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EVALUATION OF ETHYL METHANE SULFONATE (EMS) INDUCED MUTANTS OF *MORUS SPECIES* (GENOTYPE BC_{2-59}) FOR NUTRITIVE AND BIOMASS PRODUCTIVITY

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ABSTRACT

Mulberry is the primary forage for silkworm *Bombyx mori* L. (monophagous) and leaf protein is the base for synthesis of silk protein (sericin and fibroin). Nearly 70% of the leaf protein is biosynthesized into silk by silkworm. Thus the phenomenal increased biomass (leaves) and nutritive content in mulberry varieties is the principal determining factor for higher cocoon yield. Realising the biomass and nutritive significance of mulberry, the objective was prioritized for quantitative and qualitative improvement in crop. In the present investigation the active bud sprouts of mulberry genotype BC_{2-59}, in multiple sets were treated for twelve hours intermittently (every one hour) with three concentrations (0.1%, 0.3% & 0.5%) of EMS and evaluation of crop was carried out. EMS is a potent chemical mutagen and monofunctional ethylating teratogenic agent with formula $CH_3SO_3C_2H_5$, used extensively in genetic research. EMS is proved mutagenic in wide variety of genetic test systems from virus to mammal. The results revealed that the clones of M_1V_1 and M_1V_2 generation of 0.3% EMS treatment were significantly altered in their morpho-metric characters, biomass yield and phytochemical constituents. The significant variation in the morpho-metric characters such as height of the plant, number of branches, stem girth, number of leaves per plant and increased biomass were recorded among the M_1V_2 clones of 0.3% EMS treatment ($p=0.0007$). The leaf area and number per plant showed significant increase in M_1V_2 clones. The mean number of leaves (761.93) in the variants as against the control (459.60) was recorded. Nutritive parameters such as proteins, reducing sugars, minerals and chlorophyll content were also significantly altered. Moisture and moisture retention capacity(MRC) were found to be high in 0.3% EMS induced variants.

KEYWORDS: BC_{2-59}, EMS, Morpho-Metric Characters, Biomass, Nutritive, Phytochemical

ABBREVIATIONS

BC_{2-59}: *Back cross*, **EMS:** *Ethyl methane sulfonate*, **M_1V_1:** *Mutation1 Variation 1*, **M_1V_2:** *Mutation 1 Variation 2*

INTRODUCTION

Mulberry belongs to the family Moraceae and order urticales. There are several species viz; *Morus alba, M. nigra, M. indica, M. laevigata, M. bombycis*, etc. which have been used directly or crossed and induced mutations for the development of varieties. Mulberry is basically deciduous species of sub-tropical forests with arboreal habit and distributed in a wide area of tropical, sub-tropical, temperate and sub-arctic zones (Dandin and Jolly, 1986; Sanjappa, 1989).There are mulberry varieties for many environments, from sea level to altitudes of 4 000m (FAO, 1990) and from the humid tropics to semi-arid lands, such as in the Near East with 250 mm of annual rainfall and the southwestern United States (Tipton, 1994).It is of great economic importance to sericulture industry and is extensively cultivated for forage of silkworm in sericulturally important countries of both tropical and temperate regions. Although mulberry is basically a tree, it is

maintained as small bushes through repeated pruning and training for optimum production and utilization of leaves(biomass).In India the cost of mulberry leaf production amounts nearly 60% of the total expenditure of cocoon production (Das and Krishnaswami, 1965).

Silk is a protein biosynthesized by the silkworm *Bombyx mori.* L., Silk cocoon is spun by the sericigenous insect silkworm *Bombyx mori* L., which is monophagus (Ito,1960) and exclusively feeds on mulberry, hence mulberry is the sole forage for *Bombyx mori.* The preference of mulberry by silkworm is probably due to some fragrance of mulberry and the special organs the silkworm is equipped with, which respond to the taste of leaves (Oka and Ohyama, 1986). Silk fiber is obtained from cocoon through the technique of reeling. Silk is a splendid gift of nature to mankind, an inimitable natural fiber synonymous with splendor, sibilant with luster and spectacular in vision. Nearly 70% of the mulberry leaf protein is converted into silk protein through biosynthesis by silkworm. Thus mulberry leaf protein is the quintessence for synthesis of silk protein (sericin and fibroin).Obviously mulberry is the central dogma in sericulture and the increased biomass (leaves) in mulberry variety through crop improvement techniques is the principal determining factor for higher cocoon yield, hence there is an immense need for improvement of mulberry varieties in terms of nutritive value and increased biomass (leaves) for profitable production of cocoon.

