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Improvement of Morphological Characters in *Gypsophila elegans* M. Bieb Plant by Diethyl Sulfate and Detected Variation through SRAP Molecular Markers

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Abstract

Diethyl sulphate was used to induce genetic variability in *G. elegans* plant to improve morphological characters. Besides, changes in the genomic DNA between mutants and un-treated plants were investigated. The results revealed that un-treated plants gave the highest germination percentage in M_1 and M_2 (94.03 and 96.17%), respectively, compared to treated plants that recorded lowest germination percentage in M_1 and M_2 (67.03 and 77.46%) respectively, at 4000 ppm. Morphological characters like plant height, branches number/plant, number of days to flowering and flowers number /plant showed different significant variation between mutants and un-treated plants, 1000 ppm and 2000 ppm caused early flowering, recorded high values, in M_1 and M_2, 3000 ppm recorded moderate values and 4000 ppm and un-treated plants delayed flowering phase and recorded lowest values, respectively. Using DES produced many mutants in leaves and flowers morphology. Molecular marker analyses using SRAP markers, showed variation between original and mutant genotypes, the highest number of bands was produced by SRAP-4 (8bands), while SRAP-6 generated the lowest number (3 bands). Whilst, SRAP-2 displayed the highest number of polymorphism (66.67%), but SRAP-4 scored the lowest polymorphism (12.50%). Genetic diversity six primers were used product 35 bands, 12 bands were polymorphic and recorded 34.29% polymorphism and 23 bands were monomorphic. Therefore, it was confirmed that, using SRAP markers, the existence of genetic diversity at the genomic DNA level between mutants DES-treated and control, depending on DES concentration. It was concluded induced mutation by DES used to improvement morphological characters and increase genetic diversity.

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INTRODUCTION:

Gypsophila elegans which belongs to the family *Caryophyllaceae*, is a flowering plant; Gypsophila genus comprises about 150 species of annual or perennial herbaceous plants (Madhani *et al*., 2018). Common name is **(**Baby's breath-chalk plant), native to Europe and Asia, and with single flowers (five petals) on branching stems. Leaves are narrow, grey-green, opposite, lance-shaped, smooth. Stems are highly branched and swollen at the nodes. The fruit is a rounded or oval capsule that contains brown or black seeds (Korkmaz *et al*., 2012).They are suitable for mixed flower beds, borders, pots, and containers, with delicate mass of tiny blooms, ideal for cottage, gravel or rock gardens, cut flowers, flower arrangements and bouquets (Madhani *et al*., 2018). Gypsophila genus is a medicinal plant, it contains biological compounds such as triterpene, saponins, flavonoids and sterols that are important for the pharmaceutical industries (Zdraveva *et al*., 2015).

Genetic improvement through mutation breeding in ornamental plants by chemical mutagens is aimed to inducing changes in one or many characters of an otherwise outstanding variety without altering the unique part of the genotype, and it has made a main contribution to the flower's production, increasing flowers crop and economic value by containing insect and disease resistance, improving flowers quality and a shortened growing period. So, induced mutations are considered one of the best methods for the improvement of ornamental herbs, (Kayalvizhi *et al*., 2020). Mutation is a sudden genetic alteration that takes place in an organism; it may occur naturally or be artificially induced, and the mutant that results will have altered chromosomes or genes (De and Bhattacharjee, 2011). Physical and chemical methods of mutagenesis are commonly used in mutation breeding programs, and are commonly used to induce random genetic variations in plants. The response of the genetic variation in plants varies with mutagen type and dosage (Jankowicz-Cieslak *et al*., 2016). Physical mutagenesis like (gamma rays and X-rays) is one of the main method mutagens for mutation studies in plants.

Chemical mutagenesis is another means to cause mutations in plants to improve their agronomic traits (Shu *et al*., 2012). One of the most popular methods for using chemical mutagens to add more desirable character variation is induced mutation. Alkylating chemical substances like diethyl sulfate (DES), ethyl methane sulphonate (EMS), methyl methane sulphate (MMS), dimethyl methane sulphate (DMS), hydrazine, and sodium azide, can be used to chemically cause mutations. When chemical mutagens from the alkyl group interact with DNA, the nucleotide sequence may change, and a point mutation may result. These can alkylate the phosphate groups in the phosphodiester backbone, as well as the different imino- or carbonyl groups on the purine or pyrimidine bases, and therefore react with DNA. Three chemicals are particularly important: ethyl methane sulphonate (EMS), 1-methyl-1-nitrosourea, and 1-ethyl-1-nitrosourea, which together account for 64% of these variants. Another group of the base analogues (such as 5-bromouracil and maleic hydrazide), which are closely similar to DNA bases and can be incorrectly incorporated during replication, are another type (Spencer-Lopes *et al*., 2018). Diethyl sulfate is a chemical mutagen and has been one of the most powerful mutagens in ornamental plants, being a strong mutagen in plants; it affects the

