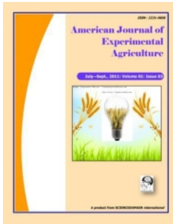

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EMS Induced Morphometric Biomass and Phytochemical Variations in *Morus* Species (Genotype RFS_{135})

H. V. Anil Kumar[1*], T. S. Muralidhar[2], Sourav Acharya[2], Manas Jyoti Das[2] and Munirajappa[3]

[1]Department of Environmental Science, DVS College of Arts and Science, Shimoga 577201, Karnataka, India.
[2]PG Department of Biotechnology, R&D Center, Dayananda Sagar College of Biological Sciences, Bangalore-560078, Karnataka, India.
[3]Department of Sericulture, Bangalore University, Bangalore-560052, Karnataka, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: In the present investigation mutations were induced by EMS in rain fed mulberry genotype RFS_{135} and evolved mutants were evaluated for crop improvement by morphometric and phytochemical studies.
Study Design: Cross-sectional study.
Place and Duration of Study: *Department of sericulture, Jnanabharathi, Bangalore University, Bangalore-560056, Karnataka, India,* between June 2006 and July 2010.
Methodology: The active bud sprouts of vegetative cuttings of RFS_{135} in multiple sets were treated for twelve hours intermittently (every one hour) with three different concentrations (0.1%, 0.3% and 0.5%).Further M_1V_1 and M_1V_2 generation clones were evaluated for biomass, nutritive and morpho-metric characters.
Results: The results revealed that concentrations of 0.1% and 0.3% EMS treatment were effective in significantly altering the morpho-metric characters, biomass yield and phyto chemical 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

**Corresponding author: Email: profanilhv@rediffmail.com;*

increased biomass was recorded among the M_1V_2 clones of 0.1% EMS treatment (p=0.0001) and 0.3% EMS treatment (p=0.0006). Further significant improvement was recorded in nutritive parameters such as proteins, reducing sugars, minerals and moisture content. Moisture retention capacity and Chlorophyll contents were also found to be high in mutant clones recovered from 0.1% and 0.3% EMS treatments.
Conclusion: 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 vegetative propagated crops.

Keywords: RFS_{135}; EMS; morphometric characters; biomass; phytochemical.

1. INTRODUCTION

Silk is a protein biosynthesized by the silkworm *Bombyx mori.* L. Silk fiber is obtained from the cocoon through process 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. Cocoon spun by the silkworm *Bombyx mori.* L. forms the raw material for production of silk and it is the end product of silkworm rearing. Silkworm *Bombyx mori* L. is monophagous [1] and exclusively feeds on mulberry, hence mulberry is the sole food of silkworm for foraging. 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 [2]. Nearly 70% of the mulberry leaf protein is converted into silk protein through biosynthesis in silkworm. Thus Mulberry leaf protein is the quintessence for synthesis of sericin and fibroin, components of silk protein. Obviously mulberry is the central dogma in sericulture and the increased biomass (leaves) in mulberry variety is the principal determining factor of higher cocoon yield. Mulberry is a perennial, deep-rooted, widely adaptable fast growing tree belonging to genus *Morus* L. under order Urticales and the family Moraceae according to Hooker.

Mulberry is basically a deciduous species of sub-tropical forests with arboreal habit and distributed in a wide area of tropical, sub-tropical, temperate and sub-arctic zones [3][4]. It is of great economic importance to sericulture industry and is extensively cultivated in sericulturally important countries of both tropical and temperate regions as forage for silkworm. Although mulberry is basically a tree, it is maintained as small bushes through repeated pruning and training for optimum utilization in sericulture. In India, the cost of mulberry leaf production amounts to nearly 60% of the total expenditure of silkworm cocoon production [5].Thus there is an immense need for improvement of mulberry varieties in terms of nutritive value and increased biomass (leaves) to ensure profitable production of cocoon.

