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ORIGINAL ARTICLE

Effect of different mutagens for induction of mutations in mulberry.

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ABSTRACT
The effect of different doses of gamma irradiation (0kR, 3kR, 6kR, 9kR, 12kR) and Ethyl Methane Sulphonate [EMS 0.0%, 0.1%, 0.2%, 0.3%, 0.4%] on mulberry [*Morus indica* L.], cvs. S_{1635}, S_{36} and V_1 have been studied with regards to survival, sprouting percentage. In M_1 generation, it is clear from the statistical analysis of morphological characters that the sprouting and survivability of mulberry genotypes were considerably influenced by the gamma irradiation. 9kR and 12 kR doses showed decrease in sprouting percentage in all cultivars. The genotype S_{1635} showed better result in EMS solution at 0.2% and 0.4% concentration w.r.t. sprouting per cent and survival per cent.
KEYWORDS: Mutation breeding, Gamma irradiation, EMS

INTODUCTION

Reduction in cost of production of silk cocoon is closely associated with qualitative and quantitative improvement of mulberry. Hence production of quality mulberry leaves is a pre requisite for the healthy growth and development of silkworm and silk production. Mulberry [*Morus sps*.] is a highly heterozygous, perennial plant and so there is a great scope to induce mutations artificially. It is also helpful to eliminate certain undesirable characters and to improve mulberry plant by qualitatively and quantitatively. Mutation breeding with physical and chemical mutagens is more widely used than conventional breeding procedures, in order to get a wide range of variants with respect to the polygenically controlled characters.

Varieties of mulberry respond differently to mutation rate and mutation spectrum after gamma ray treatment. [1]. Similar results were also reported by Sastry et al. [2] when different chemical mutagens were used for improvement of mulberry varieties.

The present investigation reports the effect of gamma irradiation and Ethyl methane sulphonate (EMS) on some morphological characters.

MATERIAL AND METHODS

A set of 50 cuttings of each variety having the 3-4 buds and about 15-20 cm long were exposed to 3 kR, 6kR, 9kR, 12 kR /hr [60 Co source] from BARC Trombay, Mumbai. Total 200 cuttings of each variety were subjected for gamma irradiation. A set of control [50 cuttings] were maintained for gamma irradiation as well as EMS was prepared with various concentrations [0.1%, 0.2%, 0.3%, 0.4% EMS] and treated for 12 hrs at 25^0c. Total 200 cuttings of each variety were selected for EMS treatment by cotton swab method. A set of control [50 cuttings] were maintained for EMS treatment. In all 1500 treated cuttings of three varieties [500 cuttings of each variety] were planted at the distance of3' X2' in split-split plot design. All recommended plant protection measures and agronomic practices were followed to raise a normal crop.

OBSERVATIONS AND DISCUSSION

In present studies, varied response of gamma irradiation and EMS solution with different doses, in respect to survival percentage, sprouting ability, height of plant, internodal distance and other morphological features have been observed.

Sprouting per cent

Sprouting is an inherent ability of plant to unfold the buds and produce the shoot proliferation. It is an important propagative parameter considered for evaluation of genotype performance. The success of well establishment of mulberry genotype depends on sprouting ability. It is an established fact that sprouting is a genetic trait of a genotype; however the role of agro-climatic conditions and moisture is a binding factor influencing the sprouting. [3].

Sprouting percentage of mulberry was significantly influenced by the genotypes. It was significantly higher in S_{1635} [91.48%] over S_{36} [77.72%] and statistically on par with V_1 genotype [86.27%]. However, the mulberry genotypes V_1 and S_{36} were found on par with each other for their sprouting per cent [86.27 and 77.72%].

The difference in sprouting of genotypes was might be associated with inherent characteristics of individual mulberry genotype. The chemical mutagenesis by the varying levels of EMS were found non significant for sprouting of mulberry genotypes. However, numerically it was more in 0.1 and 0.3 per cent EMS treatment [92.37 and 91.01 per cent] than the control and remaining doses.The sprouting per cent of mulberry genotypes was significantly reduced by the gamma irradiation over control. It was drastically reduced in gamma irradiation @12 kR [63.22 %] followed by 6 kR [79.88 %], whereas, 3 kR and 9kR were found on par [83.34 and 82.67 %] with the control treatment [95.17%].

These results revealed that a lower dose of gamma irradiation had less adverse effect on sprouting of mulberry genotypes than the higher doses. Mean varietals performance revealed that there was consistence decrease in sprouting percentage with increasing radiation dosage in all the three varieties, whereas there was non consistent decrease in sprouting percentage with increasing concentration of EMS in all the varieties under study, While comparing control and EMS treated populations in each variety, treatment at 0.4 % EMS produced comparable sprouting in V_1[91.67 %] while significantly higher sprouting [97.93%] in S_{1635} over control. This concentration of EMS might be suitable for induction of more sprouting in these two varieties, whereas at this concentration there was significant reduction in sprouting in S_{36}. The 0.1% and 0.3% EMS concentration was found suitable to obtain maximum sprouting in S_{36}.