different parts of the plants and their growth developmental phenomena by disturbing the metabolic (Owias *et al.*, 1983). Chemical mutagens have been used in many studies to induce genetic variability in ornamental plants. Molecular markers such as Sequence-Related Amplified Polymorphism (SRAP), ScoT marker, ISSR marker and RAPD marker, can selectively amplify DNA coding regions, are widely used, and have been reported to be highly stable, efficient, and suitable for direct use in different plants (Li and Quiros, 2001). (Mangaiyarkarasi *et al.*, 2014) on *Catharanthus roseus*, (Mostafa *et al.*, 2014) on *Celosia argentea*, (Radwan, 2017) on *Helichrysum bracteatum*, (Chen *et al.*, 2020) on *Chrysanthemum indicum*, (Elmenbawy *et al.*, 2020) on *Calendula officinalis*, (Habib *et al.*, 2021) on sunflower, (El-Khateeb *et al.*, 2022) on *Borgo officinalis*, (El-Gazzar *et al.*, 2023) on *Hibiscus rosa-sinensis*, and (Eid *et al.*, 2024) on *Gaillardia pulchella*. Therefore, this investigation, due to improving the morphological characteristics through induced mutation induction by diethyl sulfate (DES) in *G. elegans* was undertaken and using SRAP markers to detected variation between original and mutant genotypes. Identify the DNA polymorphisms among obtained mutants.

MATERIALS AND METHODS:

Plant materials: The seeds of *G. elegans* (local variety) were obtained from a bred strain in The Ornamental Horticulture Department Faculty of Agriculture Cairo University Egypt. The present investigation it was a field experiment conducted in this location through the two successive seasons of 2019/20 and 2020/21 for two generations (M_1 and M_2).

Seed treatment and seedling preparation: Seeds were pre-soaked in distilled water for 1 hour, batches of 300 seeds were treatment with different concentrations of DES (0.0, 1000, 2000, 3000, and 4000 ppm) for 8 hours, the seeds were sown in plastic trays filled with a mixture of peat moss, loam, and sand (1:1:1 by volume) on 5, October 2019, and 5, October 2020 for (M_1 and M_2, respectively) (Fig. 1) to produce seedling. After 8 days of sowing seeds germination began, and after 45 days of sowing, uniform Gypsophila seedlings (average 12-14 cm in height). The seedlings of each treatment were transplanted into the open field (clay loam soil), in three rows at 60 cm apart and 50 cm between the hills within each row (two plants/hill), as every plot (3.5 x 1.8 m) contained 21 hills /plot.

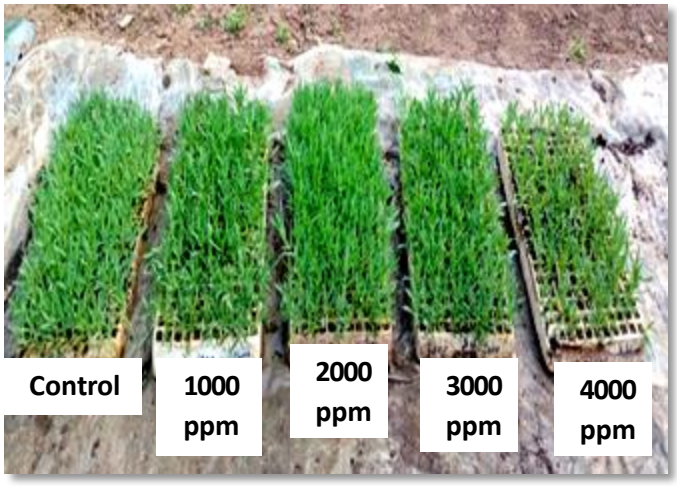

Fig. 1. Seedling stage of *G. elegans* in plastic trays after treated by different concentrations of diethyl sulphate.

The first and second mutative generations 2019/20 (M_1) and 2020/21 (M_2):

The seeds harvested from the M_1 generation were taken from individual treatments and used to raise M_2 generation plants. The mass selection of seeds in M_1 plants was done from May to June 2020, where plants that survived in each treatment were evaluated, selected, and selfed in order to obtain the second mutative generation (M_2) seeds,

according to (Sinhamahapatra and Rakshit, 1990). Mutants and changes were recorded during the vegetative growth and flowering periods. In order to prevent cross-pollination between plants and some of them, whether by wind or insects, we used a bag of paper for the flower buds before opening in order to preserve the selected characters and to grow M_2 generation (seedlings) plants. In both generations all the recommended cultural practices, namely irrigation and fertilizer, were carried out during the plant's growth and flowering period. The fertilizers were supplied for each plot as recommended, using Kristalon mineral fertilizer (N:P:K) (19:19:19). The plants were fertilized monthly after a month of transplanting (1 g/hill). Irrigation was done with tap water according to the needed amount of water, and weeding was carried out as the soil needed.

