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Ethyl Methanesulphonate (EMS) Induced Mutagenic Disorders in *Amaranthus tricolor* L.

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Abstract: ***Ethyl Methanesulfonate (EMS) is a common, powerful and one of the most effective chemical mutagen,to induce a large number of functional variations in crops. Present study was to analyze the mutagenic effectof EMS in M_1 generation in* Amaranthus*(Amaranthus tricolor L.). Seeds were treated with different doses (0.5%, 1%, 2% and 3%) of mutagen for 4 hrs and grown in gunny bags along with control. Morphological, anatomical, physiological and biochemical parameters of* Amaranthus *were analysed for 50 days at definite intervals. All parameters decreased with increase in doses of EMS. A strong deleterious effect on the germination percentage was witnessed in 3% of EMS. There was a negative correlation in length of root, shoot length, number of secondary roots and fresh weight with EMS percentage. Values of growth coefficient, relative growth rate, tolerance index, phytomass and net productivity were gradually decreasing with increasing doses of EMS. Anatomical parameters also showed marked decrease in stem. Leaf area and chlorophyll content were lowest in 3% EMS.***

Keywords: Growth coefficient, Relative growth rate, Tolerance index, Anatomy, Leaf area

1. Introduction

Mutations are the tools that are being used to study the nature and basis of plant growth and development, thereby producing raw materials for genetic improvement of crops [1], [2].Mutation induces a broad variation of morphological and yield structure parameters in comparison to normal plants [28]. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars in cereals, fruits and other crops[21].Ethyl methanesulfonate (EMS) is a common, powerful and one of the most effective chemical mutagen, to induce a large number of functional variations in crops, especially recommended when mutation is introduced to the seed materials [21]. EMS is a mutagenic,teratogenic, carcinogenic alkylating agentwhich can react directly with DNA andcause alkylation leadingto theinhibition and or stimulation of specific gene expression [13],[29]. It produces randommutation in genetic material bynucleotide substitution, particularly guanine alkylation.In plants, EMS usually causes point mutation butloss ofchromosome segment or deletion can also occur. It induces chemical modification of nucleotides, which result in mispairing and base changes.

The mutagenic effect of EMS has been reported in several plants such as *Arabidopsis*[13], [16];*Vignaunguiculata*[10],[12]; *Musa spp.* [22].

In particular, very little research on mutagenicity has been studied in *Amaranthustricolor*,thevegetable which constitute an important part of the human diet. Studies have shown that Amaranth seeds or oil is good for people with hypertension and cardiovascular disease; since regular consumption reduces blood pressure and cholesterol levels. It is also a very good source of vitamins including vitamin A, B_6 and C, riboflavin and foliate. It is also a major source of dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese[6].

The present study was undertaken to gather information on the mutagenic disorders of EMSon morphological, anatomical, physiological, biochemical and growth traits in *Amaranthus tricolor.*

2. Materials and Methods

Healthy seeds were treated with freshly prepared solutions of Ethyl methanesulfonate (EMS) for 4h with intermittent shaking. The different treatments were 0.5%, 1%, 2% and 3%. Untreated seeds were taken as control. After treatment, seeds were thoroughly washed in running water for 4h to leach out the residual of chemicals. For the studies, potting mixture was prepared according to the recommendation given by the Kerala Agriculture University, Mannuthy and filled in gunny bags.

10 sets of gunny bags were arranged in each concentration. Percentage germination was studied at 24^{th}, 48^{th}, 72^{th} and 96^{th} hrs. Plants were analyzed for various morphological, anatomical, physiological and biochemical studies at definite intervals *i.e.*, on 4^{th}, 6^{th}, 8^{th}, 10^{th}, 20^{th}, 30^{th}, 40^{th} and 50^{th} days after sowing. Data was analyzed to deduce mean (SE) and standard deviation (SD) using standard statistical procedure [31].