The latest research reveals diversified functional utilization characteristics of mulberry products and by product in the field of food processing and pharmaceutical industries. Mulberry has been used as medicinal herb since ancient times and the various parts of mulberry is a base in an ayurvedic preparation. Root extract posses 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 that monitors blood-sugar level, closely related to diabetes. Thus mulberry tea is considered as a health drink. The leaves have a diaphoretic and emollient effect. The decoction of the mulberry leaves is used as a gargle for throat inflammation. The fruits of mulberry on an average contain 12 to 20 % sugar, they are non acidic and 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. It is 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 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. 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 quality of leaves, in rain fed mulberry genotype RFS_{135}, through EMS induced chemical mutagenesis. The purpose of induced mutations is to enhance the mutation frequency rate in order to select appropriate variants for plant breeding. The mutation frequency rate of spontaneous mutations is rather very low and difficult to exploit by the plant breeders. Mutations are induced by physical (e.g. gamma radiation) and chemical (e.g. ethylmethane sulfonate) mutagen treatment of both seed and vegetatively propagated crops [6].

Ethyl Methane Sulphonate (EMS) is a potent chemical mutagen, extensively used in genetic research. It is a monofunctional-ethylating agent that has been found to be mutagenic in wide a 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 like EMS reacts with DNA in variety of ways, a broad spectrum of mutagenic effects are manifested in the population. EMS has been found more potent for mulberry [7][8]. Thus EMS was conveniently used for induction of mutations in mulberry genotype RFS_{135}. 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 0.1% and 0.3% concentrations of EMS treatment were 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 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 these vegetative parameters were mutagen dose dependent factors.

2. MATERIALS AND METHODS

Rain fed Mulberry genotype *(Morus alba)* RFS_{135}, was procured from Central Sericulture Germplasm Research Station, Hosur, Tamil Nadu, for mutagenic studies. The genotype was established at germplasm bank, Department of Sericulture, Bangalore University, following the standard procedures of the National Bureau of Plant Genetic Resources (NBPGR) [9]. The genotype was maintained following the recommended package of practices for mulberry [10]. Four set of replicates (10 cuttings in each) from disease free twigs of mulberry variety RFS_{135} were prepared following the standard procedure [11], each cutting measuring 6 to 8 inches in length with 3 to 4 active buds. 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 [12].The planted pots were maintained in the nursery with consistent care. On sprouting the buds of each set were treated intermittently every one-hour for total span 12 hours with Ethyl methane sulphonate (EMS) solution following cotton swab method. The buds were capped with sterilized cotton.EMS solution was injected to the cotton cap covering the bud using 10ml syringe [13]. 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 three concentrations of EMS solution was prepared by V/V method. 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 assessed. After 90 days, rooting ability and proliferation was evaluated. Later the saplings were transplanted to the field and and maintained them in the pit system with spacing of 3'x3' 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 and documented by photographs.

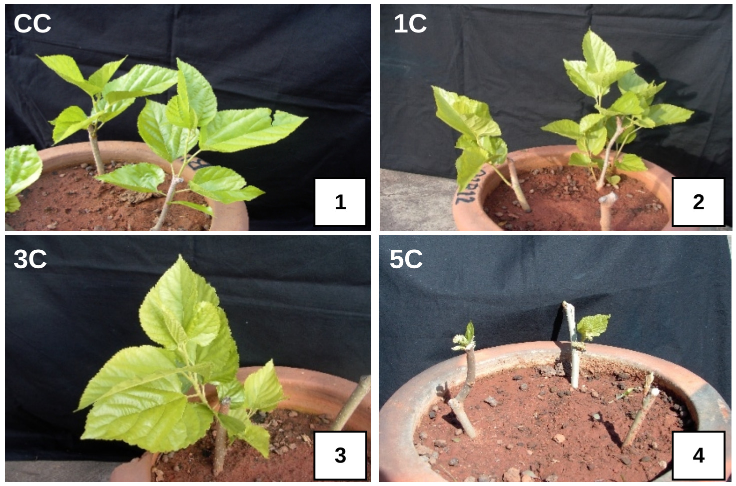
From M_1V_1 generation the beneficial variants recovered from 0.1% and 0.3% EMS treated clones of RFS_{135} were screened for further propagation of M_1V_2 populations. Cuttings screened and selected from M_1V_1 beneficial variants of RFS_{135} were raised in the nursery as per standard procedure. After three months the sapli ngs were transplanted to the field in separate blocks following RBD method with 3' x 3' spacing (pit system). The observations were recorded from 90th 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 by standard method [14].

