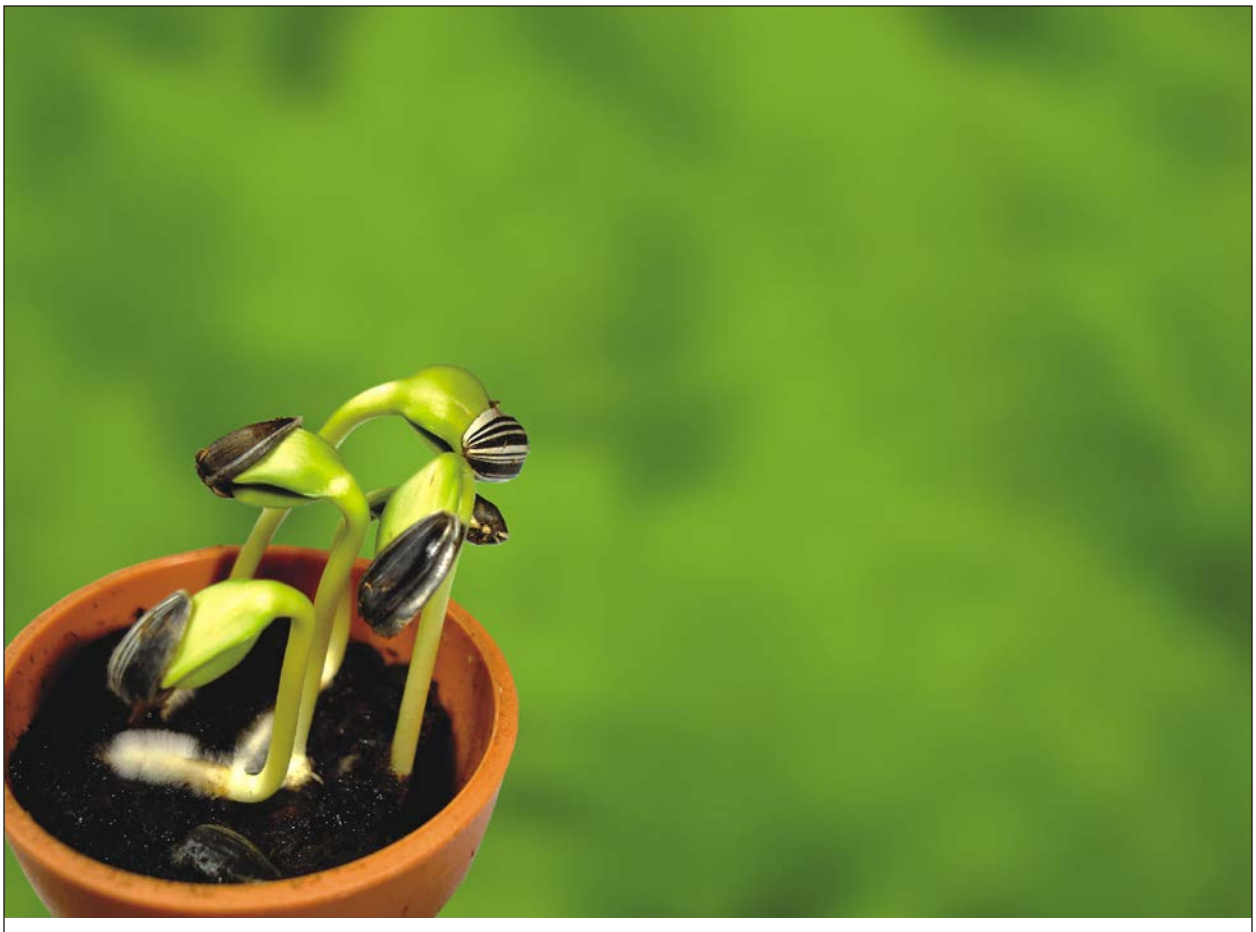

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Research Article

Effect of Dimethyl Sulphate on the Growth, Induction of Mutations and Their Identification by Peroxidase Isozyme in *Tecoma stans*

