of auxin synthesis (Gordon, 1957). It may be also due to variation in temperature, water contents and oxygen tension at the time of treatment (Nybom *et al*., 1952). The results are in conformity with the findings of Rao *et al*., (1984); Jayaramaiah and Munirajappa, (1987); Tikader *et al*., (1996). The sprouting is adversely affected by higher doses of gamma rays. Rao Eswar *et al*., (2004); 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 percent with the increase in gamma rays dosage is due to partial cell death. 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.

FIGURE1. M_5 variety irradiated at 4kR exhibited good rooting compared to control.

Rooting: The cuttings of M_5 variety irradiated at 4kR exhibited higher values (90.03%) compared to control (89.20%). Gradual reduction in rooting percentage was observed in 1kR (81.40%), 2kR (81.40%), 3kR (74.50%) and drastic reduction was noticed at 9kR (34.40%) and 10kR (32.20%) (Table – 1; Fig.1).Rooting behavior of a variety is purely a genetic character (Hartman and Kester, 1976). The present findings are in line with the reports of Fujitha and Wada (1982) who reported relatively low percentage of rooting (50%) in mutant strains of Ichinose over its control. They have also reported that spontaneous mutant of KNG produced low percentage of rooting (16% - 32%), whereas normal KNG variety showed 96% of rooting. However, Kukimura *et al*., 1975 reported the increased rooting ability of mulberry mutants.

Survivability: Survivability of plant cuttings also varied depending upon the dosage of gamma rays administered. Survivability percentage studied in the M_5 variety showed continuously declining trends. LD_{50} of M_5 was found to be between 6kR (61.20%) and 7kR (52.30%), while acute doses like 9kR (39.20%) and 10kR (29.70%) proved to be lethal for the taxa studied. The present findings are in confirmation with the results of Das *et al*., (1987); Nakajima (1972) and Jayaramaiah and Munirajappa (1987). These workers have concluded that, low doses of gamma irradiation could be used as safe and effective tools to induce variations towards improving mulberry cultivars. They also opined that the increased dosages of gamma rays (9kR and 10kR) produced semi lethality to complete lethality. Skoog (1935) and Smith and Kersten (1942) opined that, the decrease in survival percentage after radiation treatment was attributed to the destruction of auxins. Sastry *et al*., (1974) reported that, survivability in mulberry varieties S_{30} and K_2 showed that the injury was directly proportional to the concentration of mutagen. The authors also opined that, disturbances caused at the physicochemical level in cells or acute chromosomal damage or due to the combined effect of both.

FIGURE 2. M_5 variety irradiated at 4kR exhibited increased tendency in the number of branches.

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. Deshpande *et al*., (2010) were reported that, 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.

Height of the irradiated population of M_5 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 addictive effect (Thohirah Lee Abdullah *et al*., 2009). Slightly increased plant height was recorded in the population irradiated at 5kR (117.24cms). Several authors (Anon, 1977; Kearsey and Pooni, 1996) were of the view that, in some cases mutation are not stable, they will undergo recombination during meiosis. 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. H, 1995). Increased tendency in the number of branches was noticed at 4kR (Fig.2). Higher doses of gamma rays i.e., 8kR to 10kR drastically reduced the number of branches in the irradiated progeny leading to semi lethality to complete lethality. Similar results have also been reported by Rao *et al*., (1984) in M_5 and local mulberry varieties due to the effect of irradiation. 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 frequently and results in small mutated sector (Nazir *et al*., 1998). Katagiri (1976a) reported the deformation leaf and inhibition of growth at higher doses gamma rays in mulberry variety Ichinose. These effects are due to the cytological changes such as chromosomal damages, inhibited mitotic division, degeneration of nuclei, cell enlargement etc., have been reported (Sparrow *et al*., 1952., Pollard, 1964; Karpate and Chaudhuri, 1997).

FIGURE 3. Mutants exhibited enlarged leaf area at 4kR gamma irradiation.

FIGURE 4. Mutants showing curly leaves at 8kR, 9kR and 10kR gamma irradiation with reduced lamina.