The comparative studies were carried 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 consistent 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 [15],the reducing sugar estimation by Miller method [16] 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.

3. RESULTS AND DISCUSSION

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 [17]. Woody plants increase in size and complexity perennially, adding new shoots reinforcing existing stem. The numbers of primary branches are often arranged to provide a stable distribution of branch weight and expose maximum leaf area for optimal photosynthetic rates [18]. Thus there are often progressive changes in branching behavior viz., branches more or less upright to acutely orient (orthotropic) and somewhat drooping branches (Plagiotropic) [19]. 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. Wood is functionally important support tissue in woody plants and trees to maintain stability.



Figs. 1,2,3,4. Sprouting response in Mulberry genotype RFS135 (Control-CC), treated with 0.1% EMS (1C), vigorous sprouting in 0.3% EMS treatment (3C) and feeble response in 5C treated with 0.5% EMS respectively

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. Increased plant height was recorded in EMS treated mulberry genotype RFS_{135}. The 0.3% treatment of EMS was effective in increasing the plant height to 937.53 cm compared to control (662.50cm).Similarly 0.1% EMS treatment also showed increased plant height (778.06cm) (Table1). 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 [20]. Variability in plant height in chickpea through gamma irradiation has revealed that the radiation doses of 5 and10 Krad has slightly reduced plant height while other dose had no considerable effect on plant height [21]. Similar kind of variability in plant height was also observed through EMS treatments in *Capsicum annum* [22]. Mutations affecting the plant height, indicating that the mutagen doses would cause both positive and negative genetic variability in plant height have also been reported [23][24].

Increase in unit length of shoot obviously provides more scope for higher number of branches the number of branches at 0.3% EMS treated clones were 38.40 an average compared to control (21.26), while it was 28.06 at 0.1% EMS treatment. There is increase in mean number of branches per plant and mean height of the plant in mutants resulted from EMS treated population of $RFS_{135.}$ In the present study, significant variations in number of branches and plant height between the control plants and M_1V_2 variants of 0.1% EMS (p=0.0001) and 0.3% EMS (p=0.0006) treated population have been established (Table 1). Similar findings has been reported in EMS treated rapseed plants, (0.75%, 1.00% EMS solution) treatments had induced genetic variability in case of rapeseed (*Brassica napus* L.). 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 [25].

Table 1. Morphometric and biomass parameters of mulberry genotype RFS_{135}

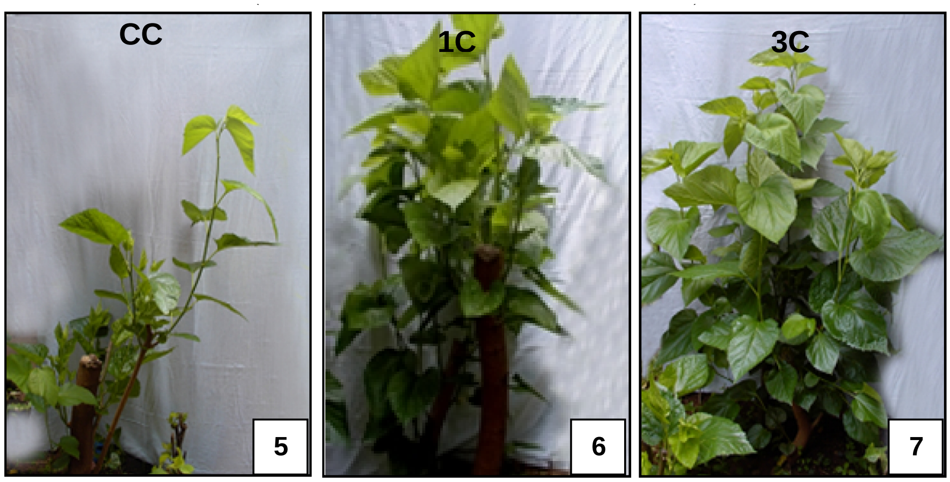
Morpho-economic parameters				**Average**		
				CC	**1C**	**3C**
Number of Leaves				660.93	872.55	989.66
Leaf Area (in cm^2)				282.64	716.91	811.51
Inter nodal Distance (cm)				5.8	3.99	3.10
Number of branches				21.26	28.06	38.40
Diameter of the stem girth (cm)				7.16	5.64	5.05
Length of Petiole (cm)				4.9	6.7	7.2
Yield per plant (kg)				9.05	11.59	13.64
Number of inflorescence				34.47	60	37.4
Length of Inflorescence (mm)				35.53	53.8	60.13
Height of the plant (cm)				662.5	778.06	937.53
ANOVA for *CC and **1C – Morphological Parameters						
Source of variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between groups	1812022.58	9.0	201335.84	15.98	0.0001	3.0204
Within groups	125959.78	10.0	12595.98			
Total	1937982.36	19.0				
ANOVA for *CC and ***3C – Morphological Parameters						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between groups	2176125.139	9.0	241791.68	10.30	0.0006	3.020
Within groups	234700.895	10.0	23470.09			
Total	2410826.034	19.0				

