SUMMARY

The serious work of many researchers in the field of mutation research have proved that the artificial induction of mutations is a time-honored method to bring about the genetic variability in the living system. As the consequence, we are now better in position to understand the applicability of induced mutagenesis to achieve the desirable alterations at genetic level. This aspect, thus generate the curiosity to know the effect and reactivity of mutagenic agents in the biological system. The findings in the present work have proved the wider scope to understand the diversity of dES and EMS induced effect on several growth governing parameters, their mutagenic sensitivity, effectiveness and efficiency.

The forage legume D. tortuosum is good plant for grazing and is known for its palatability. The plant is cultivated as a forage plant in Florida. The tender stem, leaves, pods and seeds of the plant are relished by domestic and wild animals. D. tortuosum is native of West Indies but widely distributed in Central and South America, Tropical Africa and South East Asia.

The effect of dES and EMS in D. tortuosum was assessed by studying three generations. The method of seed treatment have been employed in present investigation are according to the standard procedures described in Manual on Mutation Breeding (1977).

The experiment was designed in four parts 1) Investigation of

mutagenic sensitivity with respect to biological damage in M_1 generation. 2) Detection of mutational events and estimation of mutagenic effectiveness, efficiency and mutation rate in M_2 and M_3 generations 3) Studies on Polygenic inheritance for quantitative traits in M_2 and M_3 and 4) Protein study including protein estimation and protein banding pattern.

The mutagenic sensitivity in M_1 generation has been assessed by studying the parameters such as seed germination, seedling height, plant height at maturity, number of flowers per inflorescence, survivility at maturity, cytological studies, pollen sterility and seeds per pod.

The seed germination observed to be reduced compared to control in dES and EMS treatment. Much drastic effect was evident at higher concentration and highest treatment duration of both the mutagens. Seedling height was inversely proportional to the increasing concentrations and treatment durations. The dES treatment exhibited maximum germination injury compared to EMS. Similar type of results have been obtained on the parameters such as flowers per inflorescence and survivility at maturity. However, the gradual decline with respect to increasing concentrations and treatment durations in plant height was evident but dES shows slightly lesser magnitude of reduction compared to EMS.

The cytological observations have been made by studying mitosis and meiosis. Both the mutagens observed to be competent to

certain level to produce meiotic and meiotic chromosomal aberrations. The root tip mitotic analysis of treated material with studied concentrations exhibited varying spectra of chromosomal aberrations such as stickiness precocious movement, fragements in metaphase and laggards,bridges,fragments in anaphase. The observations revealed that the percent occurrence of individual aberrations type increases randomly but the frequency of total chromosomal aberrations increases regularly with increasing concentrations and treatment durations. The relative frequency of stickiness was high for dES compared to EMS. Where-as, EMS exhibited laggards and bridges relatively more compared to dES. The dES exhibited relatively high degree of mitotic chromosomal aberrations compared to EMS.

The meiotic analysis of dES and EMS treated population revealed the existance of stickiness, multivalents in metaphase-I; fragments, laggard, bridges in anaphase/telophase I and II and micronuclei in telophase II. The percent frequency of individual meiotic aberrations revealed irregular increase but the total meiotic chromosomal aberrations increases with increasing concentrations and treatment durations. The relative frequency of stickiness and multivalents observed to be high in dES where-as, EMS treatment showed laggards, bridges and fragments relatively more compared to dES. The dES treatment in meiosis too revealed high degree of chromosomal aberrations compared to EMS.

Both the mutagen exhibited high potentiality for sterility

induction. The EMS treatment revealed high degree of pollen sterility and reduction in the seeds per pod inspite of lower range of meiotic aberrations compared to dES.

In M_1 generation, the chlorophyll chimeral types virescens, chlorina, lutescens, and albescens have been noticed for dES and EMS. The albescens type observed to be with higher frequency and chlorina with least for both the mutagen. The dES treatment revealed high frequency of chlorophyll variations compared to EMS in M_1 generation.

The morphological variations such as leaf let margin, change in the leaf size and increase in the leaf number have been recorded. Alterations in the leaf flecking character also has been recorded in dES treatment. The frequency of morphological variants observed to be higher for dES compared to EMS.

The mutational events have been investigated in M_2 generation onward. Total of five type of chlorophyll mutations i.e, albina, virescens, chlorina, lutescens and albescens have been noticed in M_2 and M_3 generations. Among these, the albina was noticed only for EMS and lutescens for dES in M_2 generation. This trend was also observed in M_3 with only difference that lutescens again appeared in EMS.

