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Induction of Useful Mutation in Mulberry (*Morus*) Variety S_{54} by Gamma Irradiation in M_1 Generation

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Authors' contributions

Present research work was carried out in collaboration between all authors. Author HLR conducted complete analysis of the study, wrote the protocol and drafted of the manuscript. Author VNYM managed literature survey and computer word processing. Author MR designed the experiment and supervised overall experiment. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Plentiful mulberry varieties available in nature, they lack one or the other important economic trait required for silkworm *Bombyx mori* L. as food. Efforts have been made to induce phytomorphological variability in mulberry variety S_{54} using gamma rays.
Experimental Design: RBD Method with three replications/treatment was followed.
Place and Duration of Study: Mulberry garden, Department of Sericulture, Jnana Bharathi, Bangalore University and Mist chamber, Indian Institute of Horticultural Research (IIHR), Bangalore, Karnataka, India between 2006-2011.
Methodology: Gamma ray (1kR-10Kr) was used to induce variability in juvenile twigs of mulberry for various agro-botanical characters viz., sprouting, rooting, internodal distance, leaf area, plant height etc. and leaves were subjected to biochemical analysis.
Results: Mulberry variety S_{54} showed linear decrease in growth parameters with the increased gamma ray dosage and plants exhibited variability with increased rooting

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(81.33%), plant height (147.86cm) and leaf area (146.22cm^2) when compared to control in M_1 generation at 7kR. Mutants showing favourable characters were grown for M_2 generation which exhibited marked improvement in growth and yield parameters. Biochemical constituents in S_{54} mutant leaves recorded at 7kR showed increased proteins, carbohydrate, chlorophyll a and b.

Conclusion: Mulberry cuttings irradiated with gamma ray (7kR) exhibited favourable traits in rooting, plant height and leaf area over the control in M_1 generation and mutants were grown for M_2 generation and marked improvement in growth, yield and bio-chemical parameters were observed.

Keywords: Mulberry; gamma; irradiation; agrobotanical; yield; proteins; total sugars; chlorophyll.

1. INTRODUCTION

Mulberry is a fast growing, sub arboreal deciduous plant found in tropical, subtropical and temperate climates of northern hemisphere and is capable of thriving under wide range of agro-climatic conditions. Mulberry exhibits plasticity and is a versatile plant. Successful exploitation of various mutagenic agents for inducing aberration has become one of the most important lines of contemporary research. S_{54} mulberry variety leaves are well suited for rearing young age (Chawki) silkworms. Mutation induction in mulberry started towards the end of 1950's in Japan [1,2,3]. 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 [4]. Radiation is a tool for inducing variability in crop plants [5]. Investigation pertaining to the radiation effect was reviewed [6]. Radiation such as x-rays and gamma rays found to affect biological events such as survival percentage, seed germination, growth and yield of the plant [7,8,9]. Many workers used physical mutagens for induction of variability in mulberry [10,11,12]. Expose to gamma radiation is known to produce morphological mutants, physiological and biochemical mutants [13]. Mutation breeding make use of the possibility of altering the genes by exposing different parts of mulberry plants to physical mutagens [14,15]. Present investigation aims at improving morpho and phytochemical traits of already existing mulberry cultivar S_{54}.

2. MATERIALS AND METHODS

2.1 Study Area

Mulberry variety S_{54} was procured from mulberry germplasm bank maintained at Jnana Bharathi Campus, Bangalore University, Bangalore. The field experiment was conducted at mulberry germplasm bank maintained at Jnana Bharathi Campus and laboratory experiments were done at Indian Institute of Horticultural Research (IIHR), Bangalore and Moriculture Laboratory in the Department of Sericulture, Bangalore University.

2.2 Sampling and Sample Analysis

Juvenile twigs of S_{54} mulberry genotype were used for cuttings preparation, only middle part of the twigs were taken. 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-560088. Irradiation was conducted during summer months and replications were maintained for the calculation of mean values of all the parameters studied. 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 maintained for six months before transplanting them in to the main field. Transplanted twigs were planted in randomized block design (RBD) 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 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 [16,17,18].