** CC - Control; ** 1C - 0.1% EMS Treated; ***3C- 0.3% EMS Treated*

In the present findings the sprouting is prolific in 0.1% and 0.3% EMS treated clones of mulberry RFS_{135} (Figs 2&3) and declined in clones treated with 0.5% concentration of EMS (Fig 4) indicating that the ability of sprouting is dose dependent factor. Similar results have been reported in several crops. 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 [26]. Thus it infers that the genetic variabilities of agro- economic and morphometric traits among crop plants 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 studies the mutants evolved from EMS treated mulberry genotypes RFS_{135} has exhibited significant increase in the mean values in respect of increase in number of branches and number of leaves. The mean number of leaves was found to be 872.55 and 989.66 in the mutants of RFS_{135} mulberry genotype recovered at 0.1% and 0.3% EMS treatment respectively, as against the control (660.93) (Table 1).The leaf yield per plant was significantly high in the mutant clones of 0.3% EMS treatment (13.64 kg), followed by 11.59 kg at 0.1% EMS treatment compared to control (9.05 kg) (Figs. 5, 6 and 7).

The leaf yield increased by 50.64% at 0.3% and 28.0% at 0.1% of EMS treatments respectively compared to control. This significant increase in yield was due to the increased leaf area that exponentially rose to 811.51 cm^2 at 0.3% EMS treatment followed by 716.91 cm^2 at 0.1% compared to control (282.64cm^2) (Fig. 8).The increase in leaf area in EMS treated populations of RFS_{135} is quite significant. Such type of increased leaf area due to colchicine treatment in mulberry has been reported [27]. 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 that reflects the superiority of raw silk produced.



Figs. 5, 6, 7. Branching and foliage density in control and EMS treated mulberry genotype RFS_{135}

(CC- Control; 1C- 0.1%EMS; 3C- 0.3%EMS)

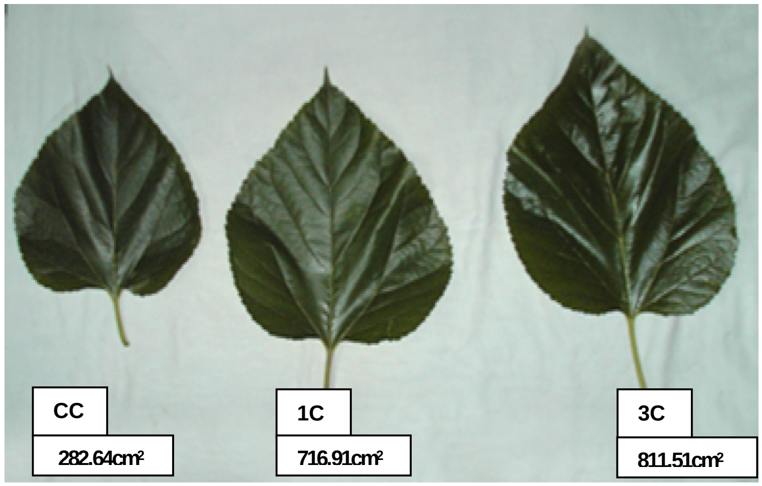


Fig. 8. Increase in leaf area in control and EMS treated mulberry genotype RFS_{135}
(CC- Control; 1C- 0.1%EMS; 3C- 0.3%EMS)

The nutritional compositions of leaves greatly influence the robustness, growth and development of silkworm larvae [28][29]. Further, it is established fact that proteins, total soluble sugars, chlorophyll, amino acids, minerals, vitamins and sterols forms important nutritional requirement of silkworm larvae[30]. In the present investigation in the M_1V_2 mutants recovered from 0.1% and 0.3% EMS treatments of RFS_{135} are quite promising.