Reduction in biological criteria [sprouting emergence] might be attributed to a drop in auxin level [4] or inhibiting auxin synthesis [5] or chromosomal aberrations or due to decline of assimilation mechanism. It can also be due to variation in temperature, water content and oxygen tension during treatment [6].

Number of days taken for sprouting differed with physical mutagen [gamma rays] and chemical mutagen [EMS].Cutting treated with EMS showed early sprouting while cutting treated with gamma irradiation showed late sprouting except 3 kR. Cuttings irradiated with 6 kR, 9 kR and 12 kR showed late sprouting. Higher doses were adversely affected the sprouting. This might be due to partial cell death, which might have resulted in decreased sprouting per cent due to gamma irradiation. Gamma rays being highly penetrative might have destroyed cells which were under going meiotic division in the bud region. Present observation is in agreement with the previous reports [7-10].

Survival per cent

The survival per cent of mulberry genotype S_{1635} was significantly higher [55.83%] than S_{36} [41.48%] and V_1 [19.38%]. While comparing the survival per cent of mulberry genotype with the treatment of chemical mutagen and physical mutagen, it was significantly higher in chemical mutagenic treatment.

Whereas irrespective of only chemical mutagenic treatment S_{1635} genotype recorded significantly higher survival per cent [77.92%] followed by S_{36} [65.86%] and the least in V_1 [26.25%]. Similar trend was found in physical mutagenic treatment. But it was significantly less as compared to chemical mutagenic treatment.

The mutagenic treatment to mulberry genotypes was significantly varied the survival per cent. Survival per cent of mulberry genotypes was higher in chemical mutagenic treatment of EMS, but it was statistically non significant. Numerically survival percentage was decreased with increased concentration of EMS, chemical mutagenic treatment to mulberry genotype.

The clones of genotypes V_1 and S_{36} treated with 0.1 per cent EMS showed high rate of survival while concentration of 0.4 % affected the survival rate. In S_{1635} and V_1 variety sprouting per cent was the highest at 0.4 per cent EMS but V_1 variety showed less survival per cent at 0.4 per cent EMS. Survival per cent was decreased with increased in the EMS dose in V_1 and S_{36}, while in S_{1635}, survival rate was higher at 0.4% EMS [81.25%].This might be due to its triploid nature which gives resistant to survive at high concentration also. The present reports are in line with the findings of Tikader et al. [11] who studied on propagation efficiency of mulberry at ploidy level and found that maximum survival per cent was in triploid followed by diploids and tetraploids. Rao [12] evaluated that triploids performed better in all the parameters as compared to other polyploid variants.

The physical mutagenic treatments by gamma irradiation to mulberry cuttings were significantly reduced in 6, 9 and 12 kR gamma irradiation [9.73, 5.56 and 2.08 per cent respectively] as compared to the control [56.94%]. The increased doses of gamma irradiation drastically reduced the survival per cent of mulberry cuttings.

Table 1 Effect of chemical, physical mutagen, genotypes, and their interaction on sprouting per cent

Variety	V_1	S_{1635}	S_{36}	**Mean**
Chemical mutagen[EMS]				
0	93.77	91.67	83.37	**89.60**
0.1%	89.60	91.67	95.83	**92.37**
0.2%	87.53	97.93	79.17	**88.21**
0.3%	89.63	95.87	87.53	**91.01**
0.4%	91.67	97.93	68.77	**86.12**
Mean of EMS	**90.44**	**95.01**	**82.93**	**89.46**
Physical mutagen [Gamma irradiation]				
0	93.77	93.80	97.93	**95.17**
3kR	89.60	93.77	66.67	**83.34**
6kR	77.10	95.87	66.67	**79.88**
9kR	85.43	89.63	72.93	**82.67**
12kR	64.60	66.70	58.37	**63.22**
Mean of gamma	**82.10**	**87.95**	**72.51**	**80.86**
Mean [EMS + Gamma]	**86.27**	**91.48**	**77.72**	**85.16**

Source		**SE (m) +**	**CD at 5%**	**CD at 1%**
Main plot		2.409078399	9.457696931	15.68560399
Chem. Mutagen		3.54556989	NS	NS
Physical .Mutagen		3.54556989	9.827818328	12.91656123
Chem. Vs Phy		1.585627059	4.39513397	5.77646179
Sub plot		3.070553595	8.511140336	11.18607016
Main x Sub		6.141107191	NS	NS
Between two subplots means at same level of main plot mean				
Main x Sub		8.872274334	NS	NS
Between two main plots means at same level of sub plot mean				