2.3 Statistical Analysis

Data collected on various parameters were tabulated using “Method of Analysis of Variance” appropriate to the experimental design [19,20].

3. RESULTS AND DISCUSSION

Sprouting percentage in the irradiated population of S_{54} mulberry variety ranged from 78.49% to 36.28% when compared to control plants (92.48%). Sprouting percentage decreases with the increase in doses of gamma rays (Table 1). Control plants sprouted 5^{th}-6^{th}day after planting and plants treated at 1kR-5kR gamma rays took 11-13 days to sprout. Higher doses (6kR-10kR) took 15 days to sprout. Drastic reduction in the sprouting percentage was observed in cuttings irradiated with 7kR-9kR. At 10kR, though the irradiated cuttings sprouted initially, they failed to grow further exhibiting complete lethality. Present results are in conformity with the findings of other workers [21,22]. Sprouting is adversely affected by higher doses of gamma rays. Sensitivity of plant material depends on the genetic constitution, DNA amount, dose employed, replication at initial stages, stage of development and genotype. Gamma rays are highly penetrating in nature, might have developed cells which are undergoing meiotic division in bud region [23]. Decrease in sprouting percentage with the increase in gamma ray dosage is due to partial cell death and also due to destruction of auxin or due to inhibition of auxin synthesis [24,25,26]. Reduction in sprouting and survival percentage of vegetatively propagated crops was reported by several workers [27,28]. Rooting percentage revealed that, control plants of S_{54} mulberry variety showed 88.33% of rooting. The irradiated hardwood stem cuttings of this taxa exhibited varied responses to different doses of gamma rays. Rooting was not affected much at 1kR and 2kR. However, rooting percentage was decreased from 3kR-9kR. It is interesting to note that at 7kR 81.33% of rooting was observed. Rooting behaviour of a variety is purely a genetic character [29]. Spontaneous mutants of KNG variety produced low percentage of rooting (16%-32%) when compared to control (96%) [30]. Survivability in S_{54} mulberry cultivar was recorded 88%. In treated population, considerable decrease in survivability percentage was observed. Data recorded in the present investigation revealed that, in lower doses (1kR and 2kR), treated population exhibited better survival rate and at higher doses considerable

decrease in survivability was noticed. At 8kR and 9kR, plants showed stunted growth, weak and feeble branches. Survivability percentage was maximum at 1kR (81.49%) and minimum at 8kR (35.18%). Destruction of auxin in treated plants may be the reason for decrease in survival percentage after radiation [31]. Series of events occurring at cellular level affect the vital macromolecules and results in physiological imbalance [32]. Low doses of gamma ray irradiation could be used as safe as well as effective method in mulberry and survivability depends on the disturbances caused at the physico-chemical level in cells or acute chromosomal damage or due to combined effect of both [33]. Retardation of root growth is one of the most common responses of plant subjected to ionizing radiation. 10kR exposure induced 100% inhibition of growth in Pinus [34]. Fasciation induced by gamma rays was observed in *Gerbera jamesonii* and this may be due to the triggering of gene responsible for hormonal action of cytokinins production due to point mutation [35].