Although globally mulberry is chiefly used to feed the silkworm but, depending on the location it is also appreciated for its fruit (consumed fresh, in juice or as preserves),as a delicious vegetable (young leaves and stems), for its medicinal properties in infusions (mulberry leaf tea), for landscaping and as an animal feed. In Peru the multiple uses of mulberry have been recognized (Zepeda,1991).In several countries like India, China and Afghanistan mulberry is utilized traditionally as a feed in mixed forage diets for ruminants. In Italy there have been several studies on the use of mulberry for dairy cows and other domestic animals. Hence mulberry leaf is not merely the sole forage for silkworm *Bombyx mori* but also proved as rich nutrient biomass for rearing of cattle, sheep, goat and rabbit.

The latest research reveals diversified functional utilization of phytochemical characteristics of mulberry and it's by product in the field of food processing and pharmaceutical industries. Mulberry has been used as medicinal herb since ancient times and it is used in an ayurvedic preparation. Root extract has hypoglycemic properties and root bark is an antihelmintic, purgative and vermifuge. Mulberry leaf is rich in gamma-aminobutylic acid proved effective against high blood pressure. The compound known as deoxynojirimycin is abundantly found in mulberry leaf responsible for monitoring blood-sugar level closely associated to diabetes. The fruits of mulberry on an average contains 12 to 20 % sugar, they are non acidic and are consumed fresh. They are used in making juice, pies and wine. Dried fruits are used in confectionery products. Recently, it is found that mulberry fruit has an anti-oxidative property. The fruits are effective to treat sore throat, depression and high fever, acting as both coolant and laxative. Mulberry grows faster than other woody plants and yields high biomass thus mulberry branches are used as raw material for paper production, the stem and stem powder is good source of media for mushroom culture.

Mulberry genotype BC_{2}-$_{59}$ is a popular irrigated clone evolved by back cross method at Central Sericulture Research and Training Institute, (Government of India) Berhampore, West Bengal. Under field conditions it yields 20,000 to 25,000 kgs per hectare/annum. The survival rate is 92.50% with rooting efficiency of 60%. The stem is erect and straight with longest shoot length of 138.00 cm and total of 1796.67 cm. The maximum number of branches per plant is 20. The inter-nodal distance is 5.55cm. The young shoot is green and mature shoot is brownish grey in colour. The leaf lamina is homophyllous, cordate to ovate in shape with serrate to dentate margin and acuminate tip. The leaf is unlobed, green, smooth and coriaceous. The petiole measures 4.84 cm in length and the leaf area is 333.00 cm^2. The 100 leaves weighs 428g. Plants are both monoecious and dieocious. Inflorescence is catkin and measures 0.84 cm length in male and 0.40 cm

in females. The male flower is small, sessile, unisexual with four perianth lobes arranged in imbricate aestivation. Stamens four, antitepalous, introse, filament slender, long, anthers dithecous, versatile and longitudinal dehiscence. Pollen small and round with smooth exine. The female flower is tiny, perianth four lobed, tetramerous, homochlamydous, actinimorphic. Gynoecium superior ovary, bicarpellary syncarpus, unilocular, single pendulous ovule on basal placenta, style short and stigma bifid. The moisture percentage of leaf is 71.49% and moisture retention capacity is 74.68%. The soluble protein is 15.71% and carbohydrate is 15.95% (Thangavelu *et al.*,1997). Realizing the significance of increased biomass(leaves) and nutritional value of mulberry leaves, the present investigation explored to achieve the enhancement in biomass coupled with nutritive improvement in leaf in the mulberry genotype BC_{2-59} through EMS induced chemical mutagenesis.

