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USING SODIUM AZIDE TO INDUCE MUTATIONS IN *DIMORPHOTHECA ECKLONIS* (L.) AND SRAP GENETIC MARKERS TO IDENTIFY THE GENETIC VARIANCE

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ABSTRACT: *Dimorphotheca ecklonis* (L.), commonly known as African daisy, is a half-hardy annual that produces large white blossoms. The flowers exhibit a striking contrast, being white with the disc florets presenting a deep blue or purple hue, a multifunctional plant that can be used in a variety of landscape designs. It is frequently utilized as a bedding plant, adding vibrant color to flower beds and borders. Additionally, it thrives in rock gardens and containers, allowing for flexible placement options. Its ability to withstand drought conditions makes it an excellent choice for low-water gardens, thus supporting sustainable landscaping practices. In the Nursery of Floricultural and Ornamental Plants, Faculty of Agriculture, Alexandria University, Egypt, the current investigation was conducted over the course of two consecutive seasons, 2022/2023 and 2023/2024. The seedlings (shoot tip) of *Dimorphotheca ecklonis* (L.) were sprayed with sodium azide at the levels of 1500, 3000, 4500, 6000 ppm and control (distilled water) to evaluate its effect on the vegetative, flowering growth and phytochemical composition, induction of mutation and genetic variability on *Dimorphotheca ecklonis* (L.) plants. Also, SRAP markers were used to evaluate the induced mutants. The levels of 6000 ppm considerably enhanced the plant height and dry weight of vegetative growth in M_2-generation, number of branches and leaves in both generations. The concentration of 6000 ppm increased significantly the leaf area in both generations. The concentrations of 6000 and 3000 ppm in the $M_1$and M_2-generations, respectively, are the latest flowering. The level of 3000 ppm in both generations substantially elevated the levels of anthocyanin and beta-carotene. Some variations in the habit of growth, leaf form and inflorescence structure were observed in both generations. SRAP marker-PCR technique was able to detect mutations in *Dimorphotheca ecklonis* (L.) plants.

Keywords: *Dimorphotheca ecklonis*, anthocyanin, phenols, beta carotene, ISSR marker

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INTRODUCTION

The Dimorphotheca genus is mainly located in Africa and Australia and belongs to the important Asteraceae family (Bailey and Bailey, 1976). *Dimorphotheca ecklonis* (L.) DC., commonly known as Cape marigold, African daisy, or star of the veldt, is a widely recognized ornamental plant grown globally. This plant is a half-hardy annual that produces large, brightly colored blossoms atop long, slender stems. Cape marigolds form a loose mound that is densely covered with flowers

during cooler seasons or in regions with cooler climates (Abdel-Wahid *et al.*, 2005).

The glandular bracts are notable, measuring between 13 to 16 millimeters in length. The flowers exhibit a striking contrast, being white on the upper side and reddish-blue on the lower side, with the disc florets presenting a deep blue or purple hue. The fruit features a distinct wrinkled surface (Munz, 1968).

Dimorphotheca ecklonis (L.) is a multifunctional plant that can be used in a variety of landscape designs. It is frequently utilized as a bedding plant, adding vibrant color to flower beds and borders. Additionally, it thrives in rock gardens and containers, allowing for flexible placement options. Its ability to withstand drought conditions makes it an excellent choice for low-water gardens, thus supporting sustainable landscaping practices.

A mutation is an alteration that can occur randomly, be inherited, and be brought on by exposure to mutagenic agents or natural processes (Öztürk *et al.,* 2020). The utilization of *in vitro* and *in vivo* methodologies in conjunction with mutation breeding research enables the production of a diverse array of mutants at extremely high frequencies (Türkoğlu *et al.,* 2023). The type and variety of the selected mutations, along with the specific region of the plant, application dosage, and duration, all influence the efficacy of the mutation (Kim *et al.,* 2020). To increase the frequency of mutations, various breeding research projects have used physical or chemical mutagens (Hewawasam *et al.,* 2004).

