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Ethyl Methane Sulphonate induced morphological variations in mulberry (*Morus*) variety M_5

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Abstract: Present investigation deal with the study of mutagenic effect of Ethyl Methane Sulphonate (EMS) on mulberry variety M_5. The results revealed that at 0.4% EMS treatment, height of the plant and leaf area were considerably increased. Stem dichotomy, fusion of leaves, increase in thickness, change in texture of leaves and occurrence of albino and xantha were common in 0.3% EMS treated plants in M_1 generation.

Keywords: M_5, EMS, Sprouting, Petiole, Internodal distance, Inflorescence

INTRODUCTION

Sericulture is a sustainable interactive chain of interdependent and interdisciplinary activities of high employment potential and rich dividend enterprise, ideally suited for unskilled labour abundant agrarian rural economy. There is an immense need for improvement of mulberry varieties in terms of nutritive value and quality to ensure profitable production of cocoon. Mulberry breeding is one of the important techniques available to breeders in their quest to develop new cultivars was worked out successfully in vegetatively propagated crops. Recent decade has witnessed intensive work on the induction of mutations by irradiation, chemicals and other mutagenic agents (Farrukh Aqil *et al.*, 2008; Sonu Goyal and Samiullah Khan, 2010). The frequency of induced mutations almost doubles those naturally occurring and they have been looked as a powerful tool for the development of new cultivars. Of all the mutagens available today, gamma rays and EMS have been found more potent for mulberry (Deshpande *et al.*, 2010; Rao *et al.*, 1984; Santhosh Lal and Pavithran, 1997).

MATERIALS AND METHODS

Indigenous mulberry variety M_5 was chosen for investigation. Stem cuttings were procured from healthy mulberry plantation aged about 8 to 10 years. The stem cuttings were planted in earthen pots filled with standard soil mixture (Red earth, sand and farm yard manure) in the proportion of 1:1:1. The mutagen effects of EMS were studied in detail in M_1 generation using five doses (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) of concentrations. A week after planting, the vegetative buds were treated with varying concentration of EMS (0.1% - 0.5%) from dawn to dusk (8am to 6pm) via cotton plugs placed on the buds (Broertjer and Van Harten, 1998) intermittently at every one hour. Ten replicas were maintained for each concentration and each replica consists of three stem cuttings. The earthen pots were placed under optimum sunlight and periodically watered. Observations were made regularly from the day of sprouting after treatment till the end of the eighth month to record the variations in the vegetative characteristics viz., sprouting, rooting behavior, branching pattern, plant height, petiole length, internodal distance, number of inflorescence, leaf shape and leaf area (Dandin and Jolly, 1986; Dandin and Kumar, 1989).

RESULTS AND DISCUSSION

In nature, many number of mulberry varieties are available and all varieties are not suitable for rearing silkworm due to lack of one or the other important agro-morphological and vegetative traits. Acclimatisation of particular variety to a particular region and season is also of paramount importance.

Effect of Ems on plant organs:

Sprouting percentage: Sprouting is an inherent capacity of a plant material to unfold the buds and produce new flush of shoots. Successful establishment of garden in vegetatively propagated plants mainly depends on sprouting ability. The EMS treated M_5 mulberry cuttings shows variations in response to different concentrations of the mutagen. Decrease in sprouting percentage was observed in plants treated with EMS compared to control. It is evident that at 0.4% concentration, higher sprouting

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Table 1. Growth and vegetative parameters of control and EMS treated M_5 mulberry variety.

Concentration	Rooting (%)	Sprouting (%)	Height of the Plant (inches)	Number of leaves	Number of inflorescence	Number of fruits	Petiole length (cm)	Internodal distance (cm)	leaf area (cm^2)
0.1	48.89	44.44	06.90	08.64	4.98	4.98	2.22	0.894	81.04
0.2	46.27	48.88	08.92	10.72	3.90	3.90	2.30	1.164	83.40
0.3	64.33	59.99	03.70	12.33	4.30	4.30	2.54	0.690	97.23
0.4	78.71	75.71	12.66	09.72	4.62	4.62	2.97	1.372	166.23
0.5	67.15	68.88	08.50	12.06	3.84	3.84	2.73	1.138	128.07
Control	91.04	89.66	04.48	08.73	3.70	3.70	2.08	1.120	69.25
SEM	--	--	--	0.58	0.92	0.45	0.04	0.07	1.24
CD at 5%	NS	NS	NS	1.26	1.08	1.10	1.58	1.06	2.36