Ethyl Methane Sulphonate (EMS) is a potent mutagen that has been extensively used in genetic research and it is a monofunctional-ethylating agent that has been found to be mutagenic in wide variety of genetic test systems from virus to mammal. The alkyl group of an alkylating agent reacts with DNA, which may lead to a change in the nucleotide sequence and hence leads to point mutation. Since the alkylating agent EMS react with DNA in variety of ways, a broad spectrum of mutagenic effects are manifested in the treated population. EMS has been found more potent for mulberry (Sastry *et al*., 1983; Yang and Yang, 1991).Thus EMS was conveniently used for induction of mutations in mulberry genotype BC_{2-59}. Observations were recorded in M_1V_1 and M_1V_2 generations for early growth parameters and later morphometric characters. The results revealed that the 0.3% concentrations of EMS treatment was effective in significantly altering the early growth parameters and later morho-economic traits. The higher concentration of 0.5% EMS reduced the survival rate and was found to be lethal but the low concentrations 0.1% and 0.3% EMS were found to be safe and optimum for treatment. Thus it infers that alterations in vegetative parameters and nutritive components were mutagen dose dependent factors.

MATERIALS AND METHODS

Irrigated Mulberry genotype (*Morus alba*) BC_{2-59}, was procured from Central Sericulture Germplasm Research Station, Hosur, Tamil Nadu,(India) for induction of mutations. The genotype was established at germplasm bank attached to the Department of Sericulture, Bangalore University, following the standard procedures of the National Bureau of Plant Genetic Resources (NBPGR) (Chahal and Gosal, 2002). The genotype was maintained following the recommended package of practices for mulberry (Krishnaswami,1978).Four set of replicates (10 cuttings in each) from disease free twigs of mulberry variety BC_{2-59} were prepared following the standard procedure, each cutting measuring 6 to 8 inches in length with 3 to 4 active buds (Dandin,1990).Each set of cuttings were planted in earthen pot filled with propagation mixture of red earth, sand and organic manure prepared in the ratio of 1:1:1 (Hartman and Kester, 1996).The planted pots were maintained in the nursery with consistent care. The three concentrations of EMS solution was prepared by V/V method. On sprouting the buds of each set were treated intermittently with definite concentration of EMS solution, every one-hour for total span of 12 hours following cotton swab method. The buds were capped with sterilized cotton and EMS solution was injected to the cotton cap covering the bud by using 10ml syringe (Broertjes and Van Harten,1988).Out of four replicates one set of cuttings were maintained as control by treating the buds with distilled water. Buds of second, third and fourth set of replicates were treated with 0.1%,0.3% and 0.5% concentrations of EMS solution respectively. The treatment was carried out from 8.00am to 6.00pm intermittently with the gap of one hour between the treatments under bright sunshine.

The treated populations were maintained in nursery for 90 days and the observations were made after 15^{th} day of treatment and sprouting ability was assesed. After 90 days, rooting ability and proliferation was evaluated. Later the saplings were transplanted to the field and and maintained in the pit system with spacing of 3ft x 3ft under necessary cultural operations and agronomic inputs, following recommended package of practices. Periodical observations were made in the first six months in M_1V_1 generation to score morphological variations, such as linear growth rate, branching

pattern, internodal distance, leaf shape, leaf count and size, number and nature of inflorescence, etc.The observed results were compared with the control (Dandin and Kumar, 1989) and documented with photographs.

The M_1V_1 beneficial variants recovered from 0.3% EMS treated clones of $BC_{2\text{-}59}$ were screened for further propagation of M_1V_2 populations. Cuttings screened and selected from M_1V_1 beneficial variants of $BC_{2\text{-}59}$ were raised in the nursery as per standard procedure. After three months the saplings were transplanted to the field in separate blocks following RBD method with 3ft x 3ft spacing (pit system). The observations were recorded from 90^{th} day to 180 days regarding agro and morpho-economic characters such as height of the plant, girth of the stem, number of branches per plant, internodal distance, number of leaves per plant, leaf size, leaf weight and moisture retention ability (Thangavelu *et al.*, 1997).