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Abstract

Background: *Tecoma stans* is an important plant as flowering perennial shrub and herbal medicine. **Methodology:** Seeds were soaked in Dimethyl Sulphate Solution (DMS) at the concentrations of 0, 200, 400, 600, 800 and 1000 ppm for 15 h to induce genetic variability. **Results:** The concentration of 600 ppm had stimulative effect on all studied traits of vegetative growth in the 1st generation only. While, the adverse effect was occurred in the M_2 generation. The concentrations of 200 and 1000 ppm increased significantly the number of florets per inflorescence in both generations. Most treatments produced changes in the leaf form in both generations. All valuable mutants were produced in the 2nd generation. The treatments of 400 and 600 ppm produced plants with lobed pinnately margins of the leaflets (M5). Florets without the orange strip in the throat were found after the concentration of 800 ppm. Florets with four petals were found using 800 ppm and plant having large number of florets in its inflorescence was also found. Dwarfed plant produced using 600 ppm. Thirteen plants with large leaves were obtained after 1000 ppm treatment (M3). Different profile among mutants was found using peroxidase isozyme. Similarity values indicated that, all mutants differed genetically from control with different genetic distances. The dendrogram tree classified the mutants to two clusters. Mutants 3 and 5 were grouped in cluster A and the other mutants and control were grouped in cluster B. **Conclusion:** Dimethyl sulphate is a powerful mutagens for inducing genetic variability and valuable mutants in *Tecoma stans*, which can be vegetatively propagated as new cultivar.

Key words: *Tecoma stans*, mutant, peroxidase isozyme, dimethyl sulphate, dendrogram tree

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tecoma stans is a flowering perennial shrub or small tree belongs to family Bignoniaceae[1]. Leaves are compound and imparipinnate with 2-5 pairs of leaflets. Leaflets are lanceolate with serrated margins. Flowers are trumpet shaped with 5 round lobes, pale to bright yellow with faint orange strips at the throat in clusters at the ends of the branches[2]. The plant has been used for a variety of purposes in herbal medicine, treating diabetes and digestive problems[3].

Induced mutation using chemical mutagen is a method to create genetic variation resulting in new varieties with better characteristics[4,5]. Dimethyl sulphate (DMS) $(CH_3O)_2SO_2$ is a chemical mutagens that act as alkylating agents and consider as the most powerful mutage in plants. Their applications on plant are easy, inexpensive and create mutation to improve their traits. They create point mutation, damage the chromosomes and thus produce tolerance in plant for numerous conditions[6,7]. Chemical mutagens has been used to produce many cultivars with improved economic value as found by Mostafa[7], Aliyu and Adamu[8], Mostafa[9], Kozgar *et al.*[10], Joshi *et al.*[11] and Kozgar *et al.*[12]. Peroxidase isozyme patterns profile and similarity value were used to determine the genetic relationships between the mutant plants[13-15].

The aim of this study was to study the effect of dimethyl sulphate on the growth of *Tecoma stans*. Also, it aimed to produce a new pattern of vegetative and flowering growth and identify them using peroxidase isozyme.

MATERIALS AND METHODS

The study was carried out at the Nursery of Ornamental Plants, Faculty of Agriculture, South Valley University, Qena, Egypt during the period of 2011-2015.

Seeds were soaked in dimethyl sulphate solutions (0, 200, 400, 600, 800, 1000 ppm) for 15 h on 17th October, 2011 for the M_1 generation. Then seeds were washed with tap water and sown in 25 cm plastic pots containing a soil mixture of clay and sand (1:1 v/v). One hundred and twenty seeds were sown for each treatment. The seeds were sown in three replications, each replication contained four pots (ten seeds in each pot). After four months from sowing, seedlings were transplanting into a 25 cm plastic pots containing the soil mixture of clay and sand (3:1 v/v). Seeds obtained from open pollinated of M_1 plants were sown on 29th October, 2013 to produce M_2 generation plants. The procedures of sowing and transplanting were made likewise the first generation.

The experimental layout was a randomized complete block design containing three replications[16]. Each replication contained six treatments and every treatment consisted of twelve plants.

Recorded data: At flowering stage, plant height, number of leaves and branches, stem diameter, leaf area, chlorophyll content, fresh and dry weights of vegetative growth, flowering date, number of florets per inflorescence and length of inflorescence were recorded. Variations in the vegetative and flowering growth were also recorded.

Peroxidase isozyme activities were studied on the leaf of the mutants and the control of *Tecoma stans*. Agar-starch-polyvinyl pyrolidin (PVP) and gel electrophoresis were carried out according to the procedures described by El-Metainy *et al.*[17] and Rida[18]. Similarity values were calculated to determine the genetic relationships between the mutant plants. Bands on agarose gels were scored as present or absent and a pairwise similarity matrix was constructed using Dice coefficient[19], followed by the Unweighted Pair Grouping Method of Average (UPGMA) method to construct the dendrogram.