Genomic DNA isolation and SRAP analyses:

Fresh young leaves of 0.5 g of control and treated plants (individuals from each treatment) were collected from *G. elegans* in the M_2 generation, were used in DNA extraction and purification by kit (Bio Basic Inc., Markham, Canada) following the manufacturer's instructions. Six SRAP primers (Table 1) were selected from (Li and Quiros, 2001) and were used to detect variation from G. pulchella original and mutant plants. The PCR reaction contained 25 µl, 10 X PCR buffer, 2 mM MgCl2, 0.2 mM dNTPs mixed, 10 pmol primers, 1.25 U Taq polymerase, and about 150 ng genomic DNA. And PCR conditions, the initial denaturing step was performed at 94°C for 5 min, followed by 5 cycles at 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min, subsequently followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min with a final extension step at 72°C for 7 min. Amplification products were separated on 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) and 0.5 µg/ml ethidium bromide at 90 V.

Table (1): Sequence of primers used in this the study. The selective nucleotide sequences for each primer are underlined.

Primers name	Sequence	
	Forward	Reverse
SRAP-1	TGAGTCCAAACCGG	GACTGCGTACGA
SRAP-2	TGAGTCCAAACCGG	GACTGCGTACGA
SRAP-3	TGAGTCCAAACCGG	GACTGCGTACGA
SRAP-4	TGAGTCCAAACCGG	GACTGCGTACGA
SRAP-5	TGAGTCCAAACCGG	GACTGCGTACGA
SRAP-6	TGAGTCCAAACCGG	GACTGCGTACGA

Data analysis

A matrix for SRAP was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the genotype. Genetic similarity coefficients were computed according to (Nei and Li, 1979). A dendrogram based on Jaccard similarity coefficients was constructed by using the un-weighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973) employing sequential, agglomerative hierarchic, and non-overlapping clustering (SAHN). All the computations were carried out using the PAST software (Hammer *et al.*, 2001). Correlation coefficients were calculated using similarity coefficients obtained from SRAP analysis.

Soil analysis: Soil analysis indicated that particle size distribution (%) was: sand: 26.7, silt: 26.2 and clay: 38.5 (texture: clay loam), pH: 7.1, and EC ds.m-1: 0.95.

Experimental data recorded: The following data were collected on *G. elegans* plants that were grown until 50 % of the flowers were opened, that is, about a month after the flowers start to appear

for each treatment: (**a)** Seed germination (%), the germination percentage of seeds was measured using the following equation:

$$\text{Germination (\%)} = \frac{\text{No.of seeds germinated}}{\text{Total No.of seeds sown for germination}} \times 100$$

(b) Vegetative characters [plant height (cm) and No. of main branches/plant]; **(c)** Flowering characters [No. of days from planting to flowering (DPF) and No. of flowers/plant]; **(d)** Plant abnormalities [leaf and flower abnormalities]; **(e)** Molecular characterization using SRAP markers of *G. elegans* mutants with DES

Statistical analysis: Data of the experiment analysis was conducted using COSTAT software; a randomized complete block design was used, with three replicates for each treatment and ten plants in each replicate. The results of the experiment were statistically analysed using (Snedecor and Cochran's, 1980), and the means were separated using (Duncan, 1980) multiple range tests and compared using the L.S.D test at 0.05 probability.

RESULTS AND DISCUSSION:

1. Seed germination (%)

It could be observed from the data in (Table 2) that the control, 2000 and 1000 ppm recorded the highest germination values, then there was a decrease in the germination percentage with 3000 and 4000 ppm, it was found that soaking the seeds in the concentrations of 2000 and 1000 ppm resulted in maximum germination percentages, giving (90.64% and 87.29%) and (95.56% and 94.21%), respectively. On contrast, using DES at 3000 and 4000 ppm reduced the seeds germination, to (83.85% and 67.03%) and (85.16% and 77.46%) in M_1 and M_2, respectively, compared to 94.03% (in M_1) and 96.17% (in M_2) for the control.

In this regard, (Kulkarni, 2011) reported that the reduction in seed germination by using DES may be owing to, one of the physiological effects of DES mutagen. (Deepika *et al.*, 2016) reported that the reduction in germination percentage may be related to disruptions in the synthesis of enzymes involved in the process of germination or the action of DES mutagens on the meristematic tissues of the radical/plumule could cause a reduction in seed germination. These results are similar to those found by (Mangaiyarkarasi *et al.*, 2014) on *Catharanthus roseus*, who applied EMS at 30, 40, 50, 60, and 70 mM, and found that the seed germination increased when EMS concentrations decreased. (Chen *et al.*, 2020) indicated that with increasing EMS concentrations, the germination rate decreased, when mutated *Chrysanthemum indicum* plants were treated by EMS at (0, 0.1, 0.2 and 0.5%) for 8 h, and (Eid *et al.*, 2024) on *Gaillardia pulchella*, they recorded a reduction in seed germination compared to the control when the seeds were soaked in four concentrations of DES from (1000-4000 ppm).