The comparative studies were carried out to tally certain common and consistent morpho-economic characters between M_1V_1 and M_1V_2 generations. The mutant plants, exhibiting maximum common and consistant morpho-economic traits at M_1V_2 generation were again carefully screened and isolated, further the leaves were subjected to phytochemical analysis. The chemo assay was carried out following the standard methods viz, quantification of proteins (Lowry *et al.*, 1951),the reducing sugar estimation by Miller method (1972) and total chlorophyll contents were estimated by DMSO(Dimethyl sulfoxide) method.

The Fisher's method was used for statistical analysis. The co-efficient of variance, co-relations, standard deviation and standard error were analyzed by standard statistical method ANOVA, using the software Microsoft Excel.

RESULTS AND DISCUSSIONS

Growth parameters such as height of the plant, number of branches per plant, stem girth, etc., are important morpho-economic traits which contributes to increased biomass. Mulberry is a perennial woody and long-standing tree. A woody plant consists of a significant amount of xylem tissue providing both structural support and transport channels for water and nutrients (Mellerowicz *et al.*, 2000). Woody plants increase in size and complexity perennially, adding new shoots reinforcing existing stem. The number of primary branches are often arranged to provide a stable distribution of branch weight and expose maximum leaf area for optimal photosynthetic rates (Burk *et al.*, 1983). Thus there are often progressive changes in branching behavior viz., branches more or less upright to acutely orient (orthotropic) and some what drooping branches (Plagiotropic) (Zimmermann and Brown, 1971).

The girth of the stem is due to development of secondary tissue or wood, which gives mechanical support while providing a bi-directional pathway for long distance transport of water and nutrients (Zimmermann and Brown, 1971; Kervella *et al.*, 1994). Wood is functionally important support tissue in woody plants and trees to maintain stability. The genetic variabilities affecting morpho-agronomic traits induced by EMS has been reported in many crops which are compared and discussed in tandem with our findings.

The 0.3% treatment of EMS was effective in increasing the plant height to 565.55 cm compared to control (461.62cm) (Table 1). Such kind of variations in plant height has been observed and recorded among population of EMS induced mutations in *Jatropha curcas.* The maximum plant height at maturity of 105 cm was recorded in 1 % EMS treatment while minimum plant height was observed (81.33 cm) in 4 % EMS treatment. Studies revealed that lower concentration of EMS had a stimulatory effect for plant height and the higher concentrations showed an inhibitory effect as compared to control (Dhakshanamoorthy *et al.*, 2010). Variability in plant height in chickpea was observed and reported due to gamma radiation (Athwal *et al.*,1970) . Similar kind of variability in plant height was also observed through EMS treatments in *Capsicum annum* (Jabeen and Mirza, 2002; 2004).The variation in plant height has also been reported (Jamil

and Khan, 2002), which revealed that the radiation doses of 5 and10 Krad has slightly reduced plant height while other dose had no considerable effect on plant height. The mutations affecting the plant height, indicating that the mutagens could cause both positive and negative genetic variability in plant height has also been reported in crop plants (Chen, Gottschalk, 1970; Okuno and Kawai, 1978).

Table 1: Mean Value of Morpho - Economic Parameters of Mulberry Genotype BC_{2-59} (Control and EMS Treated)

Morpho- Economic Parameters	Average	
	EC	3E
No of Leaves	459.60	761.93
Leaf Area (cm^2)	362.62	598.91
Inter-nodal Distance (cm)	4.55	3.32
No of Inflorescence	17.93	25.47
Number of branches	18.27	26.20
Diameter of the Stem girth (cm)	4.50	3.92
Length of the Petiole (cm)	6.86	8.54
Yield per plant (kg)	6.80	11.85
Length of inflorescence (mm)	59.53	59.87
Plant Height (cm)	461.62	565.55

Table 2: ANOVA for EC and 3E – Morpho- Conomic Parameters

Source of Variation	SS	df	MS	F	P-Value	F Crit
Between Groups	1140107.21	9	126678.579	16.129	0.0007	3.020
Within Groups	78539.07	10	7853.91			
Total	**1218646.28**	**19**				

EC – Control; 3E- 0.3% EMS Treated.