RESULTS AND DISCUSSION

The data shown in Table 1 and 2 indicated that the concentration of 600 ppm dimethyl sulphate (DMS) had stimulative effect on almost all studied traits of vegetative growth for M_1 generation. It increased the plant height, number of branches and leaves, stem diameter, leaf area, fresh and dry weights of vegetative growth. This stimulative effect might be attributed to cell division rates as well as to activation of growth hormones for example, auxin as recorded by Joshi *et al.*[11]. This stimulative effect disappeared in the M_2 generation, where all the concentrations of DMS decreased all studied traits of vegetative growth compared to control except for leaf area that increased significantly using 1000 ppm. These results can refered refer to that the ability of 600 ppm DMS on stimulation the growth just due as a physiological effect as found by El-Torky[20]. Reduced growth after mutagenesis was explained by one or more of the following reasons: The increase in destruction on growth inhibitors, drop in the auxine level inhibition of auxin synthesis and decline of assimilation mechanism as reported by Roychowdhury and Tah[21].

Dimethyl sulphate at 1000 and 800 ppm increased significantly chlorophyll content in the M_1 and M_2 generations, respectively. Plants treated with all concentrations of DMS except 1000 ppm decreased

Table 1: Effect of dimethyle sulphate concentrations on plant height (cm), number of branches and leaves, stem diameter and leaf area inboth M_1 and M_2 generations

Treatment	Plant height (cm)		No. of branches		No. of leaves		Stem diameter (cm)		Leaf area (cm^2)		Chlorophyll content (SPAD unit)	
DMS (ppm)	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
0.0	193.0^{b}	270.0^{a}	21.5^{b}	29.0^{a}	168.0^{bc}	204.6^{a}	1.4^{c}	1.6^{a}	83.0^{b}	78.9^{d}	30.6^{b}	33.3^{c}
200	200.0^{b}	223.3^{b}	22.0^{b}	16.0^{b}	200.0^{ab}	155.0^{ab}	1.3^{c}	1.4^{ab}	85.0^{b}	87.5^{bc}	28.0^{bc}	29.6^{d}
400	180.0^{b}	225.0^{b}	22.5^{b}	9.5^{b}	91.5^{d}	68.5^{c}	1.4^{c}	1.2^{bc}	83.5^{b}	68.5^{e}	24.2^{c}	36.7^{bc}
600	265.0^{a}	220.0^{bc}	36.0^{a}	12.0^{b}	232.9^{a}	109.5^{bc}	3.4^{a}	0.9^{cd}	99.5^{a}	92.1^{ab}	28.1^{bc}	37.0^{b}
800	287.3^{a}	181.6^{c}	29.0^{ab}	13.6^{b}	110.7^{cd}	173.6^{ab}	2.6^{b}	1.2^{bc}	66.0^{c}	83.9^{cd}	24.9^{c}	43.5^{a}
1000	204.0^{b}	167.0^{d}	32.0^{a}	11.5^{b}	202.1^{ab}	66.0^{c}	1.3^{c}	0.8^{d}	85.0^{b}	98.2^{a}	35.0^{a}	35.8^{bc}
$LSD_{0.05}$	43.4**	41.5**	8.9*	6.6**	64.2**	77.0*	0.2**	0.4*	9.8**	8.2**	4.4**	3.7**

Values in the same column not followed by the same letter are significantly different at the 5% level of probability, NS,*,**Not significant and significant at p = 0.05 and 0.01, respectively

Table 2: Effect of dimethyle sulphate concentrations on fresh and dry weight of vegetative growth, flowering date, inflorescence length and number of florets/inflorescence in both M_1 and M_2 generations