2. Vegetative characters

1.2 Plant height (cm)

The results in (Table 2) indicated that in M_1 and M_2, the concentrations of 1000 and 2000 ppm induced a significant increase in the plant height, and produced the tallest plants by (122.33 and 122.56 cm, with increments of 16.26% and 16.48%) and (126.30 and 127.10 cm, with increments of 14.80% and 15.52%), respectively. In contrast, the highest concentration of 4000 ppm gave the shortest plants in M_1 and M_2 (99.75cm and 105.44 cm), compared to the control (105.22 and 110.02 cm), respectively. In this concern, (Joshi *et al.*, 2011) suggested that the increase in

plant height using low concentrations of DES, it may be attributed to an increase in the rate of cell division or cell elongation. For the decrease in the plant height using the high concentration of DES, it may be due to hindering cell development and growth, as reported by (Neagu, 1984) on *Helianthus annuus*; (Badr *et al*., 2000) on *Tagetes erecta*, (El-Nashar, 2006) on *Amaranthus*. These results are similar to (Krupa-Makiewicz *et al*., 2010) they soaked the seeds of the petunia plant in DES at 0.5 and 1.0 mM, EMS at 0.5 and 1.5 mM, and MMS at 1.5 and 2.0 mM (for 60 min), and concluded that the low levels of all mutagens increased the plant height over the control. (Kapadiya *et al*., 2014) on chrysanthemum plants applied three concentrations of EMS and DES at (0.02, 0.03, and 0.04%) for 6 h and recorded that the largest concentration of both mutagens decreased plant height. (Kayalvizhi *et al*., 2017) investigated the effects of DES on tuberose, and indicated that the plant height was greater with low levels of DES. (Sedaghathoor *et al*., 2017) evaluated the effect of DES on tulip plants, and stated that the low concentrations of DES increased the plant height. (Ghosh *et al*., 2020) used EMS at 25, 30, 35, and 40 mM on *Jasminum grandiflorum.* They mentioned that the plant height was shortened with increasing EMS concentrations, and (Eid *et al*., 2024) on *Gaillardia pulchella*, treated the seeds with four concentrations from (1000 – 4000 ppm) of DES, and they found that the plant height was decreased with increasing the concentrations.

Table (2): Effect of diethyl sulphate on seed germination (%), plant height (cm), number of main branches/plant, number of days to flowering (DPF), and number of flowers /plant of *G. elegans* plant, during the M_1 and M_2 generation (2019/2020) and(2020/2021).

			Vegetative Characters		Flowering Characters	
		Seed germination	Plant height (cm)	Number of main branches/plant	Number of days to flowering	Number of flowers /plant
Control	M_1	94.03 a	105.22 c	8.32 b	111.33 a	501.30 c
	M_2	96.17 a	110.02 bc	11.37 c	108.70 a	552.85 c
1000 ppm DES	M_1	87.29 b	122.33 a	14.77 a	96.83 b	676.77 a
	M_2	94.21 a	126.30 a	15.12 b	94.50 b	680.32 a
2000 ppm DES	M_1	90.64 b	122.56 a	15.71 a	94.50 b	680.11 a
	M_2	95.56 a	127.10 a	17.15 a	91.00 b	694.10 a
3000 ppm DES	M_1	83.85 c	113.12 b	10.05 b	109.50 a	593.33 b
	M_2	85.16 b	111.25 b	12.20 c	100.33 ab	656.20 b
4000 ppm DES	M_1	67.03 d	99.75 d	7.72 b	113.83 a	437.18 d
	M_2	77.46 c	105.44 c	9.92 d	106.27 a	466.80 d

2.2 Number of main branches /plant

It is evident in (Table 2) that the concentrations of 2000 followed by 1000 ppm in M_1 and M_2 formed the largest number of branches/plant by (15.71 and 14.77 branches) and (17.15 and 15.12 branches) over the control that formed (8.32 and 11.37 branches), respectively. Whilst, with increasing the levels of DES to 4000 ppm, had a

negative effect on the formation of branches, as it produced the lowest number (7.72 branches) and (9.92 branches), compared to the control in M_1 and M_2, respectively. The previous results agreed with that obtained by (Kapadiya *et al*., 2014) they found that using the high DES concentration of (0.04%) had a negative effect on the branches formation of chrysanthemum plants; (Mangaiyarkarasi *et al*., 2014) on *Catharanthus roseus*, applied EMS at 30, 40, 50, 60, and 70 mM, and they concluded that as the concentrations decreased, the branches number increased; (El-Nashar and Asrar, 2016) on *Calendula officinalis*, They indicated that lowering mutagen concentrations (1000 and 2000 ppm) of DES had an enhanced on number of branches; (Sedaghathoor *et al*., 2017) reported that the low concentration (0.1%) of DES enhanced the growth of tulip plants; (El-Gazzar *et al*., 2023) treated *Hibiscus rosa-sinensis* plant with (EMS) and (DMS) at (0.1, 0.2, and 0.3%). They observed that the plant height, number of the leaves and branches, were decreased with increasing the concentrations of (EMS) and (DMS).