of 75.71% was recorded and the least sprouting of 44.44% at 0.1% concentration was observed when compared to control (89.66%) (Table – 1). The present results are in confirmation with the earlier workers report that different mulberry genotypes show variation in sprouting ability due to mutagen treatment (Agastian *et al.*, 1995; Eswar Rao *et al.*, 2000; Hardansau *et al.*, 1995). Decrease in sprouting percentage has been attributed to destruction of auxin or due to the inhibition of auxin synthesis and also may be due to variation in temperature, water content and oxygen tension at the time of treatment (Gordon, 1957).

Rooting percentage: Rooting behavior of a variety is purely a genetic character (Hartman and Kester, 1976) and an important criterion to be possessed by an evolved variety. Highest rooting percentage (78.71%) noticed at 0.4% concentration and least rooting percentage (46.27%) at 0.2% when compared to control (91.04%) (Table – 1). Variations in rooting behaviour may be due to nature of soil, conductivity, pH, etc. With an increase in chemical concentration, there was an increase in the rate of mutation leading to variations in rooting (Nusrat Saba and Bushra Mirza, 2002).

Dwarfing: Dwarf plants were observed in M_1 generation in the cuttings treated with EMS (0.3%). Jain *et al.*, (1968) reported stunted growth as a result of hydroxyl amine and EMS treatment in Lycopersicon. Delay in mitosis coupled with other physiological factors might have resulted in dwarfing of some of the EMS treated plants (Nyla Jabeen and Bushra Mirza, 2004).

Stem dichotomy: Stem dichotomy was observed in plants treated with EMS treatments. Dichotomy or bifurcation of stem result from the death of apical cells in EMS treated material (0.3%) and regeneration of two apices. Chandramouli (1970) and Broertjes and Van Harten (1998) observed dichotomous branching among the progenies derived from chemical mutagen treatment in maize. In the present study, it seems possible that disturbances in auxin formation and destruction of terminal meristem followed by development of two apices might have resulted in dichotomy of stem.

Leaves: The size and symmetry of leaves in M_5 mulberry variety due to EMS treatment has been altered to a considerable extent. Fusion of leaves, increase in thickness and changes in texture of leaves, occurrence of xantha and albino were common in plants treated with 3% EMS. The irregularities observed in different plant leaves by the earlier workers has been attributed to various causes such as disturbances of phytochromes, chromosome aberrations, disruption of auxin synthesis and mineral deficiencies, enlargement of palisade, spongy and mesophyll tissues etc. (Haber and Ford, 1964; Karpate and Choudhary, 1997; Mikaelsen *et al.*, 1968; Raghuvanshi and Singh, 1974). Leaf is the prime source of food for silkworm and leaf area was found to be highest (166.23cm^2) at 0.4% compared to control (69.25cm^2) (Table