On the basis of percent occurrence, the sequence of chlorophyll mutations for dES treatment was virescens > albescens > lutescens >

chlorina. However, for EMS treatment, the chlorophyll mutations occurred in different order as virescens = albescens > chlorina > albina. The frequency of total chlorophyll mutations was more in dES compared to EMS treatment and in M_3 compared to M_2 generation.

The morphological mutations isolated were Dwarf fertile mutants (dES/EMS), Dwarf sterile mutants (dES/EMS), Dwarf lethal mutants (dES/EMS), Late flowering mutants (dES/EMS), Early flowering mutants (dES/EMS), Large leaf mutant (dES), Seed coat colour mutant (dES), Bushy mutant (dES), Semidwarf fertile mutant (dES), Bushy sterile mutants (EMS), Basal branching mutant (EMS), Bifurcated stem mutant (EMS), Trichotomous branching mutant (EMS), Cytomictic sterile mutant (EMS), Mutant with ringed branching pattern (EMS), Small poded mutant (EMS) and Leafly dwarf mutant (EMS). It was observed that the total frequency of morphological mutations did not differ considerably for both the mutagen but was slightly more in EMS compared to dES. However, it could be noted that the frequency of total mutations (chlorophyll and morphological) was high in dES treatment compared to EMS. The 16 hrs treatment duration of dES and EMS have shown maximum mutation rate compared to 8 and 24 hrs of treatment duration.

The mutagenic effectiveness is the measure of mutations induced by unit concentration of mutagen. The lower concentration of dES and EMS exhibited maximum mutagenic effectiveness where-as it was reduced at higher concentrations there by indicating inversely proportional relationship with increasing concentrations. The dES treatment in D. tortuosum exhibited higher effectiveness compared to EMS.

Mutagenic efficiency which is termed as the measure of the production of desirable changes free from undesirable one, have been estimated in relation to seedling injury, lethality, percent reduction in seeds per pod, pollen sterility and mitotic and meiotic chromosomal aberrations. In relation to seedling injury, lethality, mitotic and meiotic aberrations the EMS appeared to be more efficient where-as dES possess higher efficiency in relation to seeds per pod and pollen sterility.

Mutation rate, which gives an idea of average mutations induced by particular mutagen have been estimated. The mutagen, revealing higher efficiency also shows higher mutation rate. Thus for seedling injury, lethality, mitotic and meiotic aberrations the mutagens have been rated as EMS>dES. However for seeds per pod and pollen sterility the sequence was dES > EMS.

The effect of dES and EMS on polygenic inheritance in M_2 and M_3 was studied with respect to the quantitative characters such as plant height, number of branches per plant, number of leaves per plant, leaf size, number of flowers per inflorescence, number of pods per inflorescence and number of seeds per pod. The findings have been evaluated with the help of statistical analysis.

Positive as well as negative shift in the mean with increasing variability induced by dES and EMS have been noticed in both the mutagenic treatment for the parameters such as plant height, number of branches per plant, number of the leaves per plant, leaf size and

number of flowers per inflorescence. Where as, only negative shift in the mean with increasing variance was evident for the parameters such as number of pods per inflorescence and number of seeds per pod. The effect of both the mutagens was critical for maximum number of the treatments.

The ANOVA studies revealed that the concentrations and treatment durations of dES have expressed the significant effect on number of branches per plant, number of flowers per inflorescence, number of pods per inflorescence and number of seeds per pod. Where as for leaf size, only treatment durations of dES revealed significant effect. In case of EMS, the significant effect of concentrations and treatment durations was evident on plant height, leaf size, flowers per inflorescence and seeds per pod. Where-as only treatment durations have revealed significant effect for pods per inflorescence.

The 't' test analysis for dES and EMS unearthed the significant difference between both the mutagens for the parameters such as plant height, number of leaves per plant and number of seeds per pod.

The protein study was confined to the crude protein estimation and protein banding pattern.

The level of crude protein content observed to be enhanced as well as declined in the mutant plants in comparison to control. The control exhibited 28.50% of leaf crude proteins where-as, for mutants, the protein content was varying in the range of 27.22 to 33.79%. The

maximum crude protein content (33.79%) has been observed for Large leaf mutant and minimum (27.22%) was encountered for Bifurcated stem mutant.

The seed protein banding pattern was studied by SDS-polyacrylamide gel electrophoresis. Studies on protein banding pattern exhibited induced genetic variability at protein level which appeared on electrophoretic gel by addition or delation along with the changes in the position of protein bands.

The total number of the protein bands was 18 for control where as the mutant plants show the variation in the number of protein bands in the range of 17 to 22. Of interest to note is the occurrence of one or more mutant specific bands. These specific protein bands have been encountered for Seed coat colour mutant, Bushy mutant, Semidwarf mutant and Early flowering mutant.