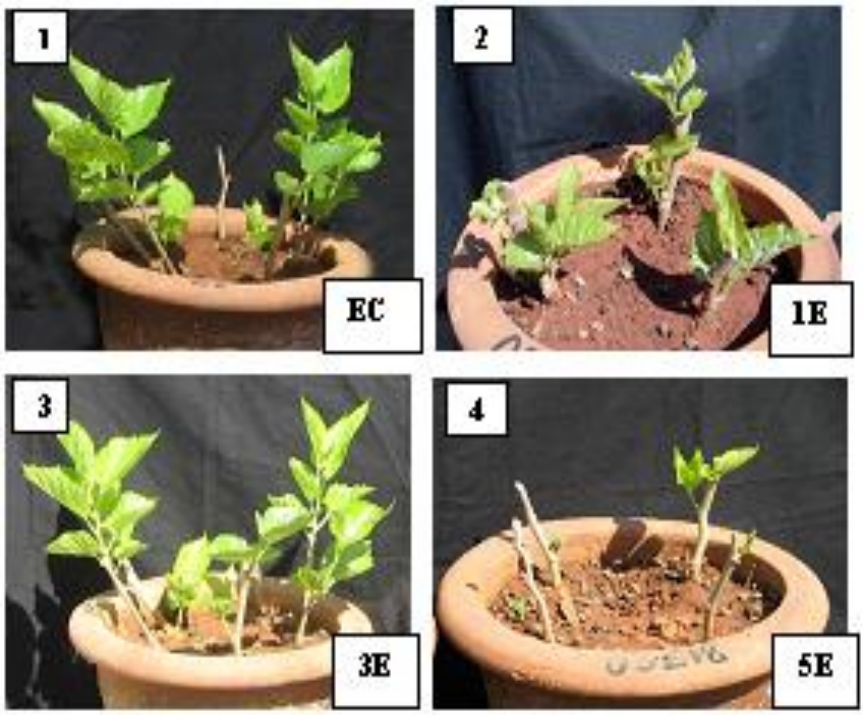
The increase in unit length of shoot obviously provides more scope for higher number of branches. The mean number of branches among clones of 0.3% EMS treated were 26.20 compared to mean of 18.27 in control. There was increase in mean number of branches per plant and mean height of the plant in EMS treated population of $BC_{2-59.}$ In the present study, significant variation in number of branches and plant height between the control plants and M_1V_2 variants of and 0.3% EMS (p=0.0007) treated population was established (Table 1; fig 5&6). Similar findings has been reported in EMS treated rapseed plants. The treatment of 0.75% & 1.00% EMS solution had induced genetic variability in case of rapeseed (*Brassica napus* L.) and evaluation for important economic character, on comparison with control had revealed that the EMS treatments shifted the mean values in positive as well as negative directions. However all the EMS treated populations showed enhancing effect on primary branches. The bidirectional response was observed in plant height and primary branches per plant. The highest heritability values for primary branches (81.06%) and grain yield(79.49%) per plant was recorded. There was considerable increase in variance for all the agro-economic traits in the studies carried out (Muhammad Aquil siddiqui *et al.*, 2009).

The effects of ethyl methane sulfonate (EMS) on seed germination, seedling survival, growth and performance of *Sesbania seban* var. *sesban*, were studied with reference to its fodder yield related traits. Results showed that seed germination, shoot length, root length and dry weight decreased with an increase in the concentration of the mutagen (0.025-0.100%). However, shoot length and root length were not much affected at lower concentrations (up to 0.025%). An inverse association of seed germination and other parameters with the mutagen dosage was observed. Seed germination, seedling survival, shoot and root length, and dry weight decreased in all the treatments compared to controls. Field results of grown plants indicated that there was a gradual decrease in height, above ground biomass and pod length among EMS

mutagenised plants, corresponding with an increase in the mutagen dose (Gupta *et al.*, 2000). Thus it infers that the genetic variabilities of agro- economic and morphometric traits among crop plants probably show strong positive or negative dose dependent co-relationship with EMS concentrations, which is in conformities with the present findings.

