

## MUTAGENIC EFFECTS OF ETHYLMETHANE SULPHONATE ON EUCALYPTUS CAMALDULENSIS X E. TERETICORNIS

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### Introduction

The direct use of induced mutations is a valuable supplementary approach to plant breeding. One of the promising advances in the field of induced mutagenesis has been the use of certain physical and chemical mutagens for inducing mutations (Gustafsson, 1960, Nilan and Konzak, 1961). The efficiency of the mutagen used depends on the radiosensitivity of the treated plant material which in turn is dependant on a large number of physiological and biological factors (Nilan, 1956, Nilan *et al.* 1965). Great strides have been made in the use of induced mutations for crop plant improvement. Although trees play an important role in our lives, the efforts put in on their genetic improvement have not kept pace with their importance. In view of the success within a short period of time of mutation breeding programme with agricultural crops (Ehrenberg, 1971, Micke, 1975), it would seem feasible to experiment with these techniques for inducing genetic diversity for the further improvement of forest trees. Thought in the past such investigations have been conducted on forest tree species (Gustafsson, 1960) but they have not been utilized for

tree improvement on a commercial scale. It is therefore, important to investigate the effectiveness of the mutagen used, and also the radiosensitivity of forest tree species. Radio Isotope Laboratory has been in such activities in the recent past to develop useful and efficient techniques to improve tree species. Results of such an endeavour on seeds of commercially important hybrid *Eucalyptus camaldulensis* x *E. tereticornis* (FRI - 5) treated with the super chemical mutagen, Ethylmethane sulphonate (Konzak *et al.* 1965), and the mutagenic effects assessed on different parameters are reported here.

### Materials and Methods

The effect of Ethylmethane sulphonate (EMS) on FRI - 5 was assessed on the basis of its effect on different parameters viz: Germination, Germination value (GV), Germination energy index (GEI), Hypocotyl and Radicle length, Cotyledon width and length, green and dry weight of the seedlings, and chromosomal abnormalities observed at anaphase. Seeds of FRI - 5 were treated with EMS solution prepared in buffer (pH-7) under different concentrations

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- 0.25, 0.50, 0.75 and 1.00%. Seeds were treated under two moisture levels. In the first case air dried seeds (Moisture content - 20.15%) were treated for 18 hours with the different concentration of EMS. The second lot of seeds was first soaked in distilled water for 8 hours (Moisture content-27.74%) and then treated with their respective concentrations of EMS solution for the required period of time. After treatment with EMS, the seeds were washed thoroughly under running water and kept for germination in the seed germinator at a temperature of  $32 \pm 2^\circ\text{C}$  and  $85 \pm 5\%$  humidity, one lot of seeds was soaked only in buffer solution (pH - 7) to serve as control. Seeds were sown in five replications per dose. One additional replication was sown for cytological study in each case. The root tips of these seeds were fixed in 1:3 Acetic Alcohol. Root tip squashes were prepared in aceto-carmin and anaphase bridge percentage scored.

Daily germination count was taken in all the treatments. After two weeks of germination, observations were recorded on seedling length, cotyledon width and length. Green weight of seedlings was recorded and subsequently the dry weight was also recorded. GV was calculated according to Czabator (1962). GEI was also calculated according to the revised formula of Groese and Zimmer (1958). Reduction percentage for the different parameters was calculated taking control as 100 for calculating LD - 50. Data

were subjected to the analysis of variance (ANOVA) for testing the significance of treatments.

### Results and Discussion

**Air dried seeds:** Sensitivity of air dried seeds of FRI-5 to the different EMS concentrations was very high. Doses beyond 0.25% had a lethal effect on seed germination. 29.41% reduction in germination was observed at 0.25% EMS and 100% reduction at 0.50% EMS. GV gave a very high reduction percentage value of 70.08% at 0.25% EMS, similarly GEI values showed that the germination index was also severely affected but to a lesser extent than GV (Table 1), highlighting the fact that not only completeness of germination, but also the speed of germination was affected. Delayed germination may be due to the inhibitory effect of the mutagen on the activity of the embryonic cells (Raghuvanshi and Singh, 1981). Decreased germination may also be attributed to the disturbances caused at physiological level of the cell (Sinha and Godward, 1972). According to Selim *et al.* (1974) reduction in germination percentage might be due to an increase in the production of active radicals in the treated cell which are responsible for total lethality. Reduction percentage observed in seedling height was 20.20% and 34.69% in hypocotyl and radicle length respectively (Table 1), clearly indicating that radicle is more sensitive to the different EMS concentrations in comparison to hypocotyl. Sensiti-

