



GAMMA-RAYS INDUCED MUTATIONS ON THE GROWTH ATTRIBUTES AND LEAF YIELD OF S₃₄ MULBERRY VARIETY *MORUS ALBA*

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ABSTRACT

S₃₄ is a drought tolerant mulberry variety cultivated under rainfed condition. In order to increase the leaf yield and to improve the quality the hard wood stem cuttings of this taxon were irradiated with γ -rays (4kR, 5kR, 6kR, 7kR, 8kR, 9kR and 10kR). Growth attributes such as sprouting performance, rooting ability, survivability, yield and phytochemical components were recorded in the irradiated population. Comparable controls were also maintained. It is observed that the lower dosages of gamma - rays i.e., 4kR and 5kR were less effective in inducing variability. At higher dosages viz., 8kR, 9kR and 10kR deformities like delayed sprouting, poor rooting, weathering of inflorescence, stunted growth, plants with weak and feeble branches, plants bearing small leaves with wrinkled and coriaceous texture were observed. On the other hand, at 6kR beneficial mutants were recorded. These variants exhibited superiority over the control in yield, phytochemical constituents and commercial characteristic features of the cocoon. The present study recommends the exploitation of these promising mutants of S₃₄ for commercial exploitation.

KEY WORDS: Gamma irradiation, Diploid, S₃₄ mulberry, Chemo-assay, Bioassay

INTRODUCTION

Mulberry plant (*Morus alba*) being cultivated widely for the culture of silkworm *Bombyx mori* L. Mulberry leaf is known to be rich in protein, starch and vitamins, helping the silkworm to meet its dietary requirements. The growth and development of silkworm is mainly depends on the nutrition composition of the food plant (Krishnaswamy *et al.*, 1985). Many varieties of mulberry have been evolved and are being cultivated to enhance the silkworm growth performance and silk production. Efforts have been made to alter the genetic structure of the mulberry through conventional and mutation breeding techniques yielding valuable results. Mutation breeding employing radiation (X-rays, Gamma rays, Fast neutrons, etc.) was initiated (Swaminathan, *et al.*, 1963, Swaminathan, 1964; Hazama, 1967, 1968a, 1968b; Katagiri and Wada, 1971; Nakajima, 1973; Fujita, 1974; Kukimura, *et al.*, 1975, Aliev, 1977) to explore the feasibility of developing new varieties of mulberry (Jayaramaiah and Munirajappa, 1987; Ramesh, 1997). Mutagenesis has been proved to be extremely useful in creating a new variability in the existing gene pool. Of late, genetically effective radiations have been widely used for induction of mutation in mulberry (Reddy, and Munirajappa, 2004; Kumar and Kumari, 2010, Udensi, *et al.*, 2012).). Gamma - rays induced diploids have shown all the symptoms such as larger leaf size, bigger leaf area, dark green colored leaves, delayed flowering, slower growth etc., when compared to control (Jayaramaiah and Munirajappa, 1987; Reddy, and Munirajappa, 2011). The present work is an attempt to improve the yield traits in the existing cultivar S₃₄ by the induction of beneficial mutation using gamma irradiation.

MATERIALS AND METHODS

Hardwood stem cuttings of S₃₄ mulberry genotypes were selected from young and healthy bushes. Each cutting measuring about 6-8 inches in length half inch in diameter bearing 3-4 active vegetative buds was preferred cuttings were irradiated with 4kR 5kR, 6kR, 7kR, 8kR, 9kR and 10kR doses of gamma rays from a Co 60 gamma rays source at Kidwai Memorial Institute of Oncology Bangalore-560021. In each dose 20 cuttings in three replications were used. The irradiated cuttings were planted in polythene bags and maintained providing necessary agricultural inputs for their further response. Comparable controls (untreated cuttings) were also maintained. Growth and Morphological characters of saplings were observed included sprouting, rooting, survivability percentage, plant height, number of branches, length of the petiole, leaf area etc., (R₁ generation). Phytochemical constituents and commercial characteristic features of cocoons were analyzed by following standard procedures (R₂ generations).

RESULTS

The stem cuttings of this taxon irradiated with different doses (4kR to 10kR) of gamma rays showed varied responses in sprouting behaviour. In general, sprouting percentage ranged from 55.00% to 75.66%. Similarly, number of days taken for sprouting differed with different doses of radiation. In control, the cuttings took 9 days for sprouting with a sprouting percentage of 85%. In the present study, it is evident that root development, decreased in a linear trend with the increase of gamma irradiation dosage.