3. Flowering parameters

1.3 Number of days from planting to flowering (DPF)

The results in (Table 2) showed that utilizing DES at 2000 as well as 1000 ppm, in M_1 and M_2 shortened the vegetative growth phase, therefore, the number of days elapsed to reach the flowering phase decreased to (94.50 and 96.83 days) and (91.00 and 94.50 days), respectively, compared to the control plants, that recorded (111.33 and 108.70 days), respectively. On the other hand, the highest DES concentration of 4000 ppm in M_1 and M_2, prolonged the vegetative growth phase, therefore, it takes more days to reach the flowering stage (113.83 and 106.27 days), respectively, compared to 2000 and 1000 ppm. In this regard, (Neagu, 1984) on *Helianthus annuus*, reported that the high levels of chemical mutagens, hindered cell development, decreased growth rate and delayed flowering phase; (Badr *et al*., 2000) on *Tagetes erecta*, and (El-Nashar, 2006) on *Amaranthus*, they stated that the physiological damage caused by increasing chemical mutagen levels may be the cause of flowering inhibition. Similar results were found by (Kapadiya, *et al*., 2014) used DES with different concentrations of (0.02, 0.03, and 0.04%) for 6 h, on chrysanthemum plants, and they revealed that the flowering was delayed by up to 7 days with high concentration. (Patel *et al*., 2018) on gladiolus, who observed that the low DES concentrations of (0.15 and 0.20%) induced early flowering, and (Ghosh *et al*., 2020) on *Jasminum grandiflorum*, applied EMS at 25, 30, 35, and 40 mM, and they indicated that the early flowering was related to low concentrations.

2.3 Number of flowers /plant

Data in (Table 2) indicated that, in M_1 and M_2 treating the plants with the DES concentrations at 2000 ppm, 1000 and 3000 ppm had appositive effect on the flower's production/plant, giving the largest values (680.11, 676.77, and 593.33 flowers) by increasing of (35.67%, 35.00% and 18.36%) in M_1 and (694.10, 680.32, and 656.20 flowers) by increasing of (25.55%, 23.06%, and 18.69%) in M_2, respectively, compared to (501.30 and 552.85 flowers) for the control. Conversely, the highest concentration of 4000 ppm decreased it to the lowest number/plant (437.18 and 466.80 flowers) by decreasing of (12.79% and 15.56%), in M_1 and M_2, respectively. These results are good in harmony with (El-Nashar and Asrar, 2016) on *Calendula officinalis*, they found that the low

DES concentration of (1000 ppm) enhanced the formation of flowers; (Kayalvizhi *et al*., 2017) on tuberose plant, reported that the low level of DES (15 mM) formed the highest number of flowers; (Sedaghathoor *et al*., 2017) on tulip plant, stated that the low levels of DES improved the production of flowers; (Elmenbawy *et al*., 2020) soaked *Calendula officinalis* seeds in three different EMS concentrations of (1000, 3000, and 10000 ppm), and concluded that flowers number decreased with increasing EMS levels; (Ghormade *et al*., 2020) on chrysanthemum, found that the low levels of EMS (0.01, 0.05, 0.1, 0.5, 1.0, and 1.5%), increased the flowers production, and (El-Gazzar *et al*., 2023) on *Hibiscus rosa-sinensis* plant, applied (EMS) and (DMS), and noticed that the low concentration (0.1%) of both mutagens increased the number of flowers.

4. The leaves and flowers abnormalities

1.4 Leaves abnormalities

The leaf abnormalities pictured in (Fig. 2) showed that using the chemical mutagen of DES produced many changes in the leaves compared to the control, such as large broad leaves with acute apex, elliptical broad leaves with rounded apex, malformed leaves with wavy edges, ovate leaves, and obtuse and leather texture leaves. The largest leaves (average 18 cm tall) were achieved with the low concentration of 1000 ppm, compared to the leaves control that recorded (15 cm in tall). It was observed that the highest concentration of 4000 ppm produced the largest number of these changes. These changes and abnormalities in the leaves may be attributed to the result of chromosomal disruptions, also may be due to the result of layer rearrangement caused by chemical mutagens, as reported by (Abd El-Maksoud, 1988). In this concern, (Srivastava *et al*., 2018) applied EMS at (0.025, 0.05, 0.1, 0.2, and 0.3%) for 6 h on orchid (*Aerides crispa*), and they observed the following leaf shapes; lanceolate leaf, straita leaves, maculate leaf, oblong, waxy, viridis leaf, short, and broader leaves; (Chen *et al*., 2020) on *Chrysanthemum indicum*, concluded that applying EMS concentrations at 0, 0.1, 0.2, and 0.5% for 8 h, produced many changes in leaf formation, and (El-Khateeb *et al*., 2022) obtained many leaf morphological changes in size, shape, margin, and petioles when soaking *Borgo officinalis* seeds in different DES concentrations of (0.1, 0.2, 0.3 and 0.4%) for 6 h.