vity of radicle length is of great significance because due to high sensitivity root development is poor which finally leads to delayed death of the seedling. Sensitivity of cotyledons to the EMS concentrations was less in comparison to other parameters, and only 9.67% and 10.52% reduction was observed in cotyledon width and length respectively (Table 1). Sensitivity of FRI - 5 seeds to EMS concentrations was found to be very high in comparison to other forest tree species, specially in the case of air dried seed treatment. When air dried seeds of *Pinus* and *Eucalyptus* species were treated with similar concentrations of EMS (Kapoor, 1981) no lethality was observed even upto 1.00% concentration in contrast to the total lethality of air dried seeds of FRI - 5 observed at 0.50% EMS. On assessing the biomass of the seedlings, slight stimulation of +7.69% was observed in dry weight at 0.25% EMS (Table 3), whereas a reduction of 9.57% was observed in green weight at the same concentration. No chromosomal observations were observed.

High sensitivity of the air dried seeds in comparison to pre-soaked seeds may be attributed to the presence of certain free, active radicals in the seeds, which in combination with EMS might be producing some lethal molecules, thereby causing total lethality of seed. On pre-soaked the seeds leaching of these radicals may be occurring so due to their non-availability lethal molecules are not formed, and thus resistance is imparted to the pre-soaked

seeds in comparison to the air dried seeds.

### **Pre-soaked seed**

Seeds of FRI-5 pre-soaked in buffer solution (pH -7) for 8 hours prior to EMS treatment were found to be more resistant to the different EMS concentrations in comparison to air dried seed treatment. Pre-soaked seeds showed 34.00% germination at the maximum concentration of 1.00% EMS (Table 2) and consequently the maximum reduction percentage over control was 48.48% at 1.00% EMS. Germination percentage showed a stimulation of +6.06% at 0.50% EMS. Accelerated germination has been reported by other workers also. Sharma and Gupta (1981) working on *Triticale* similarly reported an increase in germination on treatment with lower concentrations of EMS. They suggested that the mutagen at low concentrations inactivates those enzymes which inhibit germination. Highest reduction percentage in all parameters was observed at 1.00% EMS. GV showed a maximum reduction of 88.77% at 1.00% EMS, and GEI reported 70.20% reduction at the same EMS concentration, GV was thus the most sensitive of the germination parameters. In seedling, height maximum reduction percentage of 50.50% and 73.99% was observed in hypocotyl and radicle length respectively at 1.00% EMS, radicle thus behaving to be more sensitive than hypocotyl. Stimulation in hypocotyl and radicle length was observed at lower EMS concentration (Table 2). Stimulation in seedling height might have resulted due to the

Table 1

Mean values of different parameters after treating Dry seeds  
of FRI - 5 with Ethylmethane sulphonate (EMS)

Parameter	Actual Mean $\pm$ SE					F value after ANOVA	LD - 50 in % EMS
	Control	0.25	0.50	0.75	1.00		
G	68.00 $\pm 8.60$ 0.00	48.00 $\pm 5.83$ 29.41	0.00 (100)	0.00 (100)	0.00 (100)	49.9623***	0.325
R							
GEI	55.43 $\pm 6.23$ 0.00	31.43 $\pm 4.49$ 43.29	0.00 (100)	0.00 (100)	0.00 (100)	57.5607***	0.275
GV	96.71 $\pm 22.31$ 0.00	28.93 $\pm 6.31$ 70.08	0.00 (100)	0.00 (100)	0.00 (100)	16.8176***	0.175
HL	1.78 $\pm 0.04$ 0.00	1.42 $\pm 0.07$ 20.20	0.00 (100)	0.00 (100)	0.00 (100)	65.3661***	0.35
R							
RL	0.49 $\pm 0.08$ 0.00	0.32 $\pm 0.22$ 34.69	0.00 (100)	0.00 (100)	0.00 (100)	40.5077***	0.31
CW	0.31 $\pm 0.006$ 0.00	0.28 $\pm 0.02$ 9.67	0.00 (100)	0.00 (100)	0.00 (100)	56.5217***	0.375
R							
CL	0.19 $\pm 0.004$ 0.00	0.17 $\pm 0.009$ 10.52	0.00 (100)	0.00 (100)	0.00 (100)	479.00**	0.36
R							

G - Germination %, GEI - Germination Energy Index,  
GV - Germination value, HL - Hypocotyl length,  
RL - Radicle length, CW - Cotyledon width, CL - Cotyledon length,  
R - Reduction %

\*\* - Significant at 0.1% level.