Chemical mutagenesis represents an established technique for improving yield and quality characteristics in plants. Within this field, alkylating agents are acknowledged as particularly potent mutagens in higher plant species. Additionally, sodium azide (NaN_3) has demonstrated efficacy as a chemical mutagen capable of inducing genetic variation. Consequently, NaN_3 serves as a valuable tool for enhancing agronomic traits in various crop species. The significant contribution of mutation breeding towards increasing genetic variability for quantitative traits across diverse crop plants is well-documented in the scientific literature (Srivastava *et al.,* 2011).

Chemical mutagens result in base pair substitutions, most often from GC to AT, which change the amino acid composition and affect protein function without completely removing it, unlike deletions or frame shift mutations (Ikhajiagbe and Omoregie, 2020). Sodium azide stands out as one of the most powerful chemical mutagens present in cultivated plants. The method of inducing mutations is significantly influenced by the mutagen's concentration as well as the duration of application (Khan *et al.,* 2009). Every amount of mutagen concentration and exposure duration will result in an unusually large number of mutations compared to what would be predicted under typical conditions. However, negative consequences, such as increased seedling mortality and harm, may occur if the concentration is elevated to an extremely high level and the application period is extended (Jenks *et al.,* 2007).

Sodium azide (SA) is recognized as a potent chemical mutagen, regarded as one of the most effective mutagens in the realm of plant biology. It is simple, affordable, and produces mutations in plants to enhance their characteristics. Numerous factors, including pH, soaking time in water, temperature, azide concentration, and treatment length, affect how efficiently mutants are produced. It induces point mutations and causes chromosomal damage, thereby enhancing the plants' tolerance to various adverse conditions (Al-Qurainy and Khan, 2009). NaN_3 serves as a significant tool for improving agronomic traits in crop plants under various biotic and abiotic stresses (Türkoğlu *et al.,* 2023). Sodium azide, an ionic compound, exerts its mutagenic effects through an organic azide metabolite, analogous to L-azidoalanine. This metabolite is synthesized by the enzyme O-acetylserine (thiol)-lyase (formerly O-acetylserine sulfhydrylase) (Gruszka *et al.,*

2012; Viana *et al.,* 2019). The mechanism involves the entry of this azide molecule into the cell nucleus, where it induces point mutations within the genomic DNA, primarily characterized by G/C to A/T transitions (Mistry *et al.,* 2022).

Sequence-related amplified polymorphism (SRAP) is regarded as a straightforward and effective method, offering greater throughput and reproducibility compared to RAPDs, while also being simpler to execute than AFLPs. The SRAP method is capable of detecting numerous co-dominant loci and effectively capturing open reading frames (ORFs) (Li and Quiros, 2001).

This study aims to investigate the effects of sodium azide on vegetative and flowering growth in *Dimorphotheca ecklonis*, as well as its phytochemical composition and potential for mutation induction.

MATERIALS AND METHODS

The current study was conducted over two consecutive generations during the experimental seasons of 2022/2023 and 2023/2024, at the Nursery of Floricultural and Ornamental Plants, Fac. Agric., Alexandria Univ., Egypt.

Seeds of *Dimorphotheca ecklonis* (L.) were sown on the 15th of September 2022 in 30 cm clay pots filled with a mixture of sand and peat moss (1:1 v/v). Sodium azide was used as a spray on the shoot tips of the plants four-leaf formation. A hand sprayer was used to apply all the rates, and a wetting agent tween-20 was added to each test solution. Each plant was sprayed individually until the solution reached the point of run-off. Fresh solutions of sodium azide were prepared at concentrations of 1500, 3000, 4500 and 6000 ppm, and the control (distilled water).

For growing the M_2-generation, seeds were gathered from each treatment, and then sown on the 15th of September 2023. The processes of seeding and transplanting were conducted similarly to the initial generation.

The experimental design utilized a completely randomized design (CRD), comprising five treatments with three replicates (Gomez and Gomez, 1984). Each treatment consisted of ten plants.