The number of leaves per plant is a directly dependent factor on the number of nodes per unit length of shoot. The shoots with shorter internodes denote more number of nodes resulting in increased number of leaves. In the present work the EMS treated mulberry genotypes $BC_{2\text{-}59}$ has exhibited significant increase in the mean values in respect of increase in number of branches and number of leaves. The number of leaves (mean value) was found to be 761.93 in the variants of $BC_{2\text{-}59}$ mulberry genotype recovered at 0.3% EMS treatment, as against the control (459.0)(Table 1).The leaf yield per plant was significantly high in the clones of 0.3% EMS treatment (8.54 kg), compared to control (6.80 kg) (Table 1; Figs. 5&6).

The leaf yield increased by 30.64% at 0.3% EMS treatment compared to control. This significant increase in yield was due to the increased leaf area that exponentially rose to 598.91 cm^2 at 0.3% EMS treatment compared to control(362.62cm^2) (Figure 7).The increase in leaf area in EMS treated populations of $BC_{2\text{-}59}$ is quite significant. Similar considerable increase in leaf area by 166.94cm^2 and 165.20cm^2 has been recorded in M_1 variants of 0.4% and 0.5% colchicines treated populations respectively in M_5 mulberry variety (Ramesh and Yogananda, 2011).



Figures 1,2,3,4: Sprouting Response in Mulberry Genotype $BC_{2\text{-}59}$ (Control-EC), Treated with 0.1% EMS (1E), Vigorous Sprouting in 0.3% EMS Treatment (3E) and Poor Response in 5E Treated with 0.5% EMS Respectively

Such type of increased leaf area due to colchicine treatment and mutation effects in mulberry has been reportedin earlier works (Dwivedi *et al.*, 1985; 1989).They opined that the increase in leaf area was due to enlargement in palisade and spongy layers, both increasing in length and width. Further, they also reported increase in weight, thickness of leaf and high water content in the colchicine-induced variants.

In sericulture, the nutritive quality of the mulberry leaves ensures the quality of cocoon in turn the superiority of raw silk produced. The nutritional compositions of leaves greatly influence the robustness, growth and development of silkworm larvae (Legay, 1958; Purohit and Pavan Kumar, 1996). Further it is established fact that proteins, total soluble

sugars, chlorophyll, amino acids, minerals, vitamins and sterols forms important nutritional requirement of silkworm larvae (Ito and Arai, 1963; Nimura,1966). In the present investigations the M_1V_2 variants recovered from 0.3% EMS treatments of BC_{2-59} were quite promising in improved phytochemical constituents.

The protein content was higher at 0.3% EMS treatment (tender - 40.00%, medium - 38.77% and coarse - 38.15%) compared to control (tender - 23.99%, medium - 25.63% and coarse - 22.45%). The protein level was significantly high at 0.3% EMS treatment. The reducing sugar was also significantly high (tender - 14.33%, medium - 13.75% and coarse - 12.46%) in the variants of 0.3% EMS treatment of BC_{2-59} mulberry genotype as against the control plants of 4.33% in tender, 3.97% medium and 2.84.81% in coarse leaves.

There has been significant increase in the amount of reducing sugar in the variants of 0.3% EMS treatment in BC_{2-59} mulberry genotype (Table 3). The total chlorophyll content was also found higher than the control at 0.3% EMS treatment. It was found in the range of 3.308mg/gf.wt in tender, 3.819 mg/gf.wt in medium and 3.709 mg/gf.wt in coarse leaves. Chlorophyll content is relatively low i.e., 3.551 mg/gf.wt, 3.498 mg/gf.wt and 3.448 mg/gf.wt in tender, medium and coarse leaves respectively in control plants (Table 3). The increase in protein content in EMS treated mulberry genotype of BC_{2-59} is in conformity with the earlier findings (Shkvarnikov *et al.,* 1975; 1976).