3. Results and Discussion

Seed germination results implied that EMS adversely influenced the germination from the very low doses itself. The highest percentage germination was observed in the control. Considerable reduction in the germination percentage was witnessed in 3% of EMS treatment in all days of observation. Hundred percentage reduction in germination was seen in 3% EMS treatments after 48 and 72 hours and it was only 88% in 92 hours (Table 1). A delay in germination occurs in EMS treatments. Similar results have been reported in *Jatrophacurc*[7]and Malaysian rice [3]. Severe reduction in germination is an indication of effective mutagenesis

[5],[27].Reduced seed germination may be due to chromosomal damages or damage of meristematic tissues of the seed [20],[23].

Mutagenic treatment also affected the morphological parameters of Amaranthus in M_1.There was a negative correlation between length of root and EMS percentage. Maximum reduction was seen in 3% EMS. In 3% EMS, 64.29%, 32.73%, 51.93%, 56.92%, 61.39% and 71.7% shrinkage in root length of M_1 plants on 4^{th}, 6^{th}, 8^{th}, 10^{th}, 30^{th} and 50^{th} day respectively (Table 2& 3).The reduction in root length with increasing EMS concentration has been reported in chick pea [15] and*Coixlacryma-jobi* [32].

The high dose treatment of EMS causing growth inhibition has been ascribed to the cellcycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome [4].

Table 1: Impact of EMS on Percentage Germination

Treatments		*% germination*		
	24hrs (%)	*48hrsn (%)*	*72hrs (%)*	*96hrs (%)*
Control	0	57.2	79	94
0.5%	0	49.4	72	78.56
1%	0	28	48	59.38
2%	0	1.2	3	13.4
3%	0	0	0	11.2

Table 2: Impact of EMS on Root Length in M_1

Treatments	*4^{th} day*	*6^{th} day*	*8^{th} day*
	Root Length (cm)	*Root Length (cm)*	*Root Length (cm)*
Control	0.98 ±0.18	3.3 ±0.57	4.14 ±0.74
0.5%	0.56 ±0.30	3.1 ±0.21	4 ±0.94
1%	0.5 ±0.19	3.16 ±0.21	3.2 ±1.04
2%	0.4 ±0.21	2.42 ±0.26	2.44 ±0.38
3%	0.35 ±0.12	2.22 ±0.23	1.99 ±0.53

Table 3: Impact of EMS on Root Length in M_1

Treatments	*10^{th} day*	*30^{th} day*	*50^{th} day*
	Root Length (cm)	*Root Length (cm)*	*Root Length(cm)*
Control	5.06 ±0.65	7.33 ±0.76	10 ±0.5
0.5%	4.63 ±0.56	6.5 ±0.50	7.17 ±0.23
1%	3.72 ±0.38	4.17 ±0.76	5.83 ±0.24
2%	2.95 ±0.38	3.83 ±0.29	5.00 ±0.23
3%	2.1 8±0.25	2.83 ±0.29	2.83 ±0.20

Table 4: Impact of EMS on Shoot Length in M_1

	4^{th} day	6^{th} day	8^{th} day
Treatments	Shoot Length (cm)	Shoot Length (cm)	Shoot Length (cm)
Control	1.98 ±0.83	5.94 ±0.67	7.74 ±0.99
0.5%	1.45 ±0.28	5.7 ±0.63	7.4 ±0.96
1%	1.4 ±0.39	5.62 ±0.67	6.6 ±0.65
2%	1.14 ±0.45	4.34 ±0.74	6.08 ±0.40
3%	1 ±0.61	3.98 ±0.40	4.01 ±0.42

Table 5: Impact of EMS on Shoot Length in M_1

	10^{th} day	30^{th} day	40^{th} day	50^{th} day
Treatment	Shoot Length (cm)	Shoot Length (cm)	Shoot Length (cm)	Shoot Length (cm)
0.5%	8.76 ±0.96	14.33±2.08	22.2 ±1.03	35 ±1
1%	7.93 ±0.74	8.33 ±0.58	14.34 ±0.86	31 ±1
2%	6.87 ±0.46	6.83 ±0.76	11.04 ±0.76	23.67 ±1.53
3%	6.21 ±0.61	5.5 ±1.32	7.84 ±0.59	14 ±1
	5.49 ±0.79	5 ±0	4.82 ±0.56	11 ±1