Data recorded:

The following data were measured in both generations (M_1 and M_2).

Vegetative growth:

- Survival percentage for M_1-generation: The percentage of the survived plants was measured for each treatment in each replicate as the percentage of the survived plants which continued around the experiment relative to the number of transplanted seedlings according to the following formula:

$$\text{Survival \%} = \frac{\text{Number of survived plants}}{\text{Number of transplanted seedlings}} \times 100$$

- Seed germination percentage for M_2-generation: The following formula was used to compute the germination percentage of each treatment 30 days after seeding:

$$\text{Seed germination \%} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 100$$

- Plant height was measured from the soil surface in the pot to the highest point of the plant at the end of M_1 and M_2-generation experiments.
- Stem diameter (cm) was measured at the soil surface in the pots at the end of the flowering.
- Number of branches/plant was counted at the end of the first and second generations of experiments.
- Number of leaves/plant was counted at the end of the first and second generations of experiments.
- Dry weight of vegetative growth (g): Plants of each treatment per replicate were subjected to drying in an oven at 70 °C for 72 hours until a constant weight was achieved.

- Leaf area (cm^2) was determined after Koller (1972) by weighing two mature leaf blades taken from the 4th nodes of two plants at each treatment. Two squares with known areas taken from the leaf blades were weighed. The leaf area was calculated using the following equation, then the average was calculated for one leaf:

$$\text{Leaf area } (cm^2) = \frac{\text{Leaf weights} \times \text{square areas}}{\text{Square weights}}$$

Flowering characteristics:

- Flowering date (days) was expressed as the number of days between planting and the appearance of the first blossom on the plant.
- Number of flowers per plant.
- Fresh weight of 10 flowers (g) was recorded for each treatment in each replicate.
- Dry weight of 10 flowers (g): Flowers of each treatment per replicate were subjected to drying in an oven at 70 °C for 72 hours until a constant weight was achieved and then weighted in grams.
- Flower diameter (cm) was measured at the full opening stage for 10 flowers per treatment.
- Flower length (cm) was measured at the full opening stage for 10 flowers per treatment.
- Number of petals was expressed as the number of disk flowers per plant.
- Seeds weight (g) of 100 seeds was recorded for each treatment in each replicate.
- Flowering period was calculated as the number of blooming days between the first and the last inflorescence on each replicate in each treatment.

Chemical analysis:

Total leaf chlorophyll contents a and b (mg/g fresh weight) were determined according to Moran (1982), total leaf carotenoid content (mg/g) was determined according to Torres *et al.* (2014), total leaf soluble carbohydrates content (%) was determined according to Hedge and Hofreiter (1962). Anthocyanin content (ml/100 g) determination in dried flowers was done according to Fuleki and Francis (1968). The amount of beta-carotene (mg/g) in the dried flower was measured at the time of collecting using the technique outlined in the AOAC (1970). Phenols content (mg/g) in the leaves was determined according to AOAC (2000).

Abnormal characters:

All plants from both generations of treatments were checked and documented for abnormalities, and alterations in vegetative or blooming growth. These changes included:

- Habit of growth.
- Abnormal leaves (color and form).
- Abnormal inflorescences (color and form).
- Sequence-related amplified polymorphism (SRAP) fingerprinting technique (Khatab and Hegazi, 2015), was utilized as a molecular fingerprinting technique via five specific primers which were designed to assess the mutagenesis influence on *Dimorphotheca* samples. The primer code, combination and sequences of forward and reverse primers were illustrated as shown in Table (1).

Table 1. Illustrate the primer features of sequence-related amplified polymorphism (SRAP).