They have recorded increased protein content associated with earlier ripening and increased grain weight in the mutants of maize recovered from EMS treatment. Similar findings are reported with regard to increase in protein content in soyabeans due to x-ray treatment (Sebok, 1970). Chlorophyll content is an important and essential constituent of the leaves. It is one of the criteria for quantification of photosynthetic efficiency of plant.

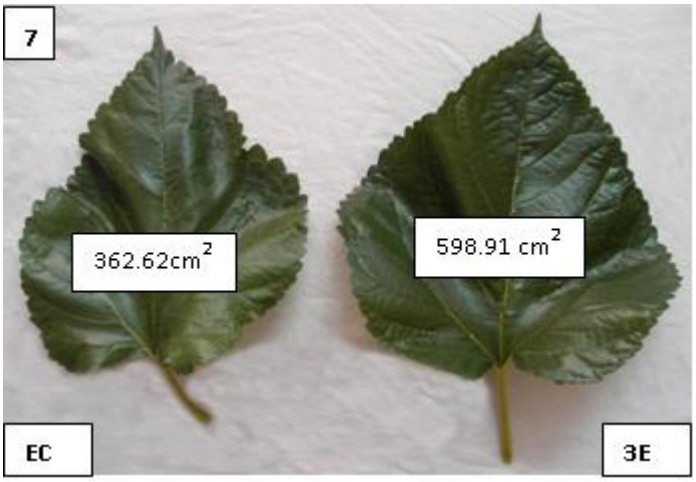
It acts as a constituent as a connecting link between the organic and inorganic components of the ecosystem and it is reported that the total chlorophyll content of fresh leaves ranged from 0.14% to 0.35% in weight (Hotta,1975).Similarly higher percentage of chlorophyll content in triploids than in tetraploidshas been found and reported (Mogli *et al.,* 1992).

Table 3: Phytochemical Composition in the Leaves of Mulberry Genotype BC_{2-59} (Control and EMS Treated)

Treatm-ents	Maturity of Leaves		Chlorophyll-a (mg/gf.wt)	Chlorophyll-b (mg/gf.wt)	Total Chlorophyll (mg/gf.wt)	Protein%	Reducing sugar %	Moisture Content %	Moisture Retention Capacity (%)
EC	Tender	Average	2.228	1.323	3.551	23.997	4.337	79.310	78.203
		S.E.M	0.001	0.001	0.003	0.061	0.070	0.009	0.034
		C.D @ 5 %	± 0.0023	± 0.0017	± 0.0058	± 0.1189	± 0.1369	± 0.0173	± 0.0663
	Medium	Average	2.194	1.304	3.498	23.633	3.947	76.117	75.753
		S.E.M	0.003	0.001	0.002	0.353	0.157	0.012	0.064
		C.D @ 5 %	± 0.0060	± 0.0017	± 0.0046	± 0.6914	± 0.3073	± 0.0236	± 0.1246
	Coarse	Average	2.164	1.284	3.448	22.455	2.843	74.323	74.387
		S.E.M	0.004	0.003	0.007	0.065	0.107	0.037	0.064
		C.D @ 5 %	± 0.0082	± 0.0051	± 0.0129	± 0.1272	± 0.2103	± 0.0728	± 0.1246
3E	Tender	Average	2.279	1.529	3.808	40.000	14.330	80.740	79.597
		S.E.M	0.002	0.001	0.003	0.361	0.068	0.012	0.177
		C.D @ 5 %	± 0.0035	± 0.0024	± 0.0058	± 0.7067	± 0.1334	± 0.0226	± 0.3477
	Medium	Average	2.307	1.512	3.819	38.777	13.750	78.850	77.690
		S.E.M	0.067	0.001	0.001	0.066	0.019	0.012	0.006
		C.D @ 5 %	± 0.1303	± 0.0024	± 0.0013	± 0.1302	± 0.0364	± 0.0226	± 0.0113
	Coarse	Average	2.220	1.489	3.709	38.153	12.467	78.877	78.887
		S.E.M	0.001	0.001	0.001	0.047	0.240	0.015	0.013
		C.D @ 5 %	± 0.0017	± 0.0011	± 0.0026	± 0.0922	± 0.4711	± 0.0285	± 0.0261

EC – Control; 3E- 0.3% EMS Treated.