Gamma rays known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetical changes in cells and tissues. Reduction in plant height may be attributed to a drop in auxin level, inhibition of auxin synthesis or decline in assimilation mechanisms [36]. Growth parameters such as height of the plants in the treated population ranged from 33.32cm to 147.86cm. Control plants revealed a mean height of 138.98cm. In general, height decreased in the plants irradiated at 5kR-9kR. However, at 7kR slightly increased height was recorded (147.86cm) than in control. At 10kR though the irradiated cuttings sprouted initially, they did not grow further. Branching pattern of treated saplings was varied depending upon the doses of gamma rays administered. Maximum branching was observed at 6kR and 7kR and it was adversely decreased at higher doses (8kR and 9kR). In the treated population, internodal distance was not affected much. However, a marginal decrease in internodal distance was noticed at 2kR (2.75cm). Leaf area was found highest at 7kR (146.22cm^2) and least at 9kR (72.67cm^2). In control plants leaf area was 144.26cm^2. Decreased leaf area was observed at higher doses (8kR and 9kR). At 8kR, mutants having boat shaped leaves with wrinkled texture were observed. At 9kR, mutants with yellow sectored leaves noticed. Petiole length remained more or less unaltered in treated populations except in the populations irradiated with 1kR (3.06cm) and 5kR (3.01cm).similarly, inflorescence length was found more or less unaltered. Number of flowers per inflorescence showed some variations with 13.33 at 5kR and 18.0 at 6kR compared to control 26.28. Deformity in inflorescence shape was observed in one of the populations irradiated at 7kR. Pollen fertility decreased with increase in gamma rays doses. Among the plants treated at 7kR beneficial mutants were selected for rising M_2 generation. Various propagation and growth parameters were recorded for M_2 generation along with control. Growth parameter like sprouting recorded an increase by 1.02% over control plants. Cuttings took 11 days to sprout. Rooting percentage was increased by 2.03% over control. Increase in survivability percentage was observed to the extent of 1.01%. Marginal increase in height and number of branches of the mutant plant was noticed. These mutants also exhibited shortened internodal distance and increased leaf area when compared to control. This accounted for an increase in yield by 13.09% than control. Plant height is a quantitative trait and is mainly controlled by polygene and each gene contributes small effects, which is called genetic addictive effect [37]. Mutations are not stable and undergo recombination during meiosis [38]. Multicellular organisms have the ability to recover from sub lethal doses of ionizing radiations and selecting the desired mutant is controlled by a single gene [39]. Reduction in plant height due to an increasing production of active radicals that are responsible for lethality or due to increasing radiation induced gross structural chromosomal changes [40]. Gamma ray inhibition of growth may be due to distraction or damage to apical meristem [41]. Survival of plants at maturity and reduction in plant height depends on the nature and extent of chromosomal damage [42,43]. In M1 generation, significant increase in

plant height at 2.5kRD and stunted growth of plant at 5kRD was observed. Slow growth in higher doses of gamma irradiation and increase/decrease in plant height at lower and higher doses respectively was due to auxin synthesis. At higher doses, delayed and stunted growth was observed in dahlia [44]. Irradiation of lower doses of gamma rays significantly improved vegetative traits while higher doses of gamma rays proved depressing for same parameters [45]. Formation of new shoots is decreased due to increased dosage of gamma rays. Gamma rays are more potent and highly penetrating, might have developed cells that were undergoing meiotic division in the bud-region. Increased gamma-ray dosage has direct negative effect on plant tissue and mutation can be lethal. Primary injuries are due to the 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 [46]. Internodal distance was found to be affected by cell number and length or both in barely [47]. Similar findings were observed in gamma irradiated Mysore local mulberry variety [48]. Somatic mutation occurs when mutant cells continue to divide, individual cell contain a patch of tissue with genotype different from rest of the body cells and also due to karyotype changes, point mutations, somatic crossing over and gene arrangement, changes in DNA amplification and segregation of pre-existing chimera tissue variation in leaves occur [49]. It was also attributed to the disturbances in phytochromes, chromosomal aberrations, mitotic inhibition, disrupted auxin synthesis, disturbances in DNA synthesis etc. [50,51,52,53]. Radiation induced pollen sterility has been reported by various researchers in crop plants [54,55].