Primers code	Primers combination	Sequences of forward and revers primer
A	me1 + me5	TGAGTCCAAACCGGATA+ TGAGTCCAAACCGGAAG
B	me1 + em2	TGAGTCCAAACCGGATA+ GACTGCGTACGAATTTGC
C	me2 + em2	TGAGTCCAAACCGGAGC+ GACTGCGTACGAATTTGC
D	me2 + me5	TGAGTCCAAACCGGAGC+ TGAGTCCAAACCGGAAG
E	me1 + me3	TGAGTCCAAACCGGATA+ TGAGTCCAAACCGGAAT

Statistical analysis:

Data were statistically analyzed by analysis of variance (ANOVA) (Steel *et al*., 1997) using SAS software Ver. 9.2 (SAS Institute Inc., 1985, Cary, North Carolina, USA). Means for the different sources of variation were applied by the least significance difference (LSD) test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Survival percentage:

The result of the survival percentage of the M_1-generation of *Dimorphotheca ecklonis* (L.) as affected by SA is shown in Fig. (1A). The survival percentage of seedlings subjected to sodium azide (SA) treatment was quantified following a one-month post-treatment period.

All concentrations of sodium azide significantly increased the survival percentage (100%) compared to the control (79.47%), but did not differ significantly between them. No significant effects were observed for the SA treatments in the M_2-generation. In both generations, there were no discernible changes in the various treatments.

