INTRODUCTION

Mutation is one of the major force of evolution. Over centuries plants have moulded themselves to the needs of nature. Man is another force mending the trends of evolution in economically important plants. Right from prehistoric days, man is trying to grow plants according to his needs. During the initial stage of human civilization his needs for crop improvement were full filled by natural variability. As time passed man's desire for better crop was increasing and so also demand for more food was escalating. This has led to the concept of hybridization as a force to generate more variability through recombination. Of course, this technique had tremendous impact on plant improvement programme. However, man's pursuit for better genotype did not stop due to rapid genetic errosion of existing genetic resources because of environmental degradation. It has become the matter of urgent concern for all human beings. Then he came out with an idea of artificially increasing variability and this has led to the concept of induced mutation.

The majority of the world population in underdeveloped and developing countries are undernourished mainly because of the inadequate and nutritionally poor supply of food. This is one of the major strategy that needs special attention so that, the quantity and quality of dietary sources can be improved by genetic alterations. Mutation induction which is the proven way to create variations, stands as a one of the tool to alter and enhance the genic status of usefull plants at will by breaking the limitations of presently known variability. By practicing the chemical and physical mutagenesis, it is possible to

sculpture the genotype and to produce the exciting, desirable and genetically stable phenotypes. Although, variations occur spontaneously too, but its rate is excessively low and also it can not be claimed that, all possible usefull variabilities will exist in the nature. Thus, the optimum performance of quantitative and qualitative food productivity can be achieved rapidly with such techniques. According to the recent report of Mutant Variety Data Bank (FAO/IAEA 1991), total of 1019 mutants of different crops have been officially released in the world including 116 in India.

The induction of the mutations in several cereal and legume crops have been resulted in the agroeconomical progress at remarkable level. The mutants characterised by disease resistance, high yielding and increased protein content are under cultivation in variety of the crops as reported by Swaminathan et al. (1969), Dhaliwal (1977) and Parodi and Nebreda (1979) in bread wheat; Hanis (1974), Doll et al. (1974) and Reinhold et al. (1980) in barley; Singh and Rao (1971) and Tanaka and Hiraiwa (1978) in rice. Mutation programme in legume has been started in early seventies. Later, it has been studied extensively by several researchers such as Sidorova et al. (1969), Gottschalk and Klein (1976), Gottschalk and Muller (1979), Blixt (1979) and Gottschalk and Wolff (1983) in Pisum sativum; Hussein and Abdalla (1979) in Vicia faba; Prasad (1976) in Vigna radiata; Nerkar (1976) in Lathyrus sativus and Gridley and Evans (1979), Tulmann et al. (1980) and Sena and Barbosa (1992) in Phaseolus vulgaris. However, there is scanty work on mutational attempt on forage plants of legumenous origin.

Although the idea of mutation was perceived by DeVaries as early as 1902, but, the work on chemical mutagens started in 1914 by T.H. Morgan who experimented with alcohol in Drossophilla (Auerbach, 1973). Since, then the lot of attempts have been made on mutational studies with the help of physical and chemical agents. Although the physical mutagenes are effective, the chemical mutagens are often known to be the most efficient point mutagenic agents (Broertjes and Vanharten, 1978; Konzak,1984).

In the present investigation the effect of two chemical mutagens, diethyl sulphate (dES) and ethylmethane sulphonate (EMS) have been studied in legume plant of forage importance Desmodium tortuosum (SW). DC. The chemical mutagens belong to alkylating group. The choice for alkylating group has been made since these mutagenic agents are most important and effective in mutation induction (Heslot, 1977).

The general causes of mutation by chemical mutagens are either the alteration or removal of DNA bases or break of DNA backbone (Freese, 1971). In particular for alkylating agents, the alkylation of DNA bases is suggested to be the principle cause of mutation. The alkylation of N-7 position of guinine figures prominantly in the mutagenic reaction (Drake, 1970; Brookes, 1990). The alkylation of guinin produces pairing error and as a result, guinine pairs with thymine instead of cytosine and transition type of mutations takes place (Kamr and Brunner, 1977).

Diethyl sulphate is although appears to be bifunctional but act as a monofunctional in the reaction (Heslot, 1977). It has structural

formula SO_2 $(OC_2H_5)_2$ with a functional group SO_2OH, molecular weight 154, melting point 24.5°C and density 1.177. It is soluble in water.

Ethylmethane sulphonate is a monofunctional alkyl mutagen with structural formula CH_3 SO_2O C_2H_5 with a functional group, SO_2OH and molecular weight 124. It is colourless liquid with boiling point 85-86°C and density 1.203. EMS is soluble in water.

