Assessment of EMS induced genetic variation in *Urginea indica* Kunth Cytotype I through the degree of changes observed in stomata in M_1 and M_2 generations

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Abstract: **Sensitivity of *Urginea indica* Kunth Cytotype I to Ethyl Methane Sulphonate (EMS) was examined by considering the epidermal parameters such as stomatal index and stomatal size. Bulbs of *Urginea indica* Kunth were treated with five different concentrations of Ethyl Methane Sulphonate (EMS) and were planted to raise M_1 and M_2 generations. The selected parameters were compared with those of control. Significant variations were observed with respect to all the parameters in both M_1 and M_2 generations. The result thus showed the effectiveness of Ethyl Methane Sulphonate to induce genetic variability in *Urginea indica* Kunth Cytotype I.**

***Keywords*: *Urginea indica* Kunth Cytotype I, stomatal index, EMS, M_1 and M_2 generations, genetic variability, induced mutation.**

I. Introduction

Urginea indica Kunth belongs to the Liliaceae family and is extremely valuable in pharmacology. It is used to cure numerous human ailments and is used extensively in human homeopathy, phytotherapy and in veterinary science. It has long been cultivated as an important economic herb due to its high medicinal potential and has been identified as promising species with excellent source of medicine with pharmaceutical and biocidal applications.

Despite extensive medicinal applications, not much work has been carried out on this plant to generate diversity in existing plant varieties, to widen the extent of adaptability and enhance productivity of this species. Genetic diversity in plant is essential for adaptation of the population to the inevitable environmental changes for their survival and growth. Moreover, it is considered as the basis of any breeding program, which is required for the crop improvement. Mutation breeding is the important method used extensively for the improvement of the plant species through the induction or elimination of the genes from elite breeding lines[1]. Mutations may arise spontaneously or they may be induced by physical or chemical mutagens. The induced mutations offer the possibility for the induction of desired changes in various attributes, which can be exploited as such or through recombination breeding[2].

Chemical mutagens are used widely to induce genetic variability in plant, because unlike physical mutagens they do not require specialized equipment and are easier to handle. Ethyl Methane Sulphonate (EMS) is considered as the most effective mutagen among the chemical mutagen as it causes point mutations in plants[3-4]. It is an alkylating agent reported to be especially effective in giving a wider spectrum of morphological characters than physical radiation and to produce random point mutation inducing a low level of chromosomal breaks and lethal effects. EMS can be used not only to increase genetic variability in plants, but also to produce mutants with high photosynthetic efficiency through the alteration of leaf anatomy.

Stomatal study is reported to be valuable at analytical level and because of uniformity in their size they are considered to be correlated with genome size[5]. Stomata are expected to show a more consistent allometric relationship with genome size owing to their uniformity in size. This research was therefore, conducted to study the effect of EMS on the leaf anatomy of *Urginea indica* Kunth. Stomatal index is an anatomical feature that is not influenced by the environment (stable), while the stomatal size keeps on changing depending upon the environmental conditions. Therefore, the effects of different concentrations of EMS on these factors were examined to find out the mutational changes induced in *Urginea indica* Kunth cytotype I, and the possibility of generation of genetically evolved varieties of this significantly important species. Moreover, in order to induce variability and utilize useful mutations for efficient plant breeding, the systematic study of viable morphological mutations in M_1 and M_2 generations is the most dependable index. Therefore, in the present research, the effects of EMS on the stomatal index and stomatal size were examined for the two generations i.e. M_1 and M_2 generations.

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II. Material and Methods

Bulbs of *Urginea indica* Kunth were collected from Birsa Agriculture University, Ranchi, Jharkhand and was named as:

- ***Urginea indica* Kunth Cytotype I**

A Chemical Mutagen, Ethyl Methane Sulphonate (EMS) was used in this research work to treat the selected cytotype of *Urginea indica* Kunth. Five different concentrations of Ethyl Methane Sulphonate (EMS) were prepared. All the concentrations were prepared under aseptic conditions. Different concentrations of Ethyl Methane Sulphonate (EMS) viz., 0.1%, 0.2%, 0.3%, 0.4% and 0.5% were prepared from the 1% stock solution of Ethyl Methane Sulphonate (EMS), which was prepared by dissolving 1 ml of Ethyl Methane Sulphonate (EMS) in 100 ml distilled water. Fresh and healthy *Urginea indica* Kunth bulbs of uniform size of the selected cytotype of *Urginea indica* Kunth, Cytotype I, were treated with different concentrations of Ethyl Methane Sulphonate (EMS) for six hours. Then the treated bulbs were thoroughly washed in running tap water to remove the residual effect of the chemicals used. The control and the treated bulbs were grown in the experimental plots in the randomized block design to rise M_1 generation. The mature leaves of *Urginea indica* Kunth Cytotype I were collected from the plants raised from the treated bulbs. The stomatal studies of these leaves were done by mechanical peeling off method. Length and width of stomata were measured and stomatal index was obtained by using the formula:

$$SI = \frac{\text{Number of stomata per unit area}}{\underset{\text{cells per unit area}}{\text{Number of Epiermal}} + \underset{\text{per unit area}}{\text{Number of Stomata}}} \times 100$$

III. Result

The effect of Ethyl Methane Sulphonate (EMS) on stomata of *Urginea indica* Kunth was studied for two generations (M_1 and M_2 generations). Both the surfaces of the leaves at apex, middle and base portions were taken to study the stomata. The results indicated that the effects of Ethyl Methane Sulphonate (EMS) showed variations in stomatal index and stomatal size at different concentrations when compared to the control in the selected cytotype of *U. indica* Kunth (table 1-4; fig. 1-5).

In *Urginea indica* Kunth Cytotype I, stomatal index was noticed to decrease at 0.1% concentration and it increased significantly at 0.3% concentration treatment of Ethyl Methane Sulphonate at all the three portions of the leaves (apex, middle and base), when compared to their respective values in control. Whereas, on the ventral surface of the leaf it was reported to decrease at 0.3% concentration at all the portions of the leaf (apex, middle and base), and increased at 0.1%, 0.2% concentrations on apex and base portions in M_1 generation (table 1; fig. 1-2).

In *Urginea indica* Kunth Cytotype I, stomatal index continued to show variations at different concentrations of Ethyl Methane Sulphonate in M_2 generation. The stomatal index in M_2 generation was reported to show increase at 0.1%, 0.2% and 0.4% while decrease at 0.3% and 0.5% at the apex and middle portion of the leaves when compared to that in M_1 generation. On the other hand, at basal portion of the leaf it showed significant increase at 0.1% (12.924 ± 0.819) and 0.5% (13.437 ± 1.044) and decrease at rest of the concentrations (0.2%, 0.3% and 0.4%) on dorsal surface in M_2 generation (table 1, 3; fig. 1-5). Similarly, on ventral surface of the leaf in *Urginea indica* Kunth Cytotype I stomatal index showed increase at 0.1%, 0.2%, 0.3% and 0.5% and considerable decrease at 0.4% in apex portion and middle portion of the leaves respectively. While at basal portion of the leaf it showed increase at lower concentrations (0.1%, 0.2% and 0.3%) and decrease at 0.4% (10.578 ± 0.703) and 0.5% (8.583 ± 1.118) concentrations in M2 generation (table 2; fig. 1-3).

When the stomatal length and width of *Urginea indica* Kunth cytotype I were compared to their respective values in control, after treatment with Ethyl Methane Sulphonate, considerable variations were observed at all the concentrations in M_1 and M_2 generations. When the length of the stomata of leaf was compared to its respective values in control, it was reported to decrease at all the concentrations at apex portion of the leaves. Increase at 0.1%, 0.2% and 0.5% and decrease at 0.3% and 0.4% concentrations at middle portion of the dorsal surface of the leaves were reported. While at the basal portion of the leaves on dorsal surface of the leaf, it was reported to increase at 0.1% (19.70 ± 0.143µ) and 0.5% (20.30 ± 0.143µ) and decrease at rest of the concentrations (0.2%, 0.3% and 0.4%) in M_1 generation. On the other hand, in M_1 generation, on ventral surface of the leaf in *Urginea indica* Kunth Cytotype I, length of the stomata was reported to decrease at all the concentrations at apex and base portions of the leaf, while at the middle portion of the leaf it was reported to increase at 0.1% and 0.5% concentration and decrease at remaining concentrations (0.2%, 0.3% and 0.4%) (table 41; fig. 166). Similarly, width of the stomata in M_1 generation was compared to their respective value in control on dorsal surface of the leaf. It was noticed that it increased at all the concentrations at apex portion of the leaf, decreased at all the concentrations except at 0.5% concentration at middle portions of the leaf. While it decreased at 0.2%, 0.3% and increased at 0.1%, 0.4% and 0.5% concentrations respectively at the base portion of the leaf.