Treatment	Fresh weight (g)		Dry weight (g)		Flowering date (day)		Inflorescence length (cm)		Number of florets/ inflorescence	
DMS (ppm)	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
0.0	287.5^{d}	708.3^{a}	140.0^{b}	275.0^{a}	473.8^{a}	444.6^{b}	19.3^{a}	11.3^{ab}	20.0^{c}	15.0^{c}
200	300.0^{d}	506.6^{ab}	150.0^{b}	205.0^{ab}	491.5^{a}	444.3^{b}	14.6^{b}	13.3^{a}	44.6^{a}	37.0^{ab}
400	392.5^{d}	280.0^{b}	167.5^{b}	127.5^{bc}	502.0^{a}	446.5^{b}	13.0^{b}	13.0^{a}	15.6^{cd}	32.0^{ab}
600	2125.0^{a}	265.0^{b}	580.0^{a}	87.5^{c}	446.5^{a}	490.5^{a}	8.1^{c}	8.5^{b}	7.6^{d}	26.0^{bc}
800	1715.0^{b}	418.3^{b}	565.0^{a}	143.3^{bc}	452.6^{a}	485.3^{a}	14.0^{b}	8.5^{b}	19.3^{c}	28.0^{abc}
1000	1342.5^{c}	246.6^{b}	515.0^{a}	88.3^{c}	473.0^{a}	479.3^{a}	19.3^{a}	14.6^{a}	32.6^{b}	42.0^{a}
$LSD_{0.05}$	254.5**	275.7*	142.9**	89.5**	NS	30.7*	4.0**	3.6*	10.4**	14.9*

Values in the same column not followed by the same letter are significantly different at the 5% level of probability, NS,*,**Not significant and significant at p = 0.05 and 0.01, respectively

significantly inflorescence length compared to control in the M_1 generation. In the M_2 generation, the concentration of 600 and 800 ppm decreased significantly inflorescence length.

The concentrations of 200 and 1000 ppp increased significantly the number of florets per inflorescence in the M_1 and M_2 generations, respectively. The concentration of 1000 ppm increased the number of florets three times than that of the control plants (42 and 15, respectively). No significant differences were found among treatments in the M_1 generation with respect to flowering date. In the M_2 generation, the high concentrations of DMS delayed flowering as shown in Table 2. This result agrees with the results of El-Mokadem and Mostafa[14].

Induction of variation: Dimethyl sulphate produced malformed leaflets in both generations using the concentrations of 200, 800 and 1000 ppm in the M_1 generation and 200, 400 and 600 ppm in the M_2 generation as shown in Fig. 1. Disappeared the orange strip from the throat of florets was found in the M_2 generation as a result of the 800 ppm DMS. Florets with four petals were obtained by 200 ppm compared to control as shown in Fig. 2.

These changes may be due to chromosomal disturbances. Also, these changes could referred to the layer rearrangement as a result of the chemical mutagens effect as reported by El-Nashar[22] and Abd El-Maksoud[23]. The treatment of 200 ppm produced one plant having inflorescences with large number of florets in the M_2 generation as shown in Fig. 3.

The concentration of 600 ppm produced dwarfed plant in the M_2 generation as shown in Fig. 4. This dwarfed growth may be due to physiological damage resulted in the alteration from normal to dwarf growth as reported by Abd El-Maksoud and El-Mahrouk[24]. The dwarfed growth was explained as a result of auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances[11].

The treatment of 1000 ppm in the M_2 generation produced 13 plants with large leaves as shown in Fig. 5. The stimulatory effect of the mutagen may be attributed to the increase in the rate of cell division or cell elongation as reported by[11]. The treatments of 400 and 600 ppm produced plants with changes in the leaf form in the M_2 generation. Their leaflets had lobed pinnately margins, while the control plants had serrate margins as shown in Fig. 5.