These findings are consistent with previous reports by Al-Halawany (1992) in

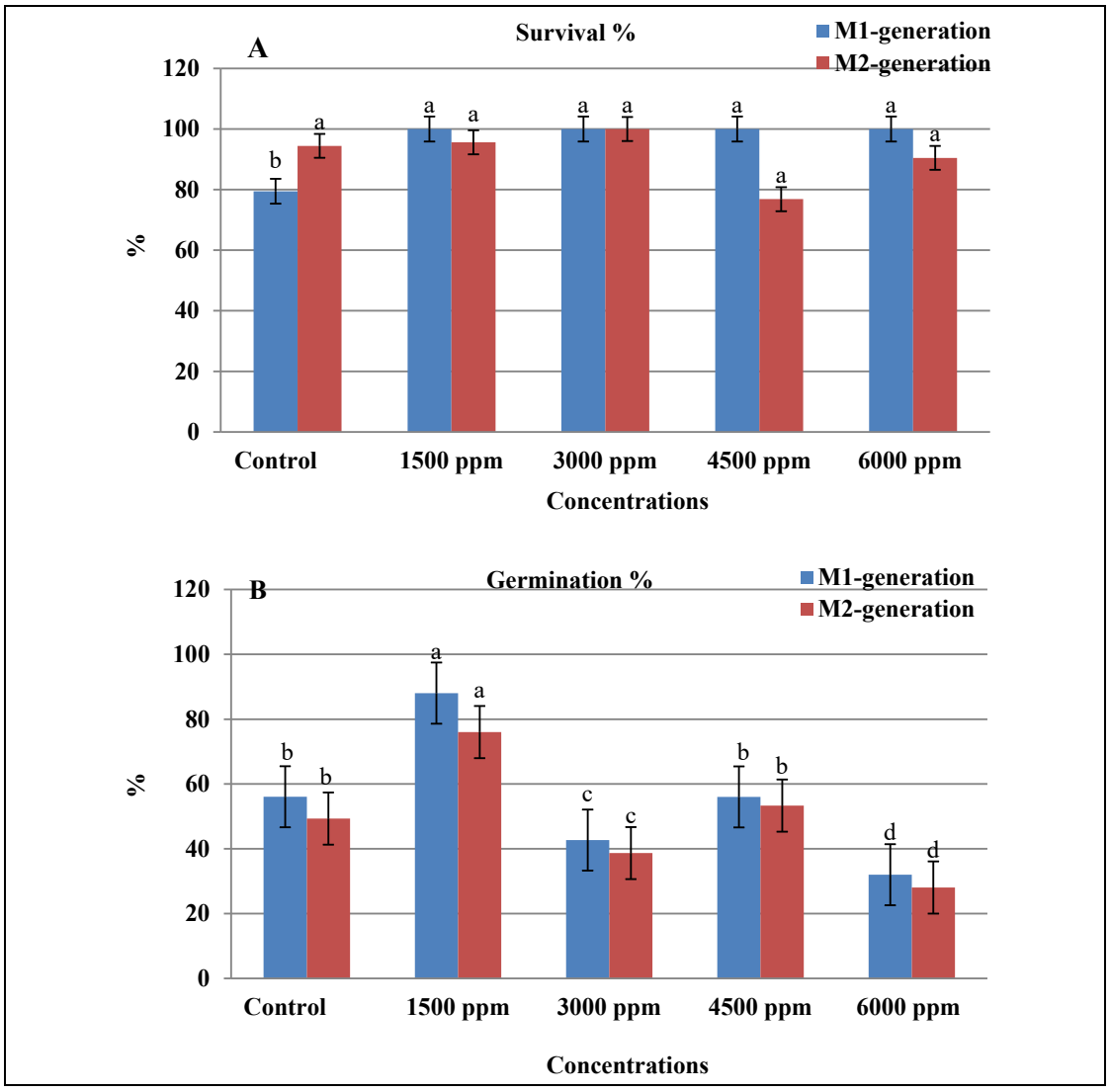

Fig. 1. Survival percentage (A) and germination (B) of *Dimorphotheca ecklonis* (L.) as affected by sodium azide at the M_1 and M_2-generations.

Catharanthus roseus and by Jakhar and Ramkrishna (2004) in *Eruca sativa*. Furthermore, Mani (1989) observed that the plant survival exhibited a dose-independent response to EMS.

Seed germination percentage:

Fig. (1B) displays the seed germination percentages for both generations. The concentration of 4500 ppm did not differ substantially from the control, although all values did differ considerably.

The low concentration of SA (1500 ppm) gave the highest percentages (88.03% and 76.00% for first and second generations, respectively) and differed significantly from the control (56.05% and 49.32%) for first and second generations, respectively. The highest dosage (6000 ppm) decreased significantly seed germination percentages to 32.01% and 28.03% for first and second generations, respectively.

The high seed germination obtained in the second generation may be related to the stimulation effect and may be attributed to environmental factors, including seed moisture content and mutagen chemical concentrations (Hussein *et al.*, 1974; Abd El-Maksoud and El-Mahrouk, 1992). The results were analogous to those documented by El-Nashar (2006) about *Amaranthus.*

The stimulatory effects observed on seed germination following exposure to low and intermediate doses of diethyl sulfate (DES) could potentially be attributed to enzymatic activation and the induction of meristematic cell division. Conversely, a proposed physiological effect of sodium azide, particularly at elevated concentrations, involves the inhibition of the synthesis for enzymes critical to the germination process.

Plant height:

The results of the plant height of the M_1-generation as affected by sodium azide are listed in Table (2), which shows that the impact of the different treatments on the plant height did not reach statistical significance in the M_1-generation. On the other hand, the M_2-generation showed significantly different results between treatments; the highest plants (19.53 cm) were recorded in the level of 6000 ppm relative to the control (14.13 cm), but no significant differences between other treatments.

Table (2) showed no clear trend for the different SA treatments, which was also reported by many other workers. i.e Potdukhe (2004) in *Cajanus cajan* and El-Nashar (2006) in *Amaranthus caudatus* and *A. hypochondriacus*.

Stem diameter:

Table (2) demonstrated that the various SA interventions did not have any significant effects on the diameter of the stem in either generation.

This result was confirmed with El-Khateeb *et al.* (2022) on *Borago officinalis*.All these results may be due to the simulative effect of the mentioned treatments or concentrations on the plant height and stem diameter, which could be related to the physiological activation of plant metabolism as a result of DES application El-Torky (1992); Abd El-Maksoud and El-Mahrouk (1992 and 1993).

The observed reductions in plant height and stem diameter may be attributed to physiological damage induced by Diethyl sulfate (DES) or sodium azide (SA), as well as their respective hydrolysis products. This interpretation aligns with the findings reported by Hussein *et al.* (1974), Abd El-Maksoud and El-Mahrouk (1992 and 1993), El-Torky (1992), and El-Nashar (2006).