Fig. 2. Leaves abnormalities shapes of *G. elegans* treated by different concentrations of DES in M_1 and M_2 generations.

(a) Control original leaves plants, (Lanceolate with acuminate apex) average 15.00 cm in tall; **(b)** 1000 ppm DES, (Large broad leaves with acute apex) average 18 cm in tall; **(c)** 2000 ppm DES, (Elliptical broad leaves with rounded apex) average 15 cm in tall; **(d)** 3000 ppm DES, (Malformed leaves with wavy edges) average 15 cm in tall;

(e) 4000 ppm DES, (Ovate leaves) average 15 cm in tall; **(f)** 4000 ppm DES, (Obtuse and leather texture leaves) average 9 cm in tall.

2.4 Flowers abnormalities

The flowers abnormalities obtained in (Fig. 3) illustrated that treating the plants by DES had an evident effect on inducing many changes in the formation and color of the flowers such as, flower with four petals, colored pink flower, flower with biforked petal, colored pink flower of a trumpet shape, and flower with deformed petal. The largest number of flowers variations was observed with the highest DES concentration of (4000 ppm). These flowers abnormalities by DES mutagen may be attributed to a deficiency or delay in the development of flowers, as well as a proliferation of inflorescence-like structures in their place, according to (Coen and Carpenter, 1993), (Nakatsuka *et al*., 2005) reported that the changes may be attributed to the result of a gene mutation that caused the floral meristem to be replaced with meristems that contain some or all of the flower's characters. In this respect, (Kolar *et al*., 2015) applied EMS on *Delphinium malabaricum* plant, and they recorded many changes in morphological of flowers. (Samatadze *et al*., 2019) mutated *Calendula officinalis* seeds with 0.04 and 0.08% of DES, and observed many changes in vegetative and floral characters. (Chen *et al*., 2020) they found that using EMS concentrations of 0, 0.1, 0.2, and 0.5% for 8 h., on *Chrysanthemum indicum* induced many changes in shape of leaves and flowers. (Elmenbawy *et al*., 2020) exposed the seeds of *Calendula officinalis* to EMS at 1000, 3000, and 10000 ppm, and several variations in flowers colour and shape were recorded, and (Radwan, 2023) on *Gaillardia pulchella*, treated the seeds with four different concentrations from (1000 – 4000 ppm), he obtained many abnormalities in the flowers color and formation, and the concentration of (4000 ppm) gave the largest number of these variations.

(a) Control original color, (Serrulate apex); **(b)** 1000 ppm DES, (Flower with four petals); **(c)** 2000 ppm DES, (Colored pink flower); **(d)** 3000 ppm DES, (Flower with biforked petal). **(e)** 4000 ppm DES, (Colored pink flower of a trumpet shape; **(f)** 4000 ppm DES, (Flower with deformed petal).

(e) Molecular characterization using SRAP markers of *G. elegans* mutants with DES

Six SRAP primers were used for identifying DNA polymorphism among *Gypsophila* plants mutated by DES and the untreated control. A total of 35 amplified fragments, ranging from 90 to 1200 bp were recorded. Twelve amplicons out of 35 fragments were polymorphic (34.29%), while 23 fragments were monomorphic (65.71%). The highest number of bands was produced by primer SRAP-4 (eight amplicons), followed by primers SRAP-1 and SRAP-3 (seven bands); while the lowest number of amplicons was generated by primer SRAP-6 (three bands). On the other hands, primer SRAP-2 displayed the highest number of polymorphism (66.67%), followed by SRAP-1 gave 57.14% polymorphism. However, primer SRAP-4 scored the lowest number of polymorphism (12.50%). On the other hand, the population treated with 1000 ppm DES scored four markers of (1100 bp) and (-182, -372, and -

500 bp), using primers SRAP-1 and SRAP-2, respectively. Besides, the individuals mutated with 2000 ppm DES scored one positive marker with molecular sizes of +195 bp, using primer SRAP-3. Furthermore, the plants mutated with 3000 ppm DES recorded one negative marker with a molecular size of -600 bp, using primer SRAP-6. Also, the control plants displayed one positive marker of 660 bp, using primer SRAP-2 (Table 3) and (Fig. 4).