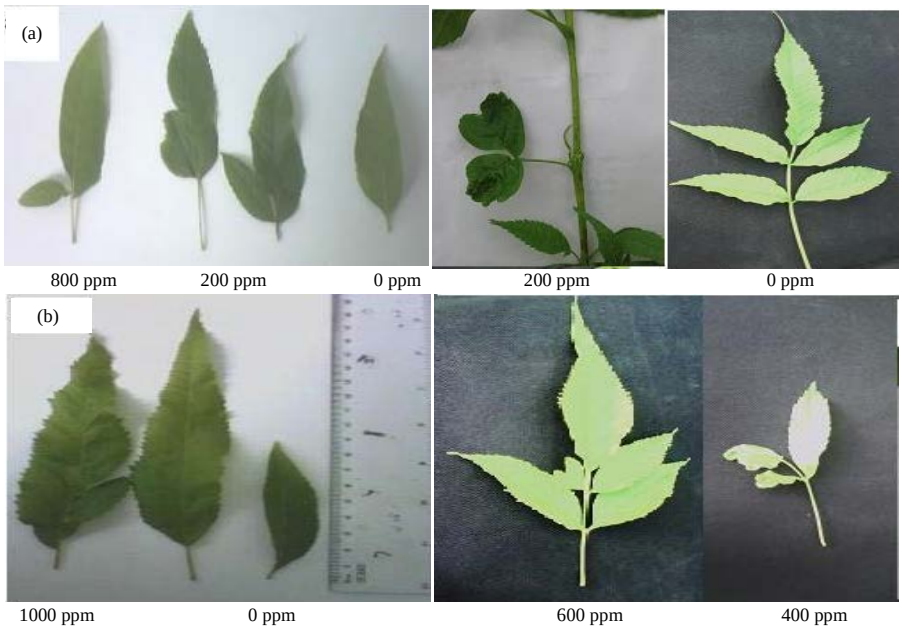

Fig. 1(a-b): Photograph showing malformed leaves of *Tecoma stans* as a result of the treatments with dimethyle sulphate in the (a) M_1 and (b) M_2 generations

Fig. 2: Photograph showing florets with four petals after 200 ppm DMS and florets without the orange strip in its throat as a result of the 800 ppm DMS compared to the control in the M_2 generation

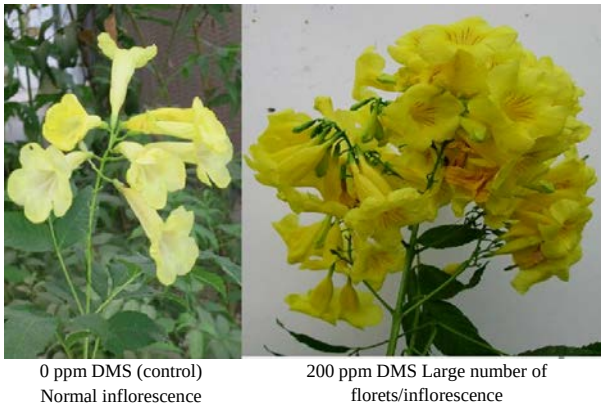

Fig. 3: Photograph showing inflorescences with large number of florets of *Tecoma stans* as a result of the treatment with 200 ppm dimethyle sulphate in the M_2 generation

Fig. 4: Photograph showing dwarfed plant (left) compared to control (right) of *Tecoma stans* as a result of the treatment with 600 ppm dimethyle sulphate in the M_2 generation

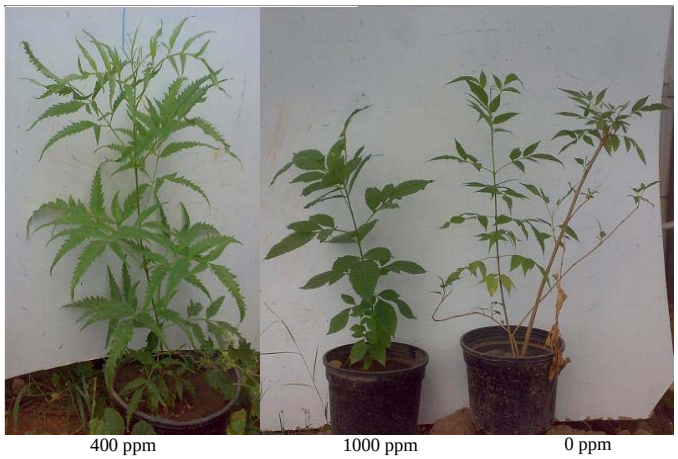

Fig. 5: Photograph showing plant with lobed pinnately margins leaflets and other with broad leaflets compared to the control of *Tecoma stans* as a result of the treatment with 1000 ppm dimethyle sulphate in the M_2 generation

Table 3: Mutants of *Tecoma stans* selected for peroxidase analysis

Mutant No.	Selected variation characteristic
M0 (control)	Normal plant
M1	Dwarfed plant
M2	Disappeared the orange strip from the throat of florets
M3	Plants with large leaves
M4	Inflorescence with large number of florets
M5	Leaflets has lobed pinnately margins

Peroxidase isozyme banding battern-using electrophoresis is a useful tool for breeders to detect the genetic differences among individuals[25]. Five mutants and the control of *Tecoma stans* were used as shown in Table 3. Different profile among mutants were found with a total number of six loci control the production of peroxidase in *Tecoma stans* as shown in Fig. 6. Two bands migrated toward the cathode (-) and the others migrated toward the anode (+) in the electrophoresis field and were designed as prx1 to prx6.