Number of branches per plant:

Treatment with all tested concentrations of sodium azide resulted in an increased number of branches per plant in both the M_1 and M_2-generations, as detailed in Table (2). The maximum observed values were associated with the 6000 ppm treatment group, giving means of 25.33 branches/plant in the M_1-generation and 30.73 branches in the M_2-generation. These values represent a notable increase compared to the control,

Table 2. Mean values of the plant height, stem diameter, number of branches/plant, number of leaves/plant, dry weight of vegetative growth (g) and leaf area (cm^2) of *Dimorphotheca ecklonis* (L.) as affected by sodium azide (SA) treatments in the M_1 and M_2-generations.

SA ppm	Plant height (cm)		Stem diameter (cm)		Number of branches/plant		Number of leaves/plant		Dry weight of vegetative growth (g)		Leaf area (cm^2)	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Control	16.56^{a}	14.13^{b}	0.473^{a}	0.722^{a}	17.66^{b}	19.36^{c}	73.36^{c}	117.33^{c}	4.41^{a}	5.21^{b}	684.37^{b}	565.62^{ab}
1500	18.70^{a}	15.76^{ab}	0.500^{a}	0.763^{a}	18.33^{b}	23.66^{bc}	128.01^{bc}	117.33^{c}	4.23^{a}	5.26^{b}	1015.90^{ab}	463.01^{ab}
3000	19.02^{a}	18.96^{ab}	0.543^{a}	0.663^{a}	19.66^{ab}	21.0^{c}	148.05^{ab}	189.29^{ab}	5.70^{a}	6.02^{b}	1621.73^{a}	877.77^{a}
4500	16.83^{a}	17.53^{ab}	0.636^{a}	0.909^{a}	24.00^{ab}	27.70^{ab}	143.33^{b}	134.62^{bc}	5.63^{a}	8.93^{ab}	579.77^{b}	811.89^{a}
6000	17.04^{a}	19.53^{a}	0.623^{a}	0.948^{a}	25.33^{a}	30.73^{a}	206.05^{a}	223.30^{a}	7.53^{a}	12.46^{a}	454.40^{b}	386.59^{b}

***Values in each column followed by the different letter (s) are significantly different at $p \leq 0.05$. Least Significant Difference**

which exhibited means of 17.66 and 19.36 branches /plant in the M_1 and M_2-generations, respectively.

The present results agree with the results of El-Khateeb *et al.* (2022) in *Borago officinalis*.

Number of leaves per plant:

As indicated in Table (2), the treatment of 6000 ppm produced the greatest number of leaves per plant in the M_1 and M_2-generations (206.05 and 223.30, respectively) when compared to the control (73.36 and 117.33, respectively).

There were also noticeable and noteworthy impacts on the branches and leaves of each plant. The results align with the findings presented by El-Nashar (2006) on Amaranthus. The physiological effects of SA and its hydrolysis products may indeed elucidate the observed increase in the branches and leaves in each plant.

Dry weight:

Table (2) demonstrated that the dried weight of vegetative growth in the M_1-generation was not significantly affected by the various SA treatments. The treatment of 6000 ppm produced the highest dry weight of vegetative development (12.46 g) in the M_2-generation when compared to the control (5.21 g), and there were notable variations between the various treatments.

It was comparable to the results of Abd El-Maksoud and El-Mahrouk (1992) on *Asparagus densiflorus*, where the dry weight increased proportionally and decreased proportionally with increasing DES concentrations. The environmental factors that predominate during the growth period of the plants, such as temperatures and/or nutrition, could be the cause of the variation in responses at different doses.

Leaf area:

In the M_1-generation, treatments of 3000 ppm SA gave the largest average of total leaf area per plant (1621.73 cm^2) compared to the control (684.37 cm^2), as shown in Table (2) with significant differences. While the treatment of 6000 ppm recorded the lowest average of total leaf area (454.40 cm^2), with no discernible variations between the various treatments and the control. In the M_2-generation, the treatment of 3000 ppm significantly expanded the leaf area (877.77 cm^2) compared to the control (565.62 cm^2), with no significant difference. While the treatment of 6000 ppm SA decreased the average of total leaf area per plant (386.59 cm^2) with no significant difference compared to the control.