TABLE-1. Effect of gamma irradiation on propagation and growth attributes of S₃₄ mulberry variety (R₁ generation)

Treatments	Sprouting (%)	Rooting (%)	Surviv-ability (%)	Height of the plant (cm)	Number of Branches	Internodal distance (cm)	Length of petiole (cm)	Leaf area (cm ²)	Number of flowers/ inflorescence	Length of inflorescence (cm)	Pollen fertility (%)
Control	85.00 ±2.88	80.00 ±2.88	80.00 ±2.88	166.26 ±2.00	5.00 ±0.57	3.71 ±0.01	3.46 ±0.17	164.14 ±3.78	36.66 ±1.76	3.12 ±0.33	82.20 ±2.54
4kR	75.00 ±2.88	75.00 ±2.88	73.33 ±4.40	158.26 ±2.96	6.00 ±1.15	3.37 ±0.19	3.33 ±0.18	171.69 ±0.34	29.33 ±1.45	2.80 ±0.10	78.36 ±1.59
5kR	71.66 ±1.66	70.00 ±2.88	63.33 ±1.66	149.50 ±5.62	5.66 ±0.88	3.54 ±0.05	3.63 ±0.28	172.51 ±0.62	32.00 ±3.21	2.80 ±0.09	72.16 ±3.93
6kR	73.33 ±1.66	85.00 ±2.88	60.00 ±2.88	172.70 ±2.86	6.33 ±1.20	3.12 ±0.22	3.10 ±0.57	174.02 ±5.15	37.66 ±2.84	2.92 ±0.04	66.39 ±5.72
7kR	70.00 ±2.88	65.00 ±2.88	56.66 ±4.40	156.16 ±3.59	5.66 ±0.88	3.83 ±0.05	3.30 ±0.20	166.86 ±0.49	27.66 ±1.45	2.52 ±0.08	57.88 ±7.65
8kR	65.00 ±2.88	60.00 ±2.88	55.00 ±2.88	140.73 ±0.92	4.66 ±0.33	3.79 ±0.07	3.83 ±0.14	160.97 ±0.27	31.00 ±2.64	2.65 ±0.15	54.42 ±5.06
9kR	61.66 ±1.66	50.00 ±2.88	51.66 ±1.66	134.20 ±2.34	4.66 ±0.33	3.84 ±0.05	3.74 ±0.11	160.99 ±0.98	31.66 ±2.18	2.54 ±0.11	48.81 ±6.77
10kR	55.00 ±2.88	45.00 ±2.88	48.33 ±1.66	125.13 ±2.48	4.33 ±0.33	3.83 ±0.06	3.63 ±0.21	159.77 ±0.64	27.00 ±1.52	2.44 ±0.08	45.86 ±5.87
SEM	2.597	4.225	4.190	4.594	1.188	0.160	0.279	2.739	3.240	0.213	3.840
CD@ 5%	5.570	9.063	8.987	9.854	2.549	NS	NS	5.875	6.951	0.458	8.236

TABLE-2. Phytochemical constituents of S₃₄ mulberry mutant recovered at 6KR gamma irradiation (R₂ generation)

Treatments	Maturity of leaves	Protein (%)	Reducing sugar (%)	Amino acid (µmoles/gf. wt.)	Chl - a (mg/gf.wt)	Chl - b (mg/gf.wt)	Total chlorophyll (mg/gf.wt)	Moisture content (%)	Moisture retention capacity (%)	
									6hrs	12hrs
Control	Tender	24.28 ± 0.496	3.38 ± 0.088	12.74 ± 0.126	2.04 ± 0.012	0.31 ± 0.005	2.41 ± 0.038	74.87 ± 0.225	76.52 ± 0.854	65.74 ± 0.897
	Medium	23.59 ± 0.01	2.73 ± 0.104	12.56 ± 0.107	2.20 ± 0.033	0.35 ± 0.005	2.53 ± 0.037	74.30 ± 0.202	74.76 ± 0.297	63.57 ± 0.413
	Coarse	22.40 ± 0.006	2.66 ± 0.143	11.98 ± 0.124	1.92 ± 0.045	0.26 ± 0.005	2.17 ± 0.034	70.66 ± 0.722	67.09 ± 0.420	57.51 ± 1.178
Mutant	Tender	25.49 ± 0.527	4.29 ± 0.156	13.35 ± 0.031	2.26 ± 0.005	0.41 ± 0.005	2.59 ± 0.061	76.09 ± 0.120	77.28 ± 0.279	66.14 ± 0.470
	Medium	24.26 ± 0.13	4.31 ± 0.011	12.89 ± 0.074	2.21 ± 0.052	0.39 ± 0.003	2.59 ± 0.023	74.67 ± 0.025	75.64 ± 0.337	63.99 ± 0.192
	Coarse	24.22 ± 0.337	3.89 ± 0.205	12.66 ± 0.049	2.07 ± 0.011	0.37 ± 0.005	2.46 ± 0.082	73.02 ± 0.041	69.02 ± 0.457	58.07 ± 0.337
SEM		0.356	0.120	0.096	0.028	0.005	0.044	0.339	0.379	0.583
CD 5%		NS	0.378	NS	0.088	0.018	0.409	0.068	NS	NS