The protein content was higher at 0.3% EMS treatment (tender-43.33%; medium- 41.59% and coarse- 39.51%) followed by 0.1% EMS treatment (tender - 37.33%, medium - 35.83% and coarse - 33.90%) compared to control (tender - 29.66%, medium - 28.43% and coarse - 25.92%). The protein level is significantly high at 0.3% EMS treatment. The reducing sugar was also significantly high (tender-12%, medium-9.26% and coarse - 7.307%) in the mutants recovered from 0.3% EMS treatment of RFS_{135} mulberry genotype (Table 2).

The 0.1% EMS treatment showed the reducing sugar of 3.16% in tender. 1.56% in medium and 1.096% in coarse leaves, whereas the control plants showed 2.16% in tender, 1.06% medium and 0.81% in coarse leaves. There has been significant increase in the amount of reducing sugar in the mutants of 0.3% EMS treatment in RFS_{135} mulberry genotype (Table 2).The total chlorophyll content was also found higher than the control at 0.3% EMS treatment. It was found in the range of 3.708 mg/gf. wt in tender, 3.696 mg/gf.wt in medium and 3.675 mg/gf.wt in coarse leaves. Whereas it was 2.909 mg/gf.wt, 2.850 mg/gf.wt in medium and 2.863 mg/gf.wt in coarse of 0.1% EMS treated variants. Chlorophyll content is relatively low i.e., 2.791 mg/gf.wt, 2.766 mg/gf.wt and 2.755 mg/gf.wt in tender, medium and coarse leaves respectively in control plants (Table 2).The increase in protein content in EMS treated mulberry genotype of RFS_{135} is in conformity with the findings of Shkvarnikov et al. [31]. These authors have recorded increased protein content associated with earlier ripening and increased grain weight in the mutants of maize recovered from EMS treatment. Similar findings were reported with regard to increase in protein content in soybeans due to x-ray treatment [32]. 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 of connecting links between the organic and inorganic components of the ecosystem. Earlier it has been reported that the total chlorophyll content of fresh leaves ranges from 0.14% to 0.35% in weight [33]. The highest percentage of chlorophyll content has been recorded in triploids compared to tetraploids [34].

Mulberry leaves are also rich in alkaloids, polyphenols, flavonoids, and anthocyanins, which have been proved to be responsible for health benefits. Anthocyanins are pigments which hold potential use as dietary modulators of mechanisms for various diseases [35]. Anthocyanins are the major flavonoids present in mulberry, probably expected to contribute significantly to their antioxidant activity. Nevertheless it is significant that alcoholic fermentation of fruits of *Morus nigra* might also contribute to antioxidant activity, due to anthocyanins, such as (cyanidin 3-glucoside and cyanidin 3-rutinoside) and two flavonoids (quercetin 3-glucoside and rutin [36].These two useful antioxidants are the phytochemical and nutrient constituents of mulberry leaves. In the present study the mutant clones of 0.3% and 0.1% EMS treatment has shown higher total chlorophyll compared to control. Since these pigments, 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). Similarly 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 has revealed significant variations. Mulberry variety Da 10 (*Morus atropurpurea* Roxb.) was screened as the most valuable cultivar considering its high content of functional components [37]. Probably the mutants recovered from 0.3% and 0.1% 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 RFS_{135}.

In the present investigations, the M_1V_2 mutants of RFS_{135} mulberry genotype recovered from 0.1% and 0.3% EMS treatments have shown significant changes in moisture content and moisture retention capacity. The leaves of M_1V_2 mutants of 0.1% EMS treatment showed the moisture content in the range of 78.66% in tender, 76.37% in medium and 74.06% in coarse leaves. The moisture retention capacity was high (73.09%) compared to control plants (69.46%).