Biochemical parameters like proteins, total soluble sugars, amino acids, phenolic content, chlorophyll-a, chlorophyll-b, total chlorophyll and moisture content were studied in both mutant and control plants of S_{54} variety. Analysis was carried out in tender, medium and coarse leaves separately. Amount of proteins found to be 10.04%, 9.49% and 9.23% respectively in tender, medium and coarse leaves of mutants when compared to control (tender 9.68%; medium 9.29%; coarse 8.87%). Medium leaves of mutant showed 9.49% of protein when compared to 9.29% of control. Whereas in case of coarse leaves of control, protein content were 8.87% much lesser than treated plants (9.23%). Total soluble sugars present in tender (8.58%) and coarse (8.17%) leaves of mutants were higher compared to control (8.34% in tender and 7.92% in coarse leaves). But in case of medium leaves, total soluble sugars were slightly lesser in mutants (8.01%) than in control (8.04%). Amino acid content was found higher in all the orders of leaves. In control variety, 64.62µmole/gf.wt., 60.49µmole/gf.wt. and 53.29µmole/gf.wt. in tender, medium and coarse leaves respectively compared to 62,57µmole/gf.wt. 58.61µmole/gf.wt. and 52.74µmole/gf.wt. of mutant population. Phenolic content in tender and coarse leaves of the control plants were higher (98mg/gf.wt., 8.02mg/gf.wt.) compared to mutant (6.78mg/gf.wt., 7.98mg/gf.wt.). However, it was higher in medium leaves of mutant (7.43mg/gf.wt.) compared to the control (7.29mg/gf.wt.). With respect to chlorophyll-b, higher amount was present in tender leaves of control (1.04mg/gf.wt) compared to mutant (0.98mg/gf.wt). In medium leaves of mutant plants it was higher (1.43mg/gf.wt) compared to control (1.29mg/gf.wt). Chlorophyll-a was higher in tender, medium and coarse leaves of mutant (2.70, 3.98, 4.01mg/gf.wt respectively) compared to 2.67, 3.52 and 3.98mg/gf.wt of control plant. Total chlorophyll was 4.01, 4.09, 5.02mg/gf.wt in control leaves population compared to three different orders of the mutant leaves such as 3.98mg/gf.wt, 5.14mg/gf.wt and 5.28mg/gf.wt. Regarding moisture content, it was higher in all three grades of leaves in mutant plants (69.4%, 65.2% and 63.4%) compared to 68.7%, 64.2% and 62.3% in tender, medium and coarse leaves respectively (Table 2).

Table 1. Effect of gamma irradiation on propagation and growth attributes of S_{54} mulberry variety at M_1 generation

Treatment	Sprouting (%)	Rooting (%)	Survival (%)	Plant height (cm)	Number of branches	Internodal distance(cm)	Leaf area (cm^2)	Petiole length(cm)	No. of flowers/ inflorescence	Inflorescence length (cm)	Pollen fertility (%)
Control	92.48	88.33	88.00	138.98	5.88	3.81	144.26	3.29	26.28	3.01	88.29
1kR	72.19	79.10	81.49	131.14	6.01	3.85	129.31	3.06	22.09	2.81	81.46
2kR	78.49	81.27	79.48	128.78	6.14	2.75	134.27	3.21	25.18	2.83	83.29
3kR	67.20	73.14	68.17	114.57	5.78	3.98	131.13	3.24	24.26	2.79	76.16
4kR	53.18	61.18	54.33	101.68	5.97	3.78	128.29	3.18	23.23	2.66	65.24
5kR	49.28	59.78	47.08	94.18	6.18	3.81	101.01	3.01	13.33	2.69	61.17
6kR	51.06	51.39	49.98	88.68	6.73	3.68	98.47	3.29	18.00	2.74	63.28
7kR	44.19	81.33	40.30	147.86	6.71	2.99	146.22	3.21	20.18	2.52	56.29
8kR	36.28	42.78	35.18	34.18	4.16	2.87	82.29	3.18	18.29	2.56	38.24
9kR	38.24	29.16	38.24	33.32	4.09	2.80	72.67	3.11	19.14	2.51	27.39
10kR	--	--	--	--	--	--	--	--	--	--	--
SEM	--	--	--	2.48	0.86	0.47	7.29	0.67	3.04	0.29	8.24
CD @ 5%	5.1	5.4	4.3	3.57	1.91	1.01	8.76	1.04	4.79	0.78	10.98

Table 2. Biochemical constituents in the leaves of S_{54} mulberry mutant recovered at 7kR gamma irradiation at M_2 generation

Treatment	Leaf maturity	Proteins (%)	Total soluble sugars (%)	Amino acids (µmole/gf.wt.)	Phenols (mg/gf.wt.)	Chlorophyll-a (mg/gf.wt.)	Chlorophyll-b (mg/gf.wt.)	Total Chlorophyll (mg/gf.wt.)	Moisture (%)
Control	Tender	9.68	8.34	64.62	6.98	2.67	1.04	4.01	68.7
	Medium	9.29	8.04	60.49	7.29	3.52	1.29	4.09	64.2
	Coarse	8.87	7.92	53.29	8.02	3.98	1.69	5.02	62.3
Mutant	Tender	10.04	8.56	62.57	6.78	2.70	0.98	3.89	69.4
	Medium	9.49	8.01	58.61	7.43	3.98	1.43	5.14	65.2
	Coarse	9.23	8.17	52.74	7.98	4.01	1.47	5.28	63.4
SEM	--	0.96	--	1.04	--	0.76	--	--	0.94
CD @ 5%	--	1.04	NS	1.58	NS	0.96	NS	NS	1.68