Generally, the low concentrations of SA increased the leaf area. The findings align with those presented by El-Nashar (2006) on *Amaranthus*. Increases and reductions in the leaf area of the mutagen-treated plants might be ascribed to the impact of chemical mutagens on the cell number and/or cell length, which can alter the leaf characters of the plants following the chemical treatment. Large leaf area means an increase in cell number, and small leaf area means a decrease in cell number and size El-Nashar (2006).

Flowering date:

The SA at 6000 ppm concentration generated the flowering after (91 day) in the M_1- generation and differed significantly from the control and SA at 1500 ppm (70.00 and 70.66 days, respectively), as shown in Table (3) while the other treatments weren't much different from the control. The treatment of 3000 ppm induced flowering (163.65 day) in the M_2-generation compared to the control (131.68 day) with a significant difference. While the other treatments weren't much different from the control.

The delaying effect on flowering of some SA treatments during the M_1 and M_2-generations was in harmony with the results stated by Hussein *et al.* (1974) and El-Nashar (2006). Conversely, findings reported by Badr *et al*. (2000) and El-Nashar (2006) indicated that elevated concentrations of the applied agent of mutagens appeared to inhibit cell growth, decrease the overall growth rate, and delay the onset of flowering.

Table 3. Mean values of the flowering date, number of flowers, fresh weight and dry of 10 flower (g), flower diameter, flower length, number of petals, seeds weight (100 seeds) and flowering period of *Dimorphotheca ecklonis* (L.) as affected by Sodium azide (SA) treatments in the M_1 and M_2-generations.

SA ppm	Flowering date (days)		Number of Flowers		Fresh weight of 10 flowers (g)		Dry weight of 10 flowers (g)		Flower diameter (cm)		Flower length (cm)		Number of Petals		Seeds weight (100 seeds)		Flowering period (days)	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Control	70.00^{b}	131.68^{b}	4.36^{b}	8.70^{b}	8.41^{b}	5.66^{c}	1.43^{b}	0.97^{b}	6.11^{a}	5.68^{b}	5.16^{a}	8.68^{a}	15.68^{a}	14.66^{a}	1.38^{c}	1.75^{a}	137^{a}	135.33^{a}
1500	70.66^{b}	147.31^{ab}	5.06^{b}	13.33^{b}	7.42^{bc}	7.41^{bc}	1.16^{b}	1.26^{ab}	5.44^{ab}	6.12^{ab}	3.67^{a}	7.06^{a}	17.63^{a}	15.03^{a}	1.57^{a}	1.66^{ab}	135^{a}	119.66^{ab}
3000	77.00^{ab}	163.65^{a}	5.73^{ab}	13.06^{b}	9.60^{a}	7.07^{bc}	2.01^{a}	1.15^{b}	5.60^{ab}	6.44^{a}	5.17^{a}	6.84^{a}	17.06^{a}	15.69^{a}	1.51^{b}	1.45^{b}	130^{a}	104.30^{b}
4500	78.33^{ab}	140.33^{ab}	7.o6^{a}	23.33^{a}	8.35^{b}	8.41^{ab}	1.39^{b}	1.28^{ab}	5.08^{b}	6.10^{ab}	4.85^{a}	7.11^{a}	15.60^{a}	15.02^{a}	1.58^{a}	1.68^{ab}	130^{a}	127.32^{ab}
6000	91.00^{a}	159.02^{ab}	5.13^{b}	19.06^{a}	6.56^{c}	10.12^{a}	1.09^{b}	1.93^{a}	5.27^{b}	6.32^{ab}	4.10^{a}	8.67^{a}	16.37^{a}	16.63^{a}	1.59^{a}	1.53^{ab}	114^{b}	111.01^{ab}

***Values in each column followed by the different letter (s) are significantly different at $p \leq 0.05$. Least Significant Difference**

Number of flowers:

The number of flowers in both generations was enhanced by all concentrations of sodium azide, as demonstrated in Table (3). The treatment with 4500 ppm registered the highest value (7.06 and 23.33 in the M_1 and M_2-generations, respectively) compared to the control (4.36 and 8.70 for M_1 and M_2-generations).