On the other hand, width of stomata at apex, middle and base portions on the ventral surface of the leaf was reported to decrease at all the concentrations of Ethyl Methane Sulphonate in M_1 generation (table 41; fig. 166).

The length and width of the stomata continued to show variations in M_2 generation also. In some portions of the leaf the length as well as width increased at certain concentrations while they were reported to decrease at certain concentrations. Thus, the size of the stomata was reported to show variations after treatment with different concentrations of EMS, both in M_1 and M_2 generations. However, neither the increase nor the decrease in the size of stomata as well as stomatal index was in linear fashion.

Table-1: Stomatal Index of dorsal and ventral leaf surfaces in *Urginea indica* Kunth Cytotype I after treatment with different concentrations of Ethyl Methane Sulphonate (EMS) in M_1 Generation

Leaf surface	Concentration	Apex portion of leaf	Middle portion of leaf	Base portion of leaf
Dorsal	**Control**	10.830 ± 1.262	14.221 ± 0.965	8.696 ± 0.9950
	0.1%	7.553 ± 0.950	4.768 ± 0.117	5.475 ± 0.483
	0.2%	7.988 ± 1.152	9.745 ± 1.561	12.841 ± 0.707
	0.3%	14.116 ± 0.961	18.577 ± 1.284	10.058 ± 0.677
	0.4%	10.815 ± 1.025	11.681 ± 1.701	9.686 ± 1.051
	0.5%	11.382 ± 1.230	9.870 ± 0.886	8.940 ± 0.714
Ventral	**Control**	14.933 ± 1.034	19.801 ± 1.025	8.755 ± 0.943
	0.1%	15.838 ± 1.125	10.921 ± 1.311	10.020 ± 0.796
	0.2%	18.441 ± 0.724	16.507 ± 0.847	13.676 ± 0.999
	0.3%	5.818 ± 0.760	7.630 ± 1.247	8.156 ± 0.830
	0.4%	23.489 ± 1.053	14.486 ± 0.962	12.195 ± 0.881
	0.5%	17.407 ± 0.873	13.335 ± 1.400	13.807 ± 1.485

Table-2: Stomatal Index of dorsal and ventral leaf surfaces in *Urginea indica* Kunth Cytotype I after treatment with different concentrations of Ethyl Methane Sulphonate (EMS) in M_2 Generation

Leaf surface	Concentration	Apex portion of leaf	Middle portion of leaf	Base portion of leaf
Dorsal	Control	10.830 ± 1.262	14.221 ± 0.965	8.696 ± 0.950
	0.1%	11.525 ± 1.389	8.991 ± 0.816	12.924 ± 0.819
	0.2%	11.150 ± 0.730	10.654 ± 1.538	8.611 ± 0.727
	0.3%	8.100 ± 0.558	7.369 ± 0.538	4.245 ± 0.511
	0.4%	23.107 ± 1.336	14.799 ± 1.294	6.774 ± 0.659
	0.5%	8.006 ± 0.959	9.600 ± 1.085	13.437 ± 1.044
Ventral	Control	14.933 ± 1.034	19.801 ± 1.025	8.755 ± 0.943
	0.1%	25.008 ± 0.863	23.912 ± 0.984	23.719 ± 0.803
	0.2%	18.691 ± 0.705	19.848 ± 0.748	20.728 ± 0.798
	0.3%	17.868 ± 1.039	8.370 ± 0.986	24.606 ± 0.786
	0.4%	10.746 ± 1.533	7.174 ± 0.698	10.578 ± 0.703
	0.5%	17.337 ± 0.922	16.991 ± 1.326	8.583 ± 1.118

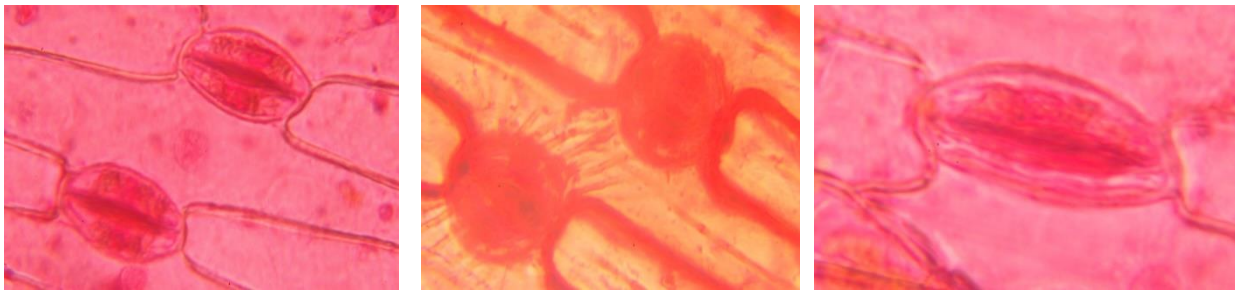
Table 3: Length and width of stomata (in µ) in dorsal and ventral leaf surfaces of *Urginea indica* Kunth Cytotype I after treatment with different concentrations of Ethyl Methane Sulphonate (EMS) in M_1 generation