The moisture content was significantly high in the M_1V_2 mutants of 0.3% EMS treatment. It is in the range of 91.49%, 88.83% and 87.56% in tender, medium and coarse respectively compared to control (tender-76.77%, medium-75.26% and coarse-73.03%). The moisture retention capacity was high (76.73%) when compared to the control plants, which was found to be 69.49% (Table 2). The present findings are in agreement with the reports of earlier worker [38][39][40][41].

Table 2. Phytochemical composition of leaves of mulberry genotype RFS_{135} (control and EMS treated)

Treatments	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(%)
Control (CC)	**Tender**	Average	1.573	1.218	2.791	29.66	2.16	76.77	72.54
		S.E.M	0.0167	0.0133	0.0244	0.031	0.022	0.3712	0.7420
		C.D @ 5%	± 0.0416	± 0.0030	± 0.0479	± 1.7328	± 0.2647	± 0.768	± 1.454
	Medium	Average	1.565	1.201	2.766	28.43	1.06	75.26	73.80
		S.E.M	0.036	0.014	0.727	0.133	0.067	0.2723	0.2423
		C.D @ 5%	± 0.0336	± 0.0055	± 0.0539	± 0.5789	± 0.0753	± 0.5635	± 0.475
	Coarse	Average	1.559	1.196	2.755	25.92	0.816	73.03	69.46
		S.E.M	0.0173	0.0047	0.0255	0.032	0.001	0.2100	0.2522
		C.D @ 5%	± 0.1934	± 0.0138	± 0.0280	± 0.7742	± 0.0945	± 0.259	± 0.494
0.1% (1C)	**Tender**	Average	1.839	1.07	2.909	37.33	3.16	78.66	74.40
		S.E.M	0.003	0.005	0.035	0.214	0.0145	0.1955	0.7496
		C.D @ 5%	± 0.0158	± 0.0281	± 0.01245	± 0.6277	± 0.1058	± 0.3833	± 1.469
	Medium	Average	1.815	1.068	2.850	35.83	1.56	76.37	72.43
		S.E.M	0.010	0.005	0.0243	0.3155	0.0208	0.1324	0.5388
		C.D @ 5%	± 0.0060	± 0.0329	± 0.0385	± 0.8125	± 0.0860	± 0.259	± 1.056
	Coarse	Average	1.803	1.06	2.863	33.90	1.096	74.06	73.09
		S.E.M	0.003	0.008	0.0259	0.0472	0.007	0.1324	0.6211
		C.D @ 5%	± 0.0044	± 0.0313	± 0.0254	± 0.8216	± 0.1445	± 0.259	± 1.217
0.3% (3C)	**Tender**	Average	2.261	1.447	3.708	43.33	12	91.49	89.54
		S.E.M	0.2074	0.0020	0.004	0.0811	0.3113	0.1227	1.0811
		C.D @ 5%	± 0.0837	± 0.008	± 0.0309	± 0.7165	± 0.8670	± 0.2407	± 2.119
	Medium	Average	2.258	1.438	3.696	41.59	9.26	88.83	84.58
		S.E.M	0.122	0.009	0.009	0.1352	0.055	0.2610	0.8423
		C.D @ 5 %	± 0.0727	± 00544	± 0.0237	± 1.1489	± 0.2087	± 0.512	± 1.659
	Coarse	Average	2.245	1.430	3.675	39.51	7.307	87.56	76.73
		S.E.M	0.065	0.021	0.242	0.0133	0.0088	0.1361	0.4890
		C.D @ 5 %	± 0.0212	± 0.100	± 0.0360	± 0.6506	± 0.2565	± 0.267	± 0.959

** CC - Control; ** 1C - 0.1% EMS Treated; ***3C- 0.3% EMS Treated*

4. CONCLUSION

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 50.64% at 0.3% and 28.0% at 0.1% of EMS treatment compared to control. This significant increase in yield was due to exponential rise in leaf area at 0.3% EMS treatment followed by 0.1%.Similarly significant rise in protein and high moisture retention capacity of leaf are other beneficial indicators of crop improvement scored at 0.3% and 0.1% EMS treatments. 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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