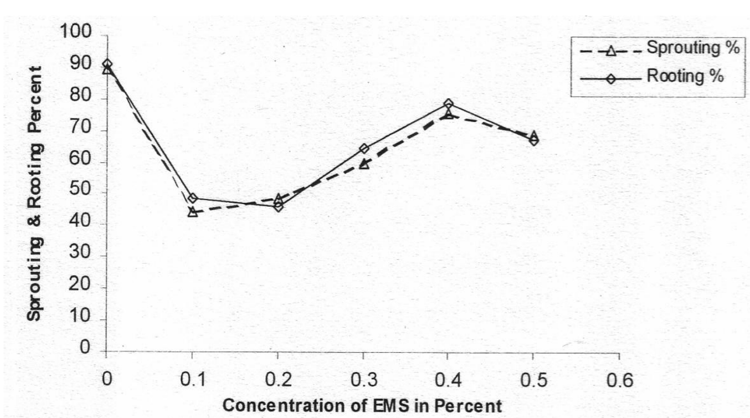

Fig. 1. *Showing sprouting and rooting percentage*

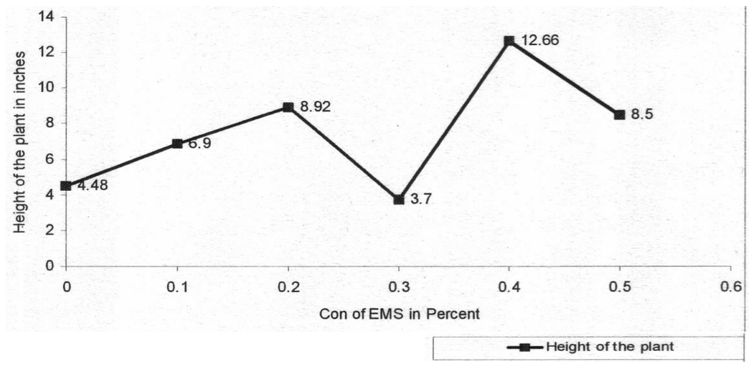

Fig. 2. *Showing plant height.*

- 1). Increased leaf area may be due to chromosomal aberrations, enlargement of mesophyll cells, disturbances in DNA synthesis etc (Dwivedi *et al.*, 1989; Singh *et al.*, 1999).

Growth parameters: Raisinghani and Mahna (1994) noticed growth abnormalities including variation in height in gamma ray irradiated vegetative shoots in *Vigna mungo*. Waghmare and Mehra (2000) recorded increased plant height and number of branches in *Lathyrus sativus* L. on treatment with 0.5% EMS. Present results revealed that, height of the plant varied depending upon the concentration of EMS treatment. Considerable increase in plant height (12.66") was noticed at 0.4% compared to control (4.48") and the lowest plant height (3.70") was recorded at 0.3% EMS concentration (Fig. 2). This is attributed to shorter internodal distance and branching pattern. Interestingly in 0.4% EMS concentration, positive correlation was seen between sprouting percentage and plant height. Konzak *et al.* (1961); Rahman and Soriano, (1973) have interpreted that, the reduced growth in treated materials are due to abnormal cytological behaviour, irregular cell enlargement and degeneration of nuclei.

Internodal distance is one of the important vegetative character, which determines the plant height, branching pattern and tillering nature. The clones of M_5 mulberry genotype treated with 0.3% EMS concentration showed significant decrease in internodal distance (0.66cm) and plant height was reduced (3.70") when compared to internodal distance of control (1.12cm). This clearly