Leaf quality is influenced by number of factors such as variety, cultivation practices, incidence of pests and diseases, method of harvesting and preservation of leaves [56]. Number of workers have reported different traits such as protein, amino acid, carbohydrate, nitrogen, chlorophyll contents and leaf moisture are responsible for mulberry leaf quality [57,58] and one single variety consists of all the nutrients at the highest level [59,60].

4. CONCLUSION

Mulberryis highly versatile and polygenic plant exhibits considerable plasticity. Juvenile twigs of S_{54} treated with varying doses of gamma ray (1kR-10kR). Plants irradiated at 7kR showed considerable variation in M_1 generation compared to control plants with respect to rooting, plant height and yield parameters. Cuttings of mutants were grown in M_2 generation and plants recovered indicated remarkable beneficial agro botanical parameters like rooting, survivability, plant height, yield and biochemical constituents over plants grown in M_1 generation. However, further systematic yield trials and evaluation of these variants over a period will establish their potentiality as cultivars.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sugiyama T, Tojyo I. Studies on the effect of irradiation on bud of mulberry cutting in the hybridization. Bull Seri ExpSta Tokyo. 1962;18(2):115-132.
2. Tojyo I. Studies on polyploidy mulberry tree I. Breeding of artificial autotetraploids. Bull. Seric. Expt. Stn. Jpn. 1966;20(3):187-207.
3. Hazama K. Induced mutations and plant breeding methods in vegetatively propagated species. J Seric SciJpn. 1967a;36(4):346-352.
4. Ramesh HL. Induction of variability for important morpho-economic traits in mulberry. Ph.D. Thesis. Bangalore University, Bangalore, India; 1998.
5. Stadler LJ. Genetic effects of x-rays in maize. ProcNatlAcad Sci. USA. 1928;14:69-75.
6. Sparrow AH, Konark CF. The use of radiation in plant breeding accomplishment and prospects. Cam Cult. 1958;425-452.
7. Mishra K, Raghuvanshi SS. Cytogenetic effect of gamma ray irradiated stored seeds of *Trigonellafoenumgraecum*. Cytologia. 1988;54:33-36.
8. Kumar N, Prasad RR. Gamma rays induced morphological variation in *Brassica rapa*. J Indian Bot Soc. 1990;69:453-454.
9. Acharya NN, Tiwari DS. Effect of gamma rays on seed germination, survival and pollen fertility of *Hamatocactussetispinus* in M_1 generation. Mysore J Agric Sci. 1995;30:10-13.
10. Aliev MO. Use of chemical mutagens combined with hybridization of mulberry forms differing in ploidy. Shelk. 1977;1:7-8