The use of alkylating agents in induction of mutation was initiated in early 1940 (Aureback, 1973) but mutagenic potentialities of some alkylating agents such as dES and EMS have been highlighted during late 1960's. Now, these mutagens are recognised as a highly potent and effective. Russian genetist Rapaport (Aureback 1973) studied for the first time the mutagenic ability of dES and later it has been introduced as a successful mutagen by many workers (Heiner et al., 1960; Gustafsson, 1969; Yagamuchi et al., 1974; Malik and Mary, 1971; Ashri,1972). The EMS is recognised as a highly potent and effective mutagenic agent in higher plants too (Froese-Gertzen et al., 1963; Nauman et al., 1976). The mutagenic efficiency of EMS was further highlighted by Siddiq and Swaminathan (1968), Santos (1969) Ismail et al. (1976), Fillippetti and Marzano (1984) and Singh and Raghuvanshi (1988; 1991).

The legumes, which are known as the important complements to carbohydrate staples are low in oil but very much important on account of their high protein content and good protein quality. Out of 70%

of plant proteins (consumed by man), 15% of proteins are derived from legume only (Gottschalk and Wolff, 1983). And thus leguminous plants play vital role in improvement of human health. In addition, legumes are crucial to balance the nature as most of them, if not all, are able to 'fix' the atmospheric nitrogen and thereby maintaining soil productivity even for long period. A leguminous crop can add upto 500 Kg of nitrogen to the soil per hectare per year (NAS, 1979)

Even though, thousands of legumes of known and unknown potentialities are documented, only few of them such as cow pea, pigeon pea. Phaseolus beans and mung beans as a pulses and vegetable legumes; Soyabean and peanuts as a industrial oil seed legume; Desmodium, Centrosema, Stylosanthus and Pueraria as a forage legume and Leucaena as a forage-fuel silviculture species are the predominantly cultivated in the tropics. Besides, there are large number of legume species which have received scanty attentions so far, possesses a good potentialities for contributing the food, fuel and forage needs.

The advantage of induced variability is not only confined to the agriculture crops but also to those plants which play vital role directly or indirectly in quality leading life. It is important to note that the life-standard leading food products (vegetarian and non-vegetarian) in developed countries are the result of qualitatively improved crops varieties and improved husabandary.

Perhaps, the most important task of mankind today is to solve the problem of world-hunger and malnutrition. To achieve this goal, the qualitative as well as quantitative improvement of human dietary system needs to be undertaken by all possible means. The conversion of forage potentialities in to animal products, like milk and meat thus, can be a interesting aspect since these are the major dietary sources in the world. The domestic animals must be suplimented with protein rich feeding material. In this context, legumes attract the attention of scientists towards the exploitation of its strong proteinacious forage potentialities. Leguminous forage plants can full fill the gap of protein rich feeding source since, the crude protein content level of forage legume is much higher than grasses. The digestable crude proteins (DCP) per 100 unit of feed has been reported as 12.0 g in legume against only 3.7 g in grasses (Minson, 1977). Milk yielding animals such as cows, buffalos, goats and sheeps require high standard of nutritious food at all the time and thus the quality of feed offered to these animals is more important and needs special attention.

There is a need to develop the promising cultivers and to explore the genic status of existing forage legumes so that more economically and commercially important varieties with nutritions quality will be available for domestic animals. Such programme will partially, if not completely full fill our efforts to improve the quality and quantity of milk and body weight of the animals, the life leading nutritious dietary sources.

The use of leguminous plants for grazing the domestic

animals is of more recent practice. It was generally belived that, legumes would not survive in tropical environment due to excessive rain fall and high temperature. Since 1966, the enthusiastic efforts of John Griffiths Davies at CSIRO laboratories, Australia have shown that the forage legumes can be successfully introduced and maintained in tropical area also.

The high proportion of forage legume currently used in the tropical countries are grown in northern Australia. Besides this, South and Central America have been particularly important sources of pasture legume following the several countries of African continent. Forage legume also have been introduced in South Asia, South-east Asia and Philippines (Skerman, 1977).

The better available land needs to be used more and more for food and cash crop. But it is also essential to improve the productivity of land which is not suitable for the cultivation so as to develop complimentary land use system. This can be achieved by converting non-utilized or wasteland as a grazzing land by planting forage legumes. This will not only give good pasture for animals but also improve soil texture and fertility. If this is practiced, then the high protein containing and soil enriching forage legumes will deserve the special place in feature agricultural system.