TABLE-3. Rearing efficiency of CB silkworm race (PMxNB₄D₂) fed with the leaves of S₃₄ mutant recovered at 6KR gamma irradiation (R₂ generation)

Treatments	Average larval weight (g)	Single cocoon weight (g)	Single shell weight (g)	Shell weight (%)	Filament length (m)	Reelability (%)	Renditta	Denier	Effective rate of rearing (%)
Mutant	3.38 ± 0.050	1.80 ± 0.021	0.29 ± 0.058	15.53 ± 0.155	757.94 ± 17.789	94.49 ± 1.119	7.81 ± 0.380	2.20 ± 0.036	93.03 ± 1.962
SEM	0.072	0.039	0.008	0.269	15.186	0.932	0.391	0.060	2.800
CD@5%	NS	0.172	0.037	1.157	65.342	4.011	NS	NS	12.048

Control plants showed 80.00% rooting which is the optimum value required for the commercial exploitation of mulberry genotypes. Rooting percentage varied from 75.00% to 45.00% in different doses of gamma irradiation. Cuttings irradiated with 4kR and 5kR gamma rays showed slightly decreased rooting ability when compared to control. Further, cuttings, which are administered with 6kR gamma rays revealed better rooting ability (85.00%). Saplings recovered from the cuttings irradiated at 6kR gamma rays showed extensive root system both in spread and depth when compared to control. In the irradiated populations, survival percentage ranged from 73.33% to 48.33% when compared to control (80.00%). At higher doses (7kR to 10kR), this genotype exhibited poor growth with feeble and weak branches. On the other hand, reduction in survivability was not significant at lower doses of gamma rays (4kR to 6kR). Survival percentage was maximum at 4kR (73.33%) and minimum at 10kR (48.33%). LD₅₀ for this taxon was found to be around 9kR since the survivability percentage was 51.66% (Table-1).



FIGURE 1-4: 1. Control plant, 2. Mutant plant (6kR), 3 & 4 cuttings irradiated at 9kR and 10kR Gamma irradiation.

Plant height and leaf area was found to be decreased with increase in dose of gamma rays showing negative correlation. The marginal reduction in plant height, stunted growth, deformities in growth like fasciation, plant with weak and feeble branches, plants bearing small leaves with wrinkled and coriaceous texture were noticed in the populations of cuttings irradiated at 9kR and 10kR. However, some of the populations irradiated with 6kR gamma rays exhibited increased plant (172.70cm) height

when compared to control (166.26cm). These variants also showed increasing leaf area with thick, succulent and dark green leaves (Fig-1). Significant results were noticed with respect to internodal distance, many irradiated saplings revealed decreased internodal distance when compared to control (Table-1).

No significant variation was observed in length of the inflorescence at any of the treatments. Inflorescence at higher dosages did not attain maturity, often-deformed inflorescence was observed. Pollen fertility lowered which gradually decreased with increased dosage of gamma rays. Total foliage yield per plant and average weight of leaves (100Nos) was greater (383.44g) when compared to control (343.46g). Initially, the average yield determined to be 0.611kg/bush compared to 0.548kg in the control counterparts in the R₂ generation.

Phytochemical Studies

Protein, reducing sugars, amino acids, chlorophyll-a, chlorophyll-b and total chlorophyll were estimated in the mutants grown in the R₂ generation. In the mutant, the amount of proteins in tender, medium and coarse leaves were 25.49%, 24.26% and 24.22% respectively which are higher than the protein percentage in the control (24.28%, 23.59 % and 22.40 %) (Table-2). Amount of reducing sugar present in tender medium and coarse leaves of induced mutant were 4.29 %, 4.31 % and 3.89 % respectively which are higher than the control (Table-2). Amino acid content was estimated to be 13.36 μ moles/gf.wt., 12.89 μ moles/ gf.wt. and 12.66 μ moles/gf.wt. respectively in tender, medium and coarse leaves of induced mutants as against the values 12.74 μ moles/ gf.wt., 12.56 μ moles/ gf.wt. and 11.98 μ moles/g f.wt. found in control (Table- 2). Chlorophyll-a was found to be 2.26, 2.21 and 2.07mg/gf.wt. respectively in tender, medium and coarse leaves of induced mutants compared to 2.04, 2.20 and 1.92mg/gf.wt. for the control. Chlorophyll-b and total chlorophyll were also found to be higher in induced mutants compared to control plants of S₃₄ mulberry variety (Table-2). Moisture percentage was found to be slightly higher in mutant with 76.09%, 74.67% and 73.02% in tender, medium and coarse leaves respectively as against 74.87%, 74.30% and 70.66% of the control. Similarly, moisture retention capacity at 6 hrs and 12 hrs of mutant was also found to be higher than the control (Table-2).