It has been shown that a negative linear relation exists between shoot length and the dosage of EMS. In all stages, peak shoot length was measured in control. More pronounced effect was viewed in 3% in all days, with 49.49%, 33%, 48.19%, 37.33%, 65.11%, 78.29% and 68.29% reduction in length of shoot on 4^{th}, 6^{th}, 8^{th}, 10^{th}, 30^{th}, 40^{th} and 50^{th} days respectively (Table- 4 & 5). Such a reduction in length of shoot arising out ofmutagenic treatments was previously reported in chickpea [15] and*Musa* spp. [22]. The reduction in length of shoot was attributed to the effects of mutagens on the physiological system [9].

The higher doses might have damaged the genetic material and also blocked cell division by decreasing the rate of physiological processes [5].

Fresh weight of whole plant decreased gradually from control to 3% in all intervals. During 10^{th} day, 14% to 43% loss in weight was recorded in various treatments. In 3% EMS, 89%, 84% and 96% depletion was noticed on 30^{th}, 40^{th} and 50^{th} day respectively (Table-6).

Table 6: Impact of EMS on Fresh Weight

	10^{th} day	30^{th} day	40^{th} day	50^{th} day
Treatments	FW/5Pt (g)	FW/Pt (g)	FW/Pt (g)	FW/Pt (g)
Control	0.08±0.01	1.43 ±0.23	3.1 ±0.15	22.49±0.50
0.5%	0.07±0.01	0.5 ±0.07	2 ±0.70	14±1
1%	0.07±0.02	0.39 ±0.05	1.5 ±0	10±1
2%	0.06±0.01	0.25 ±0.15	1 ±0.5	2.42±0.52
3%	0.05±0.15	0.16± 0.05	0.5 ±0.2	0.9±0.10

The mutational effects of EMS in the biometric parameters of plants were reported in several investigations, such as reduction in fresh and dry weights in *Zea mays* [11]; in fenugreek [18] etc.

Tolerance index is an integrated calculation of particular parameters and helpsto make a summary assessment of effect of stress factor on plant growth and development. Index values of tolerance were gradually decreasing with increasing doses of EMS on all days (Table- 7).

Present investigation also established the inhibition of relative growth rate. Maximum inhibition in growth rate was recorded in 3% (Fig- 1).

Table 7: Impact of EMS on Tolerance Index

Treatments	10^{th} day	30^{th} day	40^{th} day	50^{th} day
Control	100	100	100	100
0.5%	50	20	68.42	58.33
1%	50	10	47.37	47.22
2%	45	9	26.32	13.89
3%	20	5	5.26	5.56

Rate of growth 47.5 mg/day in control was reduced to 2.5 mg/day on 40th day. Similar trend was also observed on all days (Fig-1). The phytomass decreased with the increase in the concentration of mutagenic treatment on all days (Fig-2). In 3% EMS, ninety to 94% reduction was observed on different days of analysis.

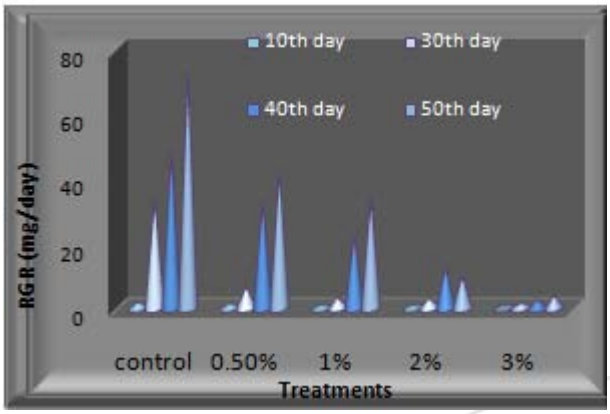


Figure 1: Impact of EMS on RGR

From lower concentration to higher concentrations, net productivity (Fig-3) also showed a linear negative trend. Previous studies in finger millet [8] were in unison with present investigation. Significant decrease in these parameters of mutant plants compared with that of the control was reported in wheat [4] and banana [30].