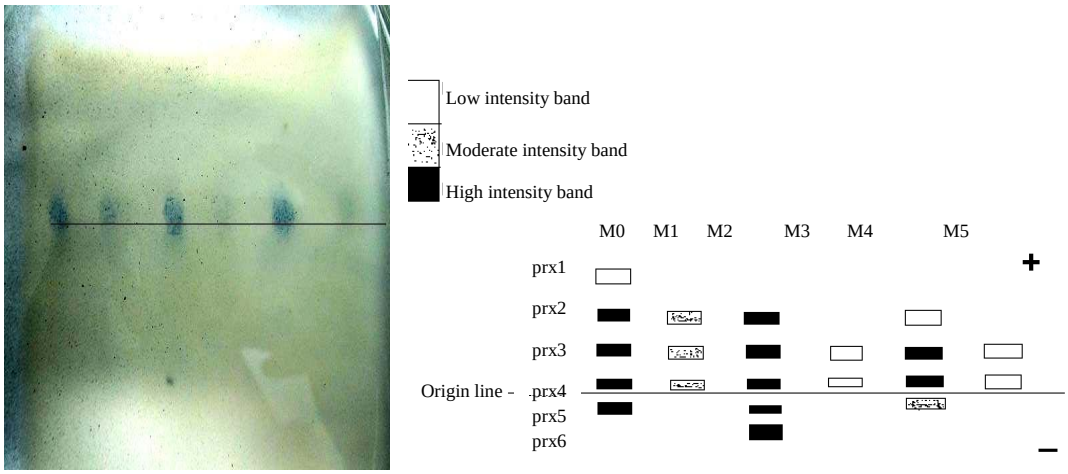

Fig. 6: Electrophoretic separation pattern of peroxidase isozyme of *Tecoma stans* mutants compared to the control

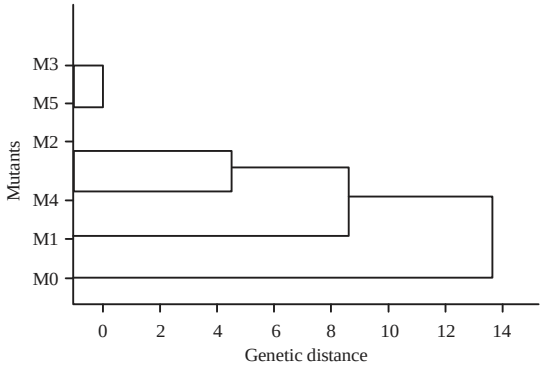

Fig. 7: Dendrogram constructed on the basis of peroxidase isozyme profile for mother plant (Control) as M0 and 5 mutants (M1, M2, M3, M4 and M5) of *Tecoma stans*

Table 4: Similarity value among all mutants of *Tecoma stans* produced by sodium azide treatments

Mutant No.	M0	M1	M2	M3	M4	M5
M0	0.0					
M1	75.0	0.0				
M2	80.0	75.0	0.0			
M3	57.1	80.0	57.1	0.0		
M4	80.0	85.7	88.8	66.6	0.0	
M5	57.1	80.0	57.1	100.0	66.6	0.0

The bands of the loci prx3 and prx4 were presented in all of the evaluated genotype but they differed in the activity (intensity). The loci prx1 was found only in the control. The genetic locus prx2 was absent in M3 and M5. Locus prx5 was disappeared from the mutants M1, M3 and M5. This may be due to the mutagenic effects[26]. The band of the loci prx6 was found only in the mutant M2. The mutagenesis treatments seemed to activate expression of some genes that resulted in the appearance of some new bands. Mutations have been identified as one of the sources of isozyme variation in higher plants. These results are almost in agreement with those of Bartosova *et al.*[13], El-Mokadem and Mostafa[14] and Malaviya *et al.*[15].

With regard to similarity values, Table 4 show that, all mutants differed genetically from control with different genetic distances. The mutant M3 was genetically related to M5 with 100% similarity value and were grouped in one cluster (A) in the dendrogram tree as shown in Fig. 7. Others mutants and the control were grouped in the cluster B.

CONCLUSION

It can be concluded that, dimethyl sulphate is a powerful mutagens for inducing genetic variability and valuable mutants in *Tecoma stans*. Breeding programme and or vegetative growth methods were needed to take advantage from the obtained useful mutants i.e., dwarfed plants as pot plant, the new type of leaves and large number of florets in the inflorescences as new cultivars.

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