11. Fujita H, Yokoyama T, Nakajima K. Re-treatment of induced mulberry mutants with gamma-rays. Technical News. 1980;23:28-29.
12. Kukimura H, Ikeda F, Fujitha H, Maeta T, Nakajima K, Katagiri K, Nakahira K, Somegou M. Genetical, cytological and physiological studies on the induced mutants with special reference to effective methods for obtaining useful mutants in perennial woody plants. In: Improvement of vegetatively propagated plants through induced mutations, Tokai, *IAEA*, Vienna. 1975;83-104.
13. Songsri P, Suriharn B, Sanitchon, Kesamala T. Effect of gamma radiation on germination and groth characteristics of *Jatropacurcas* L. J. Biol. Sci. 2011;11(3):268-274.
14. Dwivedi NK, Sikdar AK, Jolly MS. Colchicine induced variant in mulberry(*Morus alba* var. $Kanva_2$). Indian J Seric. 1987;26(2):93-97.
15. Mitra PK, Bhowmik G. Estimation of mutagenic effectiveness and efficiency of physical and chemical mutagens in *Nigella*sativa L. Ad Plant Sci. 1999;12(2):373-378.
16. Dandin SB, Jolly MS. Mulberry descriptor. Sericologia. 1986;26(4):465-475.
17. Shamachary, Jolly MS. A simple device for quick determination of mulberry leaf area in the field. Indian J Seric. 1988;27(1):51-54.
18. Sanjappa M. Geographic distribution and exploration of the genus *Morus* L. (*Moraceae*). In: Genetic resources of mulberry and utilization. Ed. By Sengupta K, Dandin SB. Central Sericultural Research & Training Institute, Mysore. 1989;4-7.
19. Sundararaj GL, Nagaraju MN, Venkataramu, Jaganath. Design and Analysis of field experiments. U.A.S., Misc., Series No. 22, Bangalore, India. 1972;424-440.
20. Singh RK, Choudhury BD. Bio-metrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi; 1979.
21. Rao P, Rao JMM, Sarojini NL. Mutation breeding in mulberry *Morus indica* L. Indian J Bot. 1984;7(1):106-111.
22. Tikader A, Vijayan K, Roy BN, Pavankumar T. Studies on propagation efficiency of mulberry [*Morus* spp.] at ploidy level. Sericologia.1996;36(2):345-349.
23. Deshpande KN, Mehetre SS, Pingle SD. Effect of different mutagens for induction of mutations in mulberry. Asian J ExpBiolSciSpl. 2010;104-108.
24. Katagiri K. Varietal differences of mutations rate and mutation spectrum after acute gamma ray irradiation in mulberry. J Seric SciJpn. 1970;39(3):194-200.
25. Skoog F. The effect of radiation on auxin and plant growth. J Cell Comp Physiol. 1935;7:227-270.
26. Gordon SA. The effect of ionizing radiation on plants, bio-chemical and physiological aspects. Quant Rev Biol. 1957;32:3-14.
27. Banerji BK, Datta SK. Induction of somatic mutation in chrysanthemum cultivar 'Anupam'. Journal of Nuclear Agriculture Biology. 1991;19:252-256.
28. Hemalatha K. Induction of mutation in carnation (*Dianthus caryophyllus* L.) through gamma rays and EMS. Ph.D. Thesis. University of Agricultural Sciences, Bangalore, India; 1998.
29. Hartman HT, Kester DE. Plant propagation-Principles and Practices. Prentice Hall of India. 1976;120-135.
30. Fujitha H, Wada M. Studies on mutation breeding in mulberry (Morus spp.) In: Induced mutation in vegetatively propagated plants. *IAEA*. Vienna. 1982;249-279.
31. Smith GF, Kersten H. Auxin in seedlings from x-rayed seeds. Amer J Bot. 1942;29:785-819.
32. Gray E. Evidence of phenotypic plasticity in mulberry (*Morus* L.). Castanea. 1990;55(4):272-281.
33. Nakajima K. Induction of useful mutation in mulberry by gamma irradiation. Japan Agricultural Research Quarterly. 1972;6(4):195-198.