Table 2

Mean values of different parameters after treating pre-soaked seeds of FRI - 5 with Ethylmethane sulphonate (EMS)

Parameter	Actual Mean $\pm$ S.E.					F value after ANOVA	LD+50/ 20 in % EMS
	Control	0.25	0.50	0.75	1.00		
G%	66.00	60.00	70.00	46.00	34.00	8.8571	0.69
R	$\pm 7.48$ 0.00	$\pm 5.47$ 9.09	$\pm 3.16$ +6.06	$\pm 5.09$ 30.30	$\pm 5.09$ 48.48	***	*
GEI	56.57	45.43	51.71	29.72	16.86	22.6553	0.76
R	$\pm 8.12$ 0.00	$\pm 4.95$ 19.69	$\pm 3.14$ 8.59	$\pm 4.38$ 47.46	$\pm 3.04$ 70.20	***	
GV	102.09	62.36	50.06	30.30	11.46	6.7224	0.49
R	$\pm 25.45$ 0.00	$\pm 10.35$ 38.91	$\pm 10.46$ 50.96	$\pm 8.77$ 70.32	$\pm 3.46$ 88.77	***	
HL	1.80	1.75	1.85	1.57	0.89	20.0180	1.00
R	$\pm 0.06$ 0.00	$\pm 0.07$ 2.78	$\pm 0.11$ +2.78	$\pm 0.13$ 12.78	$\pm 0.04$ 50.50	***	
RL	0.46	0.47	0.41	0.28	0.12	12.3708	0.84
R	$\pm 0.03$ 0.00	$\pm 0.06$ +2.17	$\pm 0.02$ 10.87	$\pm 0.03$ 39.13	$\pm 0.009$ 73.91	***	
CW	0.32	0.28	0.27	0.26	0.22	14.8571	0.775
R	$\pm 0.01$ 0.00	$\pm 0.008$ 12.5	$\pm 0.01$ 15.63	$\pm 0.01$ 18.75	$\pm 0.01$ 31.25	***	*
CL	0.17	0.18	0.14	0.14	0.11	10.7500	0.79
R	$\pm 0.008$ 0.00	$\pm 0.007$ 5.88	$\pm 0.008$ 17.65	$\pm 0.01$ 17.65	$\pm 0.006$ 35.29	***	*

G% - Germination %, GEI - Germination Energy Index  
 GV - Germination value.  
 HV - Hypocotyl length, RL - Radicle length.  
 CW - Cotyledon width, CL - Cotyledon length.  
 R - Reduction %, + - Stimulation  
 \* - LD - 20 \*\* - Significant at 1% level.  
 \*\*\* - Significant at 0.1% level.

increased synthesis of auxin, while inhibition of the latter could cause stunted growth (Dube and Dnyansagar 1981). Following treatment with EMS, hydrolysis of the mutagen, increases the pH of the solution making it toxic which might lead to seedling injury (Froese-Gertzen *et al.* 1963). Stimulation at lower concentrations was also observed in cotyledon length and width. Such observations have been reported for other forest tree species also (Kapoor, 1981). Sensitivity of cotyledon size to the different

EMS concentrations was very low, maximum reduction percentage at 1.00% EMS being only 35.29% and 31.25% in cotyledon length and width respectively (Table-2). Stimulation was also observed in the biomass of the seedlings. Green weight showed a stimulation of +8.98 and +3.99 at 0.25% EMS respectively were as in the dry weight a reduction of 3.70% was observed at 0.25% EMS with a stimulation of +7.40% and +22.22% at 0.50% and 0.75% EMS respectively (Table 3). Root tip squashes at higher

Table 3

Mean values (in mgs) of green and dry weight of seedlings after treating seeds of FRI - 5 with Ethylmethane sulphonate (EMS)