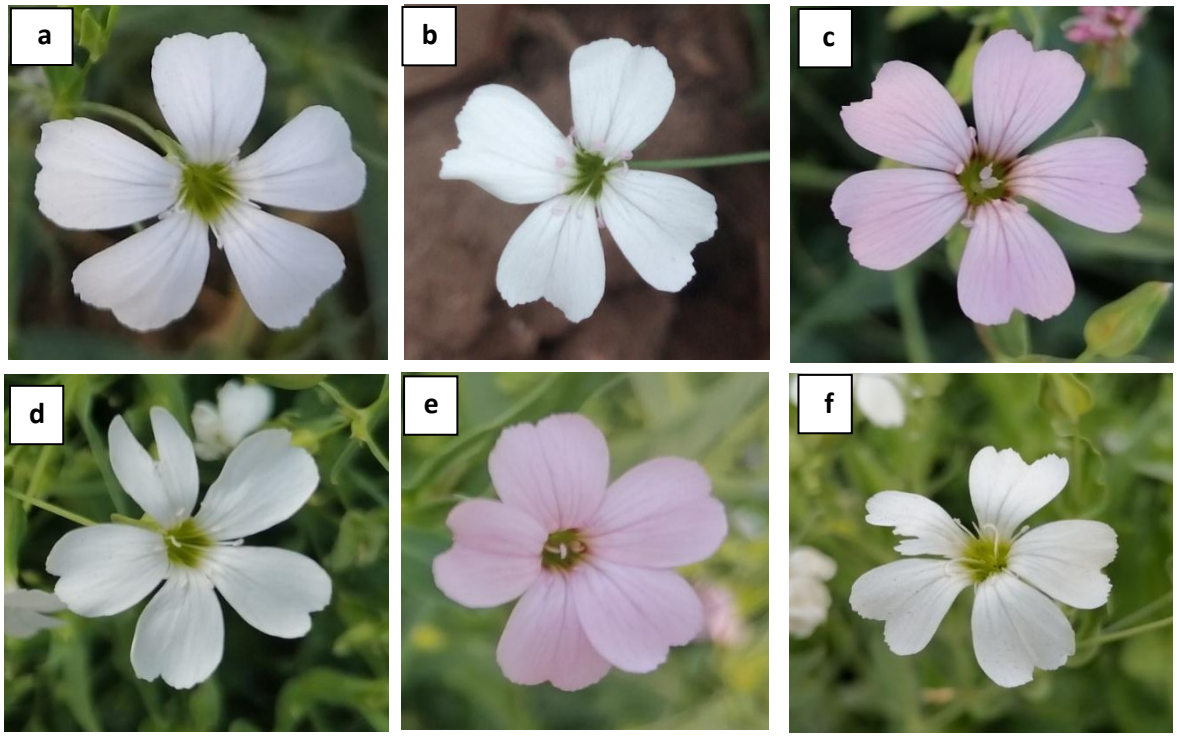

Fig. 3. Different color of flowers shapes of *G. elegans* treated by different concentrations of DES in M_1 and M_2 generations.

Table 3. SRAP analysis of *Gypsophila elegans* plants mutated by DES.

Primer	Size range	Total bands	No. of	No. of	%	Unique markers
SRAP-1	100-1100	7	3	4	57.14	1
SRAP-2	90-660	6	2	4	66.67	4
SRAP-3	195-1200	7	6	1	14.29	1
SRAP-4	110-801	8	7	1	12.50	0
SRAP-5	115-720	4	3	1	25	0
SRAP-6	240-600	3	2	1	33.33	1
Total	**90-1200**	**35**	**23 (65.71%)**	**12**	**34.29 %**	**7**

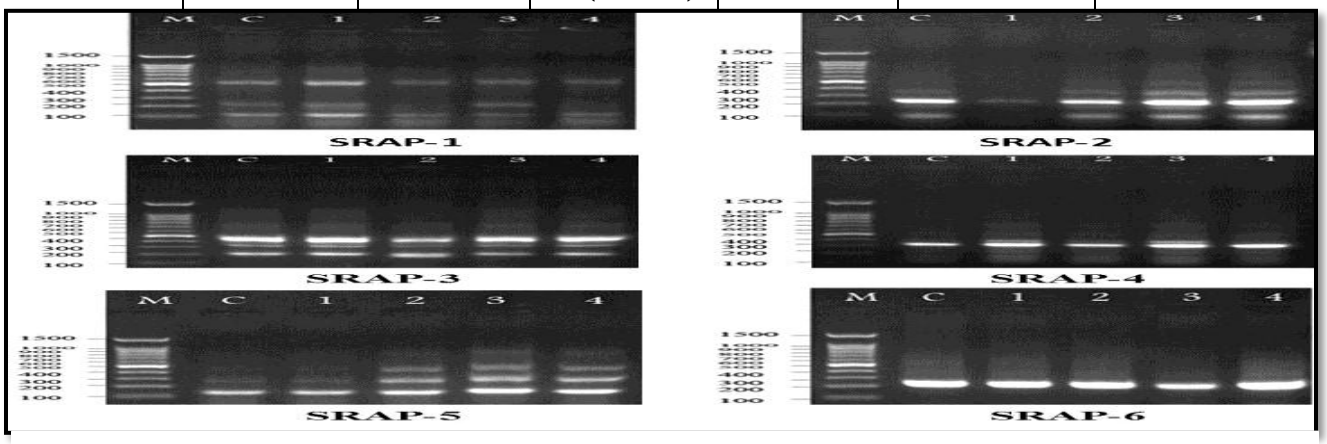

Fig. 4. SRAP-PCR analysis of *G. elegans* plants mutated with DES, using primers SRAP-1, SRAP-2, SRAP-3, SRAP-5, and SRAP-6. Lane M: 100 bp DNA ladder; lane C: The control plant; lane 1: 1000 ppm DES; lane 2: 2000 ppm DES; lane 3: 3000 ppm DES, and lane 4: 4000 ppm DES.