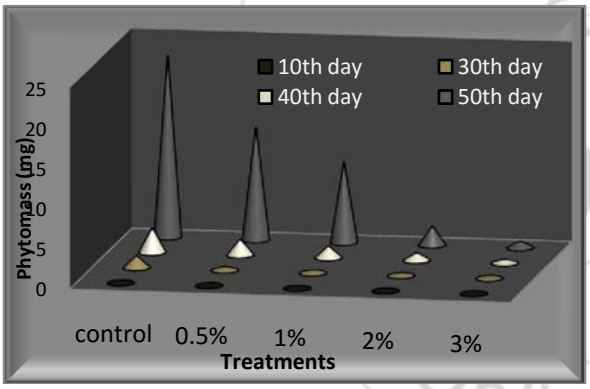


Figure 2: Impact of EMS on Phytomass

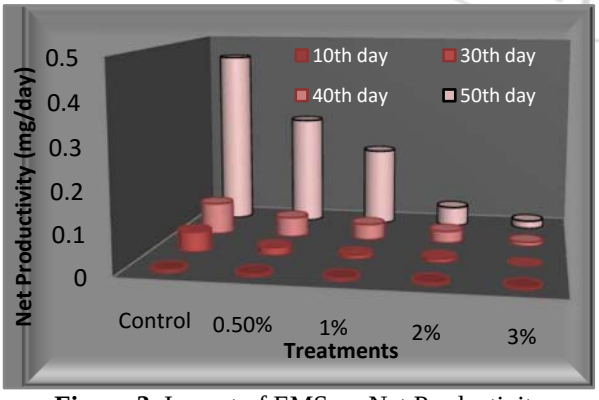


Figure 3: Impact of EMS on Net Productivity

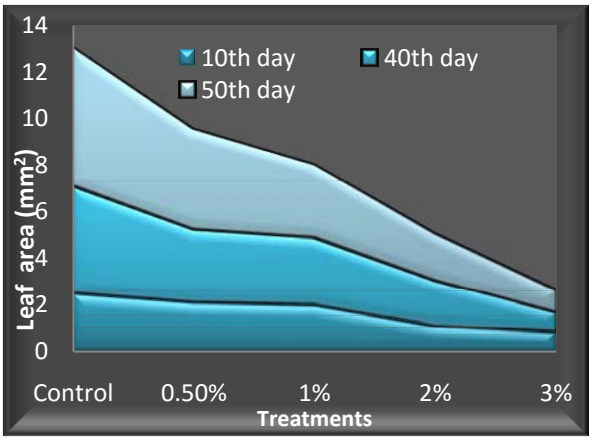


Figure 4: Impact of EMS on Leaf Area

Leaf area was observed maximum in the control plants and it progressively decreased in treatment categories (Fig-4). Reduction of 20 to 60% on 10th day; 31% to 82% on 40thday and 27% to 84% on 50th day was noticed various treatments. This result corroborated with the findings in *Capsicum annuum* [25].

The 3% EMS treated plants also recorded minimum leaves in M_1. Higher doses of EMS might have stopped the enzymes necessary for leaves initiation. Leaf abnormalities were attributed to the chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids [14].

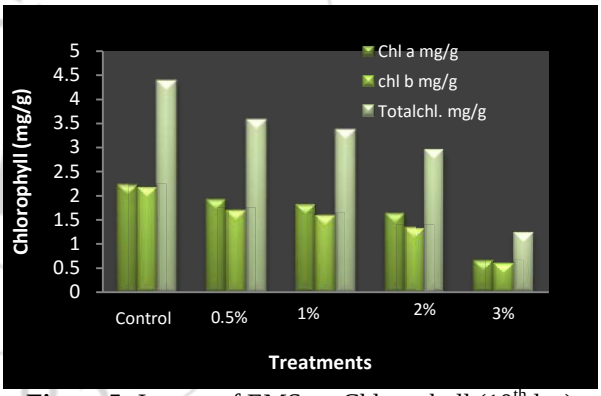
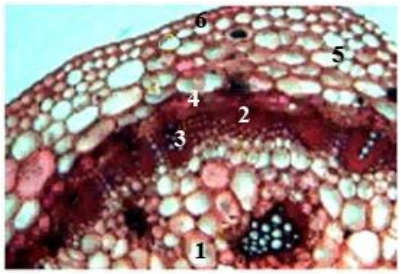