CV a (%)	14.96924728	CV r b (%)	12.49042796

Table 2 Effect of chemical, physical mutagen, genotypes, and their interaction on survival per cent

Variety	V_1	S_{1635}	S_{36}	**Mean**
Chemical mutagen[EMS]				
0	31.25	79.17	72.93	**61.12**
0.1%	39.58	68.75	72.92	**60.42**
0.2%	14.58	83.33	62.53	**53.48**
0.3%	27.08	77.08	68.80	**57.66**
0.4%	18.75	81.25	52.13	**50.71**
Mean of EMS	**26.25**	**77.92**	**65.86**	**56.68**
Physical mutagen [Gamma irradiation]				
0	33.33	75.00	62.50	**56.94**
3kR	20.83	58.33	14.60	**31.26**
6kR	2.08	18.75	8.37	**9.73**
9kR	6.25	10.42	0.00	**5.56**
12kR	0.00	6.25	0.00	**2.08**
Mean of Gamma	**12.50**	**33.75**	**17.09**	**21.11**
Mean [EMS + Gamma]	**19.38**	**55.83**	**41.48**	**38.90**

Source		**SE (m) +**	**CD at 5%**	**CD at 1%**
Main plot		1.574851971	6.182643396	10.25392298
Chem. Mutagen		2.978377799	NS	NS
Physical .Mutagen		2.978377799	8.255642062	10.85027242
Chem. Vs Phy		1.331971044	3.69203537	4.852389343
Sub plot		2.579350836	7.14959575	9.396611557
Main x Sub		5.158701671	14.2991915	18.79322311
Between two subplots means at same level of main plot mean				
Main x Sub		7.247868301	15.10418404	35.73644211
Between two main plots means at same level of sub plot mean				

CV a (%)	21.4249	CV b (%)	22.97212

The interaction effect of mulberry genotypes and mutagenic treatments were found significant for survival per cent of mulberry cuttings. The interaction between S_{1635} with chemical mutagenic treatment was found significantly superior for survival percentage of mulberry cuttings. It was significantly higher in 0.2 % EMS chemical mutagenic treatment [83.33 %] followed by 0.4 % [81.25%].Whereas, interaction effect of S_{36} and chemical mutagen EMS showed that the increased levels of EMS treatments decreased the survival per cent. The interaction effect of mulberry genotype V_1 and mutagenic treatments were found significant for drastic reduction in survival per cent.

Thus, the physical mutagenic treatment by gamma irradiation adversely affected the survival per cent of mulberry cuttings than the chemical mutagenic treatment of EMS.

Under different agro climatic conditions, Mulberry is a unique plant that shows high flexibility with differential response. The phenotypic plasticity in mulberry contributed to its ability to grow and survive under disruptive environmental conditions [13]. This opens up the opportunity to develop varieties suitable to new agricultural areas.

In case of gamma ray irradiated populations in all the three varieties mean value of survival per cent was consistently reduced. However, V_1 and S_{36} proved to be more radiosensitive. The 6kR, 9kR and 12 kR doses of gamma ray irradiation in all the varieties were significantly reduced the survival per cent

Sensitivity depends upon its genetic architecture and dose employed besides DNA amount, its replication time at initial stages and degree of hetero chromatin. Seedling survival in V_1 and S_{36} at 6kR, 9kR, and 12 kR doses was minimum or nil. However, the EMS mutagens did not show any drastic reduction in above characters in all the varieties as compared gamma ray irradiation treatments.

Mutagenic sensitivity is known to be influenced by an array of factors, such as type of mutagen and dose, treatment condition, moisture content, stage of development and genotype. The trend of physical mutagenic efficiency was different from the tread of chemical mutagen.

In general, the effectiveness decreased with the increase in dose or concentration. With increased in doses of gamma rays, the values obtained for all the biological criteria were decreased. The present reports are in line with the findings [8, 12, 14, 15 and 16]. These workers have concluded that low doses of irradiations could be used as safe and effective for mutation studies in mulberry. Sastry et al. [2], while studying the sprouting and survival ability in mulberry varieties S_{30}, S_{36}, and $K_{2,}$ showed that the injury was directly proportional to the concentration of mutagens.

CONCLUSION

Thus, the present study concludes that Gamma rays showed injurious effect to stem cuttings than that of EMS. Lower dose of gamma irradiation had less adverse effect on sprouting of mulberry genotypes than the higher doses. The higher concentrations of physical mutagens are lethal and injurious and affecting the survival per cent. While, survival per cent of mulberry genotypes was higher in chemical mutagenic treatment of EMS.

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