Leaf contributing parameters such as leaf area and length of internodes which are desirable morpho-economic traits in mulberry were also affected due to irradiation. Mutants with enlarged leaf area were observed specially at 4kR gamma irradiation (Fig.3) and mutants' showing curly leaves at 8kR, 9kR and 10kR gamma irradiation with reduced lamina (Fig.4). Similar findings affecting leaf area, shape and size have been reported in large number of genotypes from time to time through irradiation by several investigators (Hazama *et al*., 1968b, Katagiri, 1970; Kuchkarov and Ogurtsov, 1987). Dandin *et al*., (1996) have registered several leaf mutants viz., venosa, wrinkled leaf, glossy leaves etc., in case of spontaneous mutants of mulberry. The abnormalities in leaves are due to disturbances by phytochromes, chromosomal aberrations, mitotic inhibition (Moh, 1962; Mickaelsen *et al*., 1968; Abraham and Ninan, 1968). In the M_5 mulberry variety, saplings recovered from gamma irradiated cuttings showed reduced internodal distances at 4kR, 6kR and 7kR and at lower doses (1kR to 3kR) the internodal distance was not affected. The internodal length was found to be affected by cell number and cell length or both in barley (Blonstein and Gale, 1984). Similar observations, namely shortened internodes in the mutants of Mysore local mulberry variety secured by gamma irradiation have been reported by Jayaramaiah and Munirajappa (1987). Gamma rays administered in the present investigation marginally affected the reproductive parts and length of the inflorescence at maturity.

TABLE 1: Effect of gamma irradiation on propagation and growth attributes of M_5 mulberry variety at M_1 generation

Treatment	Sprouting %	Rooting %	Survival %	Height of the Plant (cm)	Branching pattern (no.)	Internodal distance (cm)	Petiole length (cm)	Leaf area (cm^2)	Number of flowers/ inflorescence	Inflorescence Length (cm)
Control	92.40	89.20	87.70	142.23	6.14	4.29	3.23	167.28	32.00	2.93
1kR	81.30	81.40	85.40	135.29	5.98	4.31	3.27	169.14	26.24	2.89
2kR	78.70	83.70	79.28	131.11	6.01	4.38	3.18	168.28	28.29	2.81
3kR	69.20	74.50	85.40	125.94	5.91	4.33	3.39	151.43	31.00	2.84
4kR	76.40	90.03	71.80	155.14	6.95	4.01	3.33	174.45	29.28	3.03
5kR	71.80	64.30	64.40	117.24	5.74	4.52	3.38	139.18	26.91	2.69
6kR	52.90	49.40	61.20	104.29	5.68	4.07	3.41	123.24	24.47	2.71
7kR	41.40	43.90	52.30	96.78	4.69	4.11	3.25	116.11	23.28	2.74
8kR	44.20	37.50	49.40	89.59	4.81	4.41	3.19	121.18	19.14	2.58
9kR	29.40	34.40	39.20	66.23	4.14	4.39	3.01	112.18	20.18	2.81
10kR	21.50	32.20	29.70	54.29	4.07	4.59	3.09	98.70	16.29	2.56
SEM	--	0.98	--	4.29	0.91	0.48	0.39	4.78	--	0.69
CD at 5%	NS	1.62	NS	6.21	1.08	0.79	0.88	6.59	NS	0.94

CONCLUSION

Mulberry variety M_5 has been subjected to different doses of gamma irradiation (1kR – 10kR) and various morphological and growth parameters were recorded. In the present investigation, promising mutants were identified in M_5 variety at 4kR. At M_2 generation, shortened internodal distance and increased leaf area were observed. However, the propagation and growth parameters such as rooting ability and root proliferation, height of the plant, branching pattern etc., were not of much promising nature. Gamma ray is a potent physical mutagen which could induce variability in mulberry variety M_5. The higher yields in the mutants derived from M_5 was found to be increased by 11.09%. Comparable results showing shortened internodes coupled with increased leaf area were recorded in radiation induced mutants.

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