Various commercial characteristics like ERR, Larval weight, Cocoon weight, Shell weight, Shell weight percentage, Filament length, Reelability percentage, Renditta and Denier were recorded. Larvae fed with the leaves of induced mutants showed an average weight of 3.38gms, which was higher than control (3.26gms). Average cocoon and shell weight were found to be 1.80gms and 0.29gms respectively in case of mutants compared to control (1.67gms and 0.26gms). Higher shell weight percentage was also encountered in the mutants. Similarly filament length was found to be higher in the mutants (757.94m) compared to control (685.125m). Renditta and Denier were also found to be better in the leaves of induced mutants of S₃₄ mulberry variety. (Table-3).

DISCUSSION

Gamma irradiation studies revealed that the lower dosages (4kR & 5kR), in general were less productive in the induction of mutations whereas at the higher dosages (8kR, 9kR & 10kR) deformity and inhibition of growth leading to semi lethality to complete lethality were observed. The morphological leaf mutants like oblong leaf with wrinkled lamina, variants with slender branches bearing small deformed leaves and mutants with increased lamina (Broad leaves) were secured in irradiated populations. Similar findings have been reported in large number of mulberry genotypes obtained through irradiation by several workers (Gehan, G. Mostafa, 2011; Luvaha *et al.*, 2008; Imran Kozgar *et al.*, 2011; Katagiri, 1970; Tarar, 1970; Kuchkarov and Ogurtrou, 1987; Dandin *et al.*, 1996; Sawhney, *et al.*, 1977). On the other hand, the moderate dosage like 6kR was found to be fruitful in the induction of beneficial mutants. Hazama *et al.*, (1968) studied the varietal defences to radio sensitivity and bud mutations of mulberry trees in gamma irradiation populations and found the inhibition of height and branching pattern. Similar results have also been reported by Rao *et al.*, (1984), Jayaramaiah (1987) and Ramesh (1997) in S₅, S₅₄ and local mulberry varieties due to the effect of irradiation. Katagiri (1976a) reported deformation in leaf and inhibition of growth at higher doses of gamma rays in mulberry variety Ichinose. Induced beneficial mutants of S₃₄ variety exhibited constructive improved trend in the amount of protein, reducing sugar, amino acid and chlorophyll content which is important from the dietary point of silkworm (Ito and Arni, 1963, Ito and Nimura, 1966). Protein in the main constituent of mulberry leaf which plays a vital role in the development of silk gland (Anfinsen, *et al.*, 1958; Fukuda, *et al.*, 1959; Qader, 1987; Bongale, *et al.*, 1993, 1995; Bose, *et al.*; 1991). It has been proved that the silkworm derives about 70% of proteins from the mulberry leaves for the biosynthesis of silk (Fukuda, *et al.*, 1956; Kaware, 1975). Amino acid and reducing sugar are also required for the synthesis of fibroin and sericin (Vijayan, *et al.*, 1997; Dorcus and Vivekanandan, 1997; Sastry, *et al.*; 1988; Muniswamy Reddy and Munirajappa, 2005). Effect of moisture content on silkworm rearing is well documented (Ito, 1963). Low leaf moisture is known to affect the growth and development of silkworm larvae.

The effective rate of rearing, larval weight, cocoon weight, shell weight, shell percentage and silk reeled (average filament length, renditta, denier, reelability percentage) data related to worked out the beneficial mutant mulberry leaves fed with the cross breed silkworm larvae (PM×NB₄D₂). The mean values for these parameters with regard to both the beneficial mutants compared to their respective control counterparts were found to be in the humanizing trend reflecting the superiority of mutants obtained in the present investigation. The percentage of improvement recorded in the larval weight (6.90%) filament length (8.90%) reelability (7.02%) renditta (7.45%) denier (6.54%) and effective rate of rearing (11.01%) with regard to mutants recovered from S₃₄ mulberry variety. The present study clearly establishes the domination of the induced mutants secured in the present work. Comparable results have also been reported by the

earlier workers (Sastry, *et al.*, 1969, Mastfaev, 1968, 1970, 1971; Badalov, 1971; Das and Prasad, 1974; Sikdar, 1993; Bongale, Chaluvachari, 1995; Ramesh, 1997, 2001 and Chopra, 2005).

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