Leaf Surface	Concentration	Apex portion of leaf		Middle portion of leaf		Base portion of leaf	
		Length (µ)	Width (µ)	Length (µ)	Width (µ)	Length (µ)	Width (µ)
Dorsal	Control	19.50 ± 0.287	13.10 ± 0.153	18.50 ± 0.400	15.30 ± 0.368	19.00 ± 0.903	14.30 ± 0.540
	0.1%	17.90 ± 0.758	14.50 ± 0.320	19.50 ± 0.287	14.80 ± 0.272	19.70 ± 0.143	16.70 ± 0.281
	0.2%	18.10 ± 0.429	13.90 ± 0.326	19.10 ± 0.381	13.50 ± 0.545	17.70 ± 0.200	14.00 ± 0.369
	0.3%	17.80 ± 0.364	13.60 ± 0.250	16.10 ± 0.218	14.50 ± 0.320	18.30 ± 0.504	14.70 ± 0.281
	0.4%	18.00 ± 0.312	15.50 ± 0.251	17.80 ± 0.519	15.00 ± 0.241	18.90 ± 0.354	15.20 ± 0.233
	0.5%	17.60 ± 0.207	15.20 ± 0.272	18.70 ± 0.396	16.70 ± 0.656	20.30 ± 0.143	14.90 ± 0.294
Ventral	Control	19.90 ± 0.293	17.00 ± 0.034	19.00 ± 0.481	16.10 ± 0.293	20.50 ± 0.209	16.20 ± 0.303
	0.1%	19.40 ± 0.375	14.90 ± 0.406	19.50 ± 0.287	15.70 ± 0.200	18.40 ± 0.467	14.20 ± 0.390
	0.2%	17.60 ± 0.286	13.90 ± 0.259	16.60 ± 0.152	13.90 ± 0.093	18.80 ± 0.336	13.90 ± 0.168
	0.3%	17.10 ± 0.354	14.80 ± 0.233	16.40 ± 0.318	13.60 ± 0.152	17.60 ± 0.318	12.90 ± 0.531
	0.4%	15.70 ± 0.442	10.10 ± 0.218	12.90 ± 0.294	8.90 ± 0.452	16.80 ± 0.519	9.80 ± 0.233
	0.5%	18.50 ± 0.349	13.70 ± 0.420	19.60 ± 0.207	15.10 ± 0.531	19.30 ± 0.244	15.40 ± 0.400

Table 4: Length and width of stomata (in µ) in dorsal and ventral leaf surfaces of *Urginea indica* Kunth Cytotype I after treatment with different concentrations of Ethyl Methane Sulphonate (EMS) in M_2 generation

Leaf Surface	Concentration	Apex portion of leaf		Middle portion of leaf		Base portion of leaf	
		Length (µ)	Width (µ)	Length (µ)	Width (µ)	Length (µ)	Width (µ)
Dorsal	Control	19.50 ± 0.287	13.10 ± 0.153	18.50 ± 0.400	15.30 ± 0.368	19.00 ± 0.903	14.30 ± 0.540
	0.1%	17.90 ± 0.758	14.50 ± 0.320	19.50 ± 0.287	14.80 ± 0.272	19.70 ± 0.143	16.70 ± 0.281
	0.2%	18.10 ± 0.429	13.90 ± 0.326	19.10 ± 0.381	13.50 ± 0.545	17.70 ± 0.200	14.00 ± 0.369
	0.3%	17.80 ± 0.364	13.60 ± 0.250	16.10 ± 0.218	14.50 ± 0.320	18.30 ± 0.504	14.70 ± 0.281
	0.4%	18.00 ± 0.312	15.50 ± 0.251	17.80 ± 0.519	15.00 ± 0.241	18.90 ± 0.354	15.20 ± 0.233
	0.5%	17.60 ± 0.207	15.20 ± 0.272	18.70 ± 0.396	16.70 ± 0.656	20.30 ± 0.143	14.90 ± 0.294
Ventral	Control	19.90 ± 0.293	17.00 ± 0.034	19.00 ± 0.481	16.10 ± 0.293	20.50 ± 0.209	16.20 ± 0.303
	0.1%	17.66 ± 0.348	14.21 ± 0.188	18.10 ± 0.244	15.09 ± 0.198	19.10 ± 0.260	13.80 ± 0.390
	0.2%	17.61 ± 0.250	14.30 ± 0.281	19.88 ± 0.168	14.23 ± 0.234	18.00 ± 0.313	13.90 ± 0.219
	0.3%	16.82 ± 0.538	11.84 ± 0.306	19.56 ± 0.438	12.16 ± 0.260	19.30 ± 0.132	14.20 ± 0.436
	0.4%	16.19 ± 0.219	13.87 ± 0.234	18.15 ± 0.234	15.59 ± 0.252	17.40 ± 0.286	14.70 ± 0.371
	0.5%	18.31 ± 0.281	13.53 ± 0.349	18.87 ± 0.254	14.70 ± 0.280	19.00 ± 0.342	14.90 ± 0.295