Parameter	Condition of seed	EMS %				
		Control	0.25	0.50	0.75	1.00
GW	DS	4.49	4.06	0.00	0.00	0.00
	R	(0.00)	(9.57)	(100)	(100)	(100)
	PS	4.26	4.63	4.43	3.96	2.71
	R	(0.00)	(+8.68)	(+3.99)	(7.04)	(36.38)
DW	DS	0.39	0.42	0.00	0.00	0.00
	R	(0.00)	(+7.69)	(100)	(100)	(100)
	PS	0.27	0.26	0.29	0.33	0.26
	R	(0.00)	(3.70)	(+7.40)	(+22.22)	(3.70)

DS  
GW  
R

-  
-  
-

Dry seeds  
Green weight  
Reduction %

PS  
DW  
+

-  
-  
-

Pre-soaked seeds  
Dry weight  
Stimulation

Table 4

Bridges observed at anaphase in cells of root tips squashed after treating pre-soaked seeds of FRI - 5 with Ethylmethane sulphonate (EMS)

EMS	Total dividing cells scored	Total Bridges scored	Bridges %
Control	95	0	0.00
0.25	95	0	0.00
0.50	90	1	1.11
0.75	90	2	2.22
1.00	85	3	3.55

EMS concentrations showed anaphase bridges, percentage of which increased linearly along with increasing EMS concentration, from 1.11% at 0.50% EMS through to 3.55% at 1.00% EMS (Table 4). Such abnormalities have been reported earlier in other forest tree species also (Haque *et al.* 1982). Linear increase in abnormal cell percentage with increase in EMS concentration might be one of the factors affecting seedling growth and biomass of seedlings, since it has been stated by many workers (Sinha and Godward, 1972) that chromosomal aberrations and gross cytological changes in call caused by mutagenic effect may result in the poor survival and reduced seedling growth.

Similar investigations have been carried out on FRI - 4 (*E. tereticornis* x *E. camaldulensis*) using EMS (Renu and Kapoor, 1984). On comparing the results it was observed that the relative sensitivity of the two reciprocal hybrids differed slightly, although the trend followed was similar.

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#### Summary

Seeds of *Eucalyptus* hybrid FRI - 5 (*E. camaldulensis* x *E. tereticornis*) were treated with

Ethylmethane sulphonate (EMS) (0.25, 0.50, 0.75, 1.00%) at two moisture levels. Effect of EMS on the hybrid was assessed by measuring different parameters viz. Germination percentage, Germination value, Germination Energy Index, Radicle and hypocotyl length, cotyledon width and length; Green and Dry weight, Chromosomal aberrations at anaphase. Air dried seeds were observed to be highly sensitive in comparison to the pre-soaked seeds. In general reduction percentage increased with the increasing doses. Stimulation was observed in a few parameters at lower concentrations. Germination value and Radicle length were the most sensitive parameters. This study has revealed the possibility of advancing new techniques and methods for inducing/manipulating genetic changes in tree species.

#### युकेलिप्टस कैमेलड्युलेसिस X यु. टेरेंटिकानिस

पर इथाइलमिथेन सल्फोनेट का उत्प्रेरक प्रभाव

रेणुस्वरूप ब एम. एल. कपूर

वाराणसी

संकर युकेलिप्टस एफ आर आई — 5 [यु. कैमेलड्युलेसिस X यु. टेरेंटिकानिस] के बीजों को दो नमी स्तरों पर इथाइलमिथेन सल्फोनेट [0.25, 0.50, 0.75, 1.00%] से उपचारित किया गया। विभिन्न परिमाणों अर्थात् अंकुरण प्रतिशत, अंकुरण ऊर्जा, अंकुरण ऊर्जा निर्देशांक, भ्रूणमूल और अधोबीजपत्र लम्बाई, बीजपत्र की चौड़ाई और लम्बाई, हरा और शुष्क भार मापनोत्तर पित्रयसूत्र स्थान को नापकर संकर पर इथाइलमिथेन सल्फोनेट का प्रभाव मापन किया गया। पानी में डुबाए बीजों की तुलना में बाष्प शुष्क बीज अत्यधिक प्रभविष्णु पाए गए। कम संकेन्द्रण रखने पर कुछ परिमाणों पर प्रेरण होता देखा गया। अंकुरण जहाँ और भ्रूणमूल

की लम्बाई सबसे अधिक प्रभविष्णु परिमाण पाए गए। इस अध्ययन से कुछ जातियों में जननिक परिवर्तन प्रेरित करने/लाने की नई प्रविधियाँ और तरीके निकालने की संभावनाएं दिखाई हैं।

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