Figures 5, 6: Branching and Foliage Density in Control and EMS Treated Mulberry Genotype BC_{2-59} (EC- Control; 3E- 0.3%EMS)



Figures 7: Increase in Leaf area in Mutant Clone of EMS Treated Mulberry Genotype BC_{2-59} (EC- Control; 3E- 0.3%EMS)

Anthocyanins are pigments which hold potential use as dietary, which is the major flavonoids (quercetin 3-glucoside and rutin) present in mulberry, is probably expected to contribute significantly to their antioxidant activity (Pérez-Gregorio *et al.*, 2011). These two useful antioxidants are the phytochemical and nutrient constituents of mulberry leaves.In the present studies the mutant clones of 0.3% EMS treatment has shown higher total chlorophyll compared to control(Table 3). Since these pigments considered as the chief phytochemical constituents has been affected quantitatively by mutagen EMS. probably EMS might also induce such variations quantitatively in the profiles of phenolic constituents and affect antioxidant activity (DPPH).

Similar studies carried out in 8 mulberry species of China for varietal differences in nutritional functional compounds of fruits and leaves for alkaloids, polyphenols, flavonoids, and anthocyanins, as concentrations of 1-deoxynojirimycin (DNJ), resveratrol, oxyresveratrol,cyanidin-3-O-β-glucoside (Cy-3-glu), cyanidin-3-O-β-rutinoside(Cy-3-rut),rutin have revealed significant variations. Mulberry variety Da 10 (*Morus atropurpurea* Roxb.) was screened as the most valuable cultivar considering its high content of functional components (Wei Song et al., 2009). Probably the mutants recovered from 0.3% EMS treatment might certainly show changes in nutritional functional components,further studies would be focused to explore the possibilities for extraction and quantitative analysis of such useful functional nutritional components from the recovered mutants of mulberry genotype BC_{2-59}. In the present investigations, the M_1V_2 mutants of

BC_{2-59} mulberry genotype recovered from 0.3% EMS treatments have shown significant changes in moisture content and moisture retention capacity. The moisture content was significantly high in the M_1V_2 mutants of 0.3% EMS treatment. It is in the range of 80.74%, 78.85% and 78.87% in tender, medium and coarse respectively compared to control (tender - 79.31%, medium - 76.11% and coarse – 74.32%).The moisture retention capacity was high ie 79.57% in tender, 77.69% in medium and 78.88% in coarse leaves, when compared to the control plants, which were found to be 78.20% in tender, 75.75% in medium and 74.38% in coarse leaves respectively (Table 3). The present findings are in agreement with the earlier findings (Dwivedi *et al.,* 1987; Bose, 1989; Sikdar, 1990; Susheelamma et al., 1991).

CONCLUSIONS

The foregoing observations depict the effective usefulness of the Ethyl methane sulphonate (EMS) as potential mutagen for induction of mutations in mulberry genotypes. The results throw light upon the optimum concentrations and efficiency for production of broad spectrum of variations. The leaf yield increased by 30.64% at 0.3% of EMS treatment compared to control. This significant increase in yield was due to exponential rise in leaf area at 0.3% EMS treatment. Similarly significant rise in protein and high moisture retention capacity of leaf are other beneficial indicators of crop improvement scored at 0.3% EMS treatment. Thus wide array of EMS induced mutations broadened the scope for critical evaluation of biomass, morpho-economic characters,as well as phytochemical constituents of the beneficial mutants obtained. This suggests that EMS at lower concentrations below 0.5% can be safely used for crop improvement in mulberry and can also be tried in other vegetatively propagated crops.

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