Fig.1-3: Effect of Ethyl Methane Sulphonate and Monosodium glutamate on the stomata of *Urginea indica* Kunth Cytotype I



Change in Shape and Size of Stomata

Fig.4: Column graph showing Stomatal Index of dorsal leaf surface after treatment with Ethyl Methane Sulphonate in *Urginea indica* Kunth Cytotype I in M_1 and M_2 generations

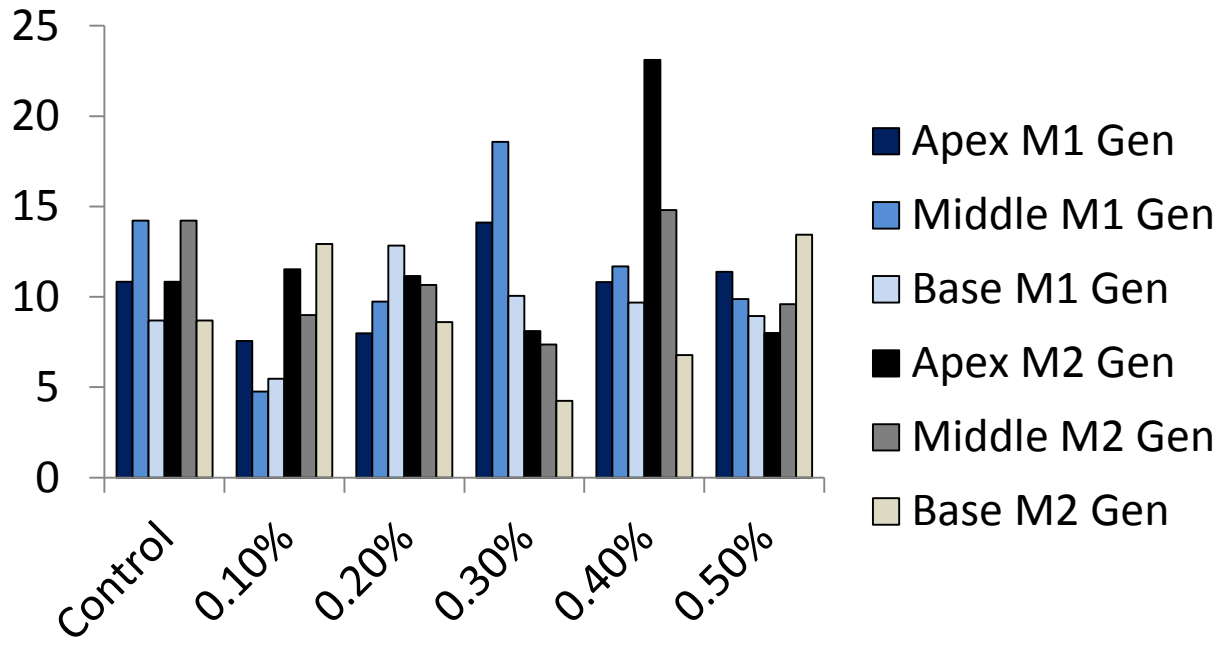
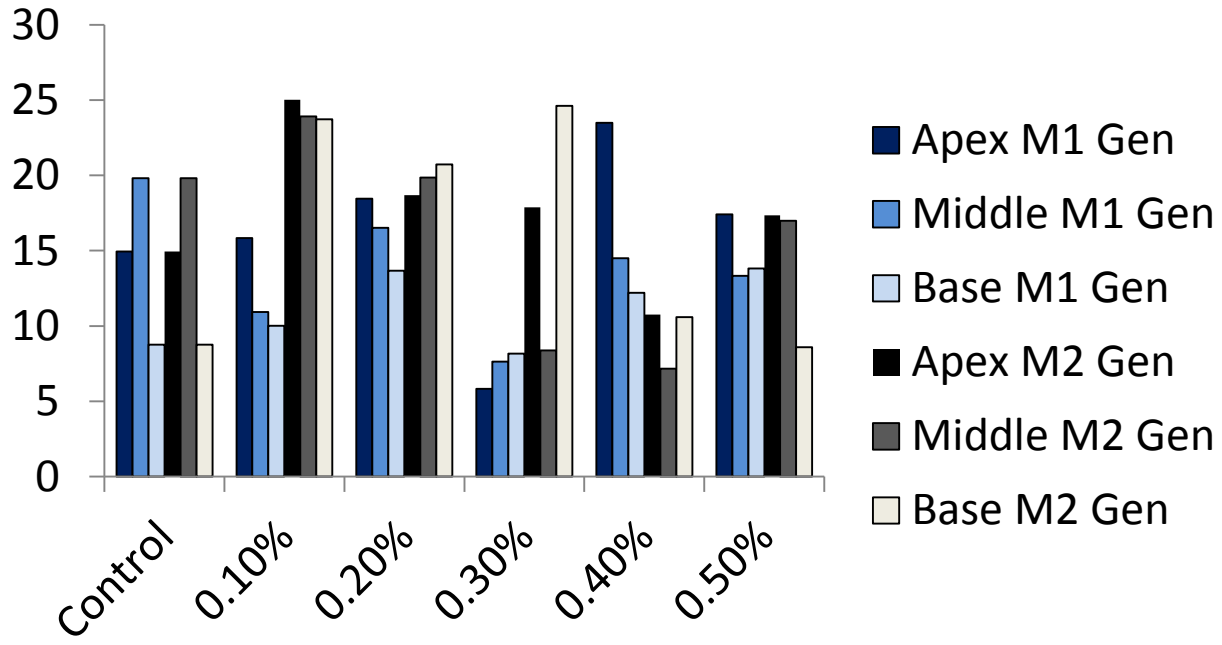