Figure 5: Impact of EMS on Chlorophyll (10thday)

Chlorophyll studies provide one of the most dependable indices of mutagenic treatments. Chlorophyll a, b and total chlorophyll diminished proportionately with increasing doses of EMS (Fig-5).In 3% EMS, 71%, 73% and 72% reduction was calculated in chlorophyll a, b and total chlorophyll respectively. EMS treatment diminished the total chlorophyll content as reported in safflower [26] and fenugreek [18]



Control

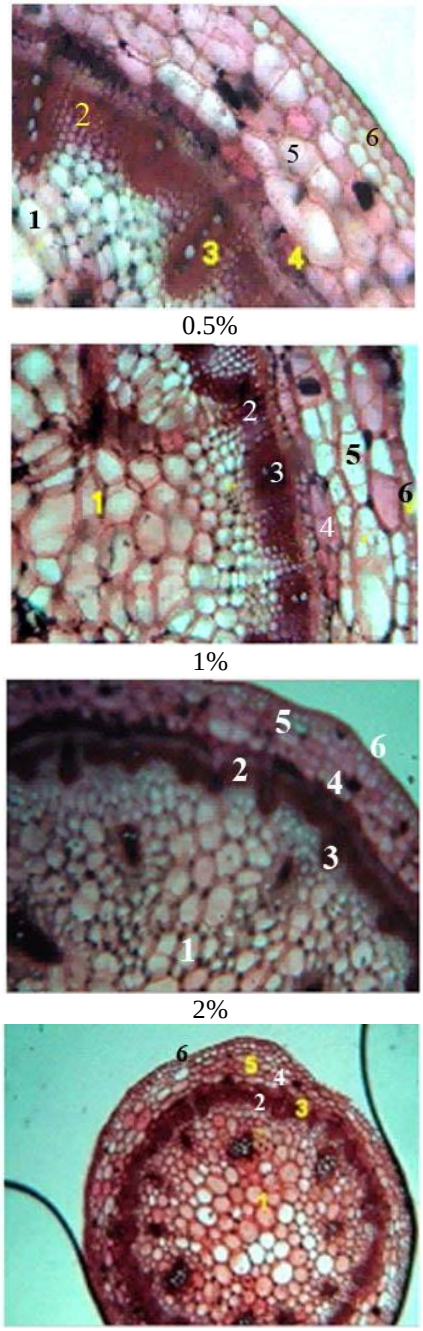


Plate 1: Anatomy of the Stem- 50th Day

1-pith; 2- Lignified cells; 3- Sec. xylem; 4- Sec. phloem;5-Cortex; 6-Epidermis.

Anatomical parameters registered a markeddiminution along with increasing dose of treatment in stem on 50th day of analysis (Plate -1).

On 50th day, the girth of stem shrinked considerably in 3%, with near about 40-60% shrinkage in the circumference of the stem. This shrinkage could be easily detected by the reduction in number, size, area and volume of ground tissue, cortical region, pith and secondary xylem and secondary phloem. All these reductions were in accordance with the applied dose of the EMS.

Prominent changes were found in the number, size and volume of parenchymatous tissue. Lignified cells and secondary xylem tissues also reduced considerably. The secondary xylem cells were disorted in size and shape. Size of the vessels alsogot reduced.

The mutagenic action of EMS results from its reaction with DNA by alkylating the phosphate groups [17]. Alkylation of a phosphate can cause breakage of the linkage betweendeoxyribose and phosphate.Incorporation of alkyl group into a base may result in the formation of a gap in the DNA template[19]and subsequent replication defects leading to mutations [24]. This changes in the genetic information oftenharmful to cells and can result in deleterious effects.

4. Conclusion

It is obvious from the current findings that with an increase in the EMS concentration, there is also an increase in the rate of mutation leading to deleterious effects.It was deleterious even at 1% EMS treated four hours. No desirable mutation occurred in any treatments. Maximum deleterious effects were noticed in the higher concentration i.e., 3% EMS.

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