34. Thapa CB. Effect of acute exposure of gamma rays on seed germination and seedling growth of Pinuskesiyagord. Our Nature. 2004;2:13-17.
35. Singh S, Dhyani D, Kumar A. Expression of floral fasciation in gamma ray induced *Gerbera jamesonii*mutants. J. Cell & Plant Sci. 2011;2:7-11.
36. Girija A, Dhanavel D. Mutagenic effectiveness and efficiency of gamma rays, ethyl methyl sulphonate and combined treatments in *Vignaungniculata*L. Walp. J. Mol. Sci.2009;4:68-75.
37. Thohirah Lee Abdullah, JohariEndan, MohdNazir B. Changes in flower development, chlorophyll mutation and alteration in plant morphology of *curcuma alismatifolia*by gamma irradiation. American J. Applied Sciences. 2009;6(7):1436-1439.
38. Anon. Manual on mutation breeding. Technical report series. IAEA, Vienna.1977;(119):169-192.
39. Brunner H. Methods of induction of mutations. Plant breeding Unit, Joint FAO/IAEA Programme, *IAEA* Laboratories, Seibessdorf, Austria; 1995.
40. Selim AP, Hussein HAS, Kishawaf IIS. EMS and gamma ray induced mutation in *Pisum sativum* L. II Effect of EMS and gamma rays on M_1 generation seedling height fertility. Egyp J Genet Cytol. 1974;3:172-192.
41. Patel JD, Shah JJ. Effect of gamma irradiation on seed germination and organization of shoot apex in *Solanummelongena* and *Capsicum annum*. Phytomorphology. 1974;24:174-180.
42. Banerji BK, Datta SK. Induction and analysis of gamma ray induced flower head shape mutation in *Chrysanthemum*. Indian journal of Agricultural Sciences.2002;72:6-10.
43. Tiwari AK, Srivastava RM, Kumar Vijai, Yadava LB, Mishra SK. Gamma rays induced morphological changes in *Gladiolus*. Progressive Agriculture. 2010;10:75-82.
44. Dwivedi AK, Banerji BK. Effect of gamma irradiation on *Dahlia*. Journal of Ornamental Horticulture. 2008;11:148-151.
45. Sumira J, Parween T, Siddiqui TO. Gamma radiation effect on growth and yield attributes of *Psoraleacorylifolia*L. with reference to enhanced production of Psoralen. Plant Growth Regulators. 2011;64:163-171.
46. Nazir MB, Mohamad O, Affida AA, Sakinah A. Research highlights on the use of induced mutations for plant improvement in Malaysia. Malaysian Institute for Nuclear Technology Research (MINT), Bangi; 1998.
47. Blonstein AD, Gale MD. 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. 1984;19-29.
48. Jayaramaiah VC, Munirajappa. Induction of mutations in mulberry variety (Mysore Local' by gamma-irradiation. Sericologia. 1987;27(2):199-204.
49. Kearsey MJ, Pooni HS. The genetic analysis of quantitative traits. Plant genetic group school of Biological Science, University of Birmingham, U.K. Chapman and Hall, ISBN: 0412609800. 1996;381-395.
50. Matsumura S, Fuji. Induction of bud sprouts by x-rays and gamma-rays. Ann rep Nat Inst Genet Japan. 1957;8:94-95.
51. Abraham A, Ninan CA. Genetic improvement of the coconut palm: Some problems and possibilities. Indian J Genet and Plant Breeding. 1968;28A:142-153.
52. Mickaelsen K, Ahnstrom G, Li WC. Genetic effects of alkylating agent in barley. Influence of past-storage, metabolic state and pH of mutagen solution. Heriditas. 1968;59:353-374.
53. Kuchkarov U, Ogurtsov KU. Spontaneous mutants of mulberry. Shelk. 1987;6:3-4.
54. Yamaguchi. Classifications of Japanese upland rice varieties by inter varietal hybrid sterility. Japan J Breeding. 1963;13(4):217-23.

55. Singh A, Roy RP. X-ray irradiation studies on *Trigonellafoenum-graecum* L. J Genet Iber. 1991;23:49-66.
56. Krishnaswami S, Roy D, Mukherjee SK. Yield and nutritive value of mulberry leaves as influenced by planting system, spacing and frequency of pruning. Indian J Seric. 1970a;9(1):38-42.
57. Chaluvachari, Bongale UD. Bioassay moulting response of silkworm *Bombyx mori* L. in relation to leaf nutritive constituents in mulberry (*Morus* spp.) genotypes.Indian J Seric.1996;35(2):160-162.
58. Bongale UD, Chaluvachari, Mallikarjunappa RS, NarahariRao BV, Anantharaman MN, Dandin SB. Leaf nutritive quality associated with maturity levels in fourteen important varieties of mulberry (*Morus spp.*). Sericologia. 1997;37(1):71-81
59. Sujathamma P, Dandin SB. Leaf quality evaluation of mulberry (*Morus* spp.) genotypes through chemical analysis. Indian J Seric. 2000;39:117-121.
60. Bose PC, Bindroo BB. A comparative biochemical study of seven promising mulberry (*Morus alba L.)* varieties under rainfed condition of sub-tropical region. Indian J Seric.2001;40:171-173.

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