Cluster analysis

The genetic identity values among *Gypsophila* plants mutated by DES and the control ranged from 0.71 to 0.97 (Table 4). The lowest genetic similarity was between the individuals mutated with 1000 and 2000 ppm DES (0.71%), while the highest genetic identity was found between the population treated with 2000 and 4000 ppm DES (97%) (Table 4). A dendrogram indicated four different groups. The first group (I) involved 2000 and 4000 ppm DES mutants. The second group (II) included 3000 ppm DES mutants. The third group (III) contained only the control. The fourth group (IV) composed of individuals mutated with 1000 ppm DES (Fig. 5).

Table 4. Distance matrix depended on Jaccard similarity coefficients in *Gypsophila elegans* plants mutated by DES.

DES conc.	Control	1000 ppm DES	2000 ppm DES	3000 ppm DES	4000 ppm DES
Control	1.00				
1000 ppm DES	0.79	1.00			
2000 ppm DES	0.84	0.71	1.00		
3000 ppm DES	0.79	0.76	0.87	1.00	
4000 ppm DES	0.87	0.73	0.97	0.90	1.00

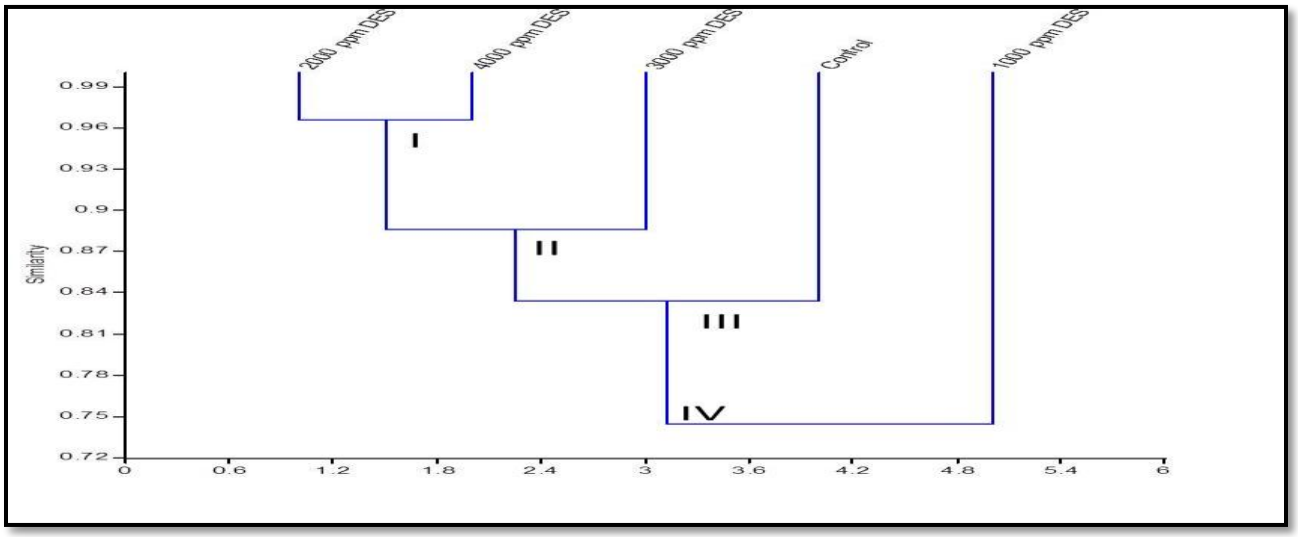

Fig (5) Dendrogram of *Gypsophila elegans* plants mutated by DES based on Jaccard's similarity coefficients, compared with the control.

CONCLUSION:

This research highlights the genetic improvement of *G. elegans* plant by DES. The results revealed that the low concentrations of 1000 and 2000 ppm had a significant increase in vegetative, flowering characters and caused early flowering in M_1 and M_2, compared to the control. In contrast, the highest concentration of 4000 ppm decreased these characters, and delayed the flowering phase). Also, it was observed that all DES concentrations induced many mutations in the shape and structure of leaves and induced color mutants and deformation of the flowers compared to control. On the other hand, SRAP markers are important tool to detected variation by DES detecting the mutagenic effects of DES. Also, it will help to discriminate between different genotypes showing mutations in morphological and floral characteristics.

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