Due to the significance of pasture legume utilization, the number of research institutes like Commonwealth Scientific and Industrial Research Organization (CISRO), Australia, IRI Research

institute in Matao, Institute de Zootecnica in Nova, Odessa, Brazil and at Kitale in Kenıya are leading the extensive research programme for commercial exploitation, evaluation and classification of a pasture legume.

As a matter of fact, the agricultural cıvilization is depend upon its ability to retain the soil fertility. In this respect, the pasture legume based land-use system appears to be better economical approach and deserve greater consideration. There is urgency to realise the importance of nutrıtional and agro-economical sıgnificances of legume based forage plants. These plants need to be studied much extensively in a attempt to improve nutritional standard of our dietary constituents. With this view, the forage system was selected as a experimental material in the present investigation.

Desmodium species are cultivated as a forage in tropical countries for their fodder excellancy. Several species of Desmodium such as D. sandwicense, D. canum, D. barticulatum, D. uncinatum and D.intortum are cultivated in Australia for forage (Skerman, 1977). Shah and Singh (1986) reported increase herbage production by introducing D.uncinatum with Setaria sphacelata in north-western Himalayan part of India. Studies on the flowering, pollination and pollen tube formation have been reported in D.uncinatum, D. sandwiscense and D. intortum by Hutton (1960). The hybridization aspect has been studied by McWhirter (1966) and Hutton and Gray (1967) in D. sandwicense, D. intortum and D. uncinatum. Surange and Deokule (1987) reported pharmacognostic studies in D. latifolium and D. velutium.

Yong et al. (1989) studied the chemical constituents in D. triquestrum and isolated three crystalline compounds namely friedenin I, epifridelin II and Stigmasterol. Bir (1990) reported diploid chromosome number 2n = 22 in D. tortuosum. In the present experiment, the attempt has been made to investigate the effect of two chemical mutagens, dES and EMS in D. tortuosum.

D. tortuosum belongs to family papillionaceae and has been described by different name in different flora as D. spirale (Oliver, 1871) D. perpureum (Fawcett and Rendle, 1920), Meibomia tortuosa (Britton and Millspaugh, 1920) and D. tortuosum (Skerman, 1977).

D. tortuosum is a native of West Indies and widely spread in Central and South America, Tropical Africa and South-east Asia, The plant is also distributed in Cuba, Canada, Jamaica, and Greneda (Britton and Millspaugh, 1920). It is also found in Hawaii at 900 m elevation (Oliver, 1871).

Desmodium tortuosum (SW.) DC is cultivated as a forage plant in Florida (Skerman et al., 1988). The plant is highly palatable and good for grazzing. The tender twigs, stem, leaves and tender pods and seeds are eaten by domestic as well as wild animals.

Desmodium tortuosum is errect (with average height of 64.8 to 66.8 cm), short lived annual herb. It is woody at the base. Cylindrical stem posseses alternately arranged branches (average number 9.1 to 9.8 per plant). The plant bears unifoliate leaves at early seedling stage but at maturity leaves are trifoliate

with lanceolate or oval leaflet. The leaflets are characteristically possess maroon coloured flecking. Central leaflet is larger than two laterals. Stipules, linear deciduous; bracts, minute and inflorescence are of recemose type. The main inflorescence shows total of 40 to 44 number of flowers, where as inflorescences on lateral branches show 16 to 18 number of flowers. The calyx of the flower is pale azure or purple coloured with deep cleft. Carolla slightly exceeding the calyx. The pods are 5 to 6 seeded. The kidney shaped seeds are twistedly arranged and separated by narrow space. One to two seeds per pod are bigger in size and show good germination compared to remaining seeds which are underdeveloped and small in size.

The present investigation in D. tortuosum has been under taken with following objectives.

- To assess the mutagenic sensitivity of dES and EMS with respect to the biological damage in M_1 generation.
- To evaluate the effect of dES and EMS on fertility.
- To determine the quantum of mitotic and meiotic chromosomal aberrations induced by dES and EMS.
- To identify and study the chlorophyll mutations in treated progeny.
- To detect and study the morphological alterations in treated progeny.
- To estimate the mutagenic effectiveness, efficiency and mutation rate of dES and EMS in D. tortuosum.

- To study the nature of induced polygenic inhertance with respect to some quantitative traits.

- To estimate the protein content in control and selected morpho-mutants.

- To study the electrophoretic characterization of protein bands in control and mutant plants.