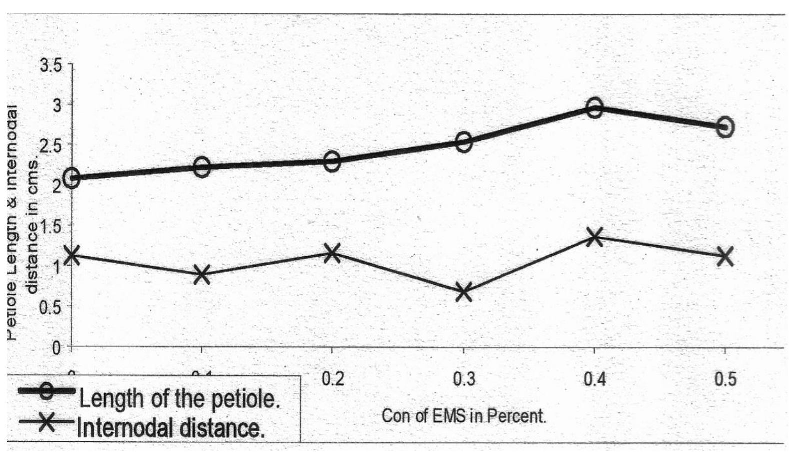

Fig. 3. *Showing petiole length and internodal distance.*

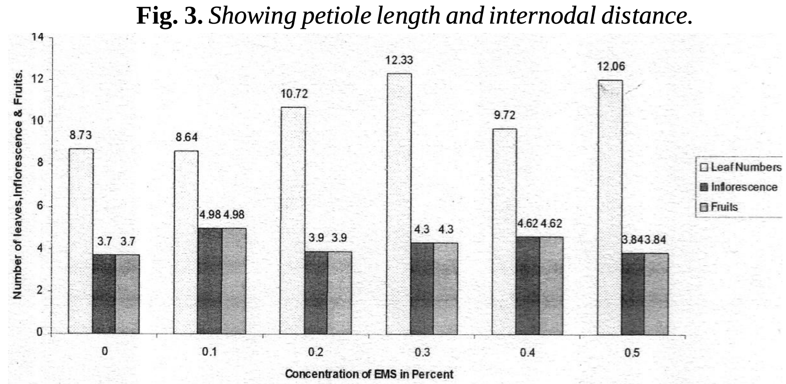

Fig. 4. *Showing number of leaves, inflorescence and fruits.*

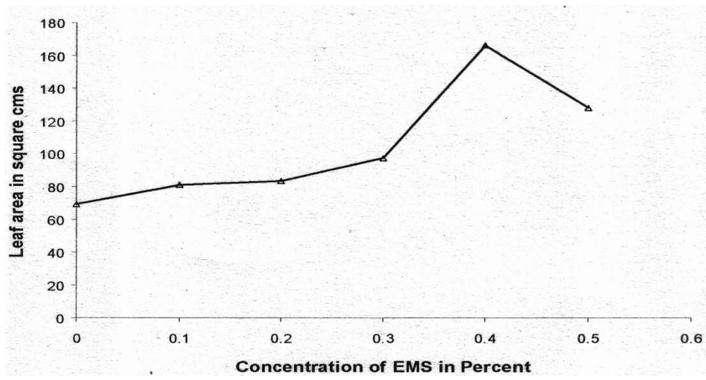

Fig. 5. *Showing leaf area.*

indicated the effect of EMS on modification of internodal distance. Similar types of changes in internodal distance were reported by Jayaramaiah and Munirajappa (1987) in mutants of Mysore local mulberry variety recovered through gamma irradiation. The internodal distance was found affected due to cell number and cell elongation or both in Barley (Blonstein and Gale, 1984). Santhoshlal and Pavithran (1997) reported the change in internodal distance affecting the plant height in EMS treated Rice variety. Ritcha Mehra Chaudhary and Suresh Kumar Sinha (1999) recovered the mutants from EMS treated *Brassica juncea* with reduced plant height affected by internodal distance. It is evident from the results that, shortest petiole length of 2.22cm was recorded at 0.1% EMS concentration and highest petiole length of 2.97cm at 0.4% EMS concentration compared to control (2.08cm) (Fig. 3). EMS treated mulberry genotypes have exhibited significant increase in respect of number of branches and number of leaves with reduced internodal distance (Kuniaki Fukui, 2005). Present findings revealed the similar results that, more number of leaves/plant (12.33) were noticed at 0.3% EMS concentration and less number of leaves/plant (8.64) at 0.1% EMS concentration when compared to control (8.73) were noticed. Similarly, more number of inflorescence and fruits (4.98) were recorded at 0.1% EMS concentration followed by 4.62 at 0.4% EMS concentration and the least number of inflorescence and fruits (3.84) were recorded at 0.5% EMS concentration when compared to control (3.70) (Graph – 4).

Leaf area is the most significant vegetative character and its impact is direct on the leaf yield of the plant. Leaf area of 166.23cm^2 recorded at 0.4% EMS concentration an increase of 2.4 fold compared to control 69.25cm^2 and approximately 2 fold increases than 0.1% and 0.2% EMS concentrations (81.04cm^2 and 83.40cm^2 respectively) (Graph – 5). Increase in leaf area in EMS treated population is quite significant compared to control and it reflects on the biomass of the plant. Such an increased leaf area is due to mutagen treatment in mulberry has been reported by Dwivedi *et al.*, (1989). Anilkumar (2000) and Van Harten (1998) opined that the increase in leaf area may be due to enlargement in palisade and spongy layers both in length and width and also due to chromosomal aberrations. The reduction in size of the inflorescence due to EMS treatment was observed. Such floral mutants have been reported in EMS treated *Brassica juncea* L. (Bhat *et al.*, 2001).

Conclusion

EMS (ethyl methane sulphonate) is the potent mutagen to induce variability in mulberry *Morus* L. It has been observed during the investigation that lower concentration of EMS does not pose any problem to the mulberry (0.1% - 0.2%) and at higher concentration (0.3% -0.5%), various agronomical and morphological variations such as early sprouting, height of the plant, leaf area, shorter internodal distance and stem dichotomy were observed. The sensitivity of mulberry *Morus* L. variety M_5 for chemical mutagen lies between 0.3% to 0.4% of EMS.

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