Fig.5: Column graph showing Stomatal Index of ventral leaf surface after treatment with Ethyl Methane Sulphonate in *Urginea indica* Kunth Cytotype I in M_1 and M_2 generations



IV. Discussion

Epidermal anatomy has long been considered as the diagnostic character in the phylogenetic studies of higher plants. The effects of mutagens on these characters can be considered as the reliable tool to measure the degree of genetic variability induced by the mutagen[6-7]. Therefore, stomatal index and stomatal size of *Urginea indica* Kunth Cytotype I were examined after treatment with different concentrations of Ethyl Methane Sulphonate for two generations (M_1 and M_2).

The chemical mutagen Ethyl Methane Sulphonate was reported to affect the size and stomatal index. It did not affect the stomata size and index in a linear fashion. The stomatal index was found to show variations at different concentrations. It was reported to decrease at 0.1% concentration, and increase significantly at 0.3% concentration at all the examined portions on dorsal surfaces. While on ventral surface of the leaf, the stomatal index was reported to increase significantly at all the concentrations except at 0.3% concentrations in apex and middle portions of the leaf and found to decrease at all the concentrations in basal region. Stomatal

index is an anatomical feature that is not influenced by the environment (stable) as compared to stomatal width and the stomatal density. However, changes in the stomatal index shows the genetic influence of the EMS on the plant.
Reduction in the length and width of stomata at almost all the portions of the leaf on dorsal and ventral surface of the leaf of *Urginea indica* Kunth Cytotype I were noticed. The reduction in size of stomatal aperture has been correlated to enhanced carbon dioxide level in the atmosphere[8]. Generally it is assumed that increase in the size of stomata, there is a decrease in stomata per unit area. Stomatal density may be a useful indicator of draught tolerance. The distribution of stomata and their number are important factor for determination of susceptibility or resistance[9]. The size of stomata is regarded as the cytological criteria, which may show changes due to mutagenic effects[10].
The variations observed in the stomatal index and stomatal size after treatment showed their sensitivity to Ethyl Methane Sulphonate. This in result may indirectly enhance the photosynthetic efficiency in the plant, which influences the entry of CO_2 into the plant[11].

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