Fresh weight of 10 flowers:

Table (3) displays the fresh weight's mean values of 10 flowers of the various SA treatments. In the M_1-generation, the largest fresh weight of 10 flowers (9.60 g) was recorded at the treatment of 3000 ppm, while the smallest one (6.56 g) was for the treatment of 6000 ppm, compared to the control (8.41 g), with significant differences. In the M_2-generation, the largest fresh weight of 10 flowers (10.12 g) was recorded at a concentration of 6000 ppm, relative to the control (5.66 g), with a significant difference and also the smallest amount.

These findings contradicted those indicated by Al-Saheal and Gamil (1982); and El-Nashar (2006).

Dry weight of 10 flowers:

The ten-flower dry weight mean values as affected by SA treatment are shown in Table (3). In the M_1-generation, the largest dry weight (2.01 g) was recorded at the concentration of 3000 ppm, relative to the control (1.43 g), with a significant difference. While the smallest one (1.09 g) was found at the treatment of 6000 ppm.

In the M_2-generation, the largest dry weight of 10 flowers (1.93 g) was recorded at 6000 ppm, relative to the control (0.97 g), with a significant difference and also the smallest one.

These findings contradicted those presented by Al-Saheal and Gamil (1982) and El-Nashar (2006).

Flower diameter:

As shown in Table (3), flower diameter was reduced employing all sodium azide doses in the M_1-generation. Treatment with 4500 ppm resulted in the lowest observed mean flower diameter (5.08cm).

On the other hand, the M_2-generation showed significant differences with the highest average of flower diameter, which was noticed at the treatment of 3000 ppm (6.44 cm) and the control (5.68 cm). Kannan *et al.* (2002) found that the higher rates of EMS reduced the flower diameter in *Jasminum sambac*.

While the opposite occurred in the M_2-generation, flower diameter was raised by employing all tested concentrations of sodium azide, the largest flower diameter (6.44 cm) was recorded at 3000 ppm, relative to the control (5.68 cm), with a significant difference.

Stimulation and reduction in flower diameter and length might be ascribed to the impact of the chemical mutagens on the size of the petals. Alterations in both cell number and cell length may occur within the petal tissues of treated plants. Larger flowers had larger petals with raise in the number of cells and/or cell size. Small flowers had smaller petals with a decrease in cell number and/or cell size (Abd El-Maksoud, 1988 and El-Nashar, 2006).

Flower length:

Table (3) demonstrated that there were no discernible impacts on flower length in both generations from the various SA treatments.

Number of petals:

Table (3) demonstrated that the number of petals in both generations did not exhibit any significant effects for the various SA treatments.

Seeds weight (100 seeds):

Within the M_1-generation, treatments involving sodium azide (SA) concentrations of 1500, 4500, and 6000 ppm resulted in significantly higher 100-seed weights (1.57 g, 1.58 g, and 1.59 g; respectively) compared to the control group (1.38 g).

In the M_2-generation, the weight of seeds (100 seeds) was diminished across all concentrations of sodium azide, as indicated in Table (3).

Flowering period:

In the M_1-generation, the lowest flowering period (114 days) was recorded at treatments of 6000 ppm and differed significantly from the control (137 days) and other treatments (Table, 3). Conversely, within the M_2-generation, the 3000 ppm treatment exhibited the shortest flowering period (104.30 days), which was significantly different from the control (135.33 days). No significant differences from the control were observed for the other treatments regarding this parameter.

These observations are consistent with the findings reported by El-Nashar (2006) concerning the *Amaranthus* plant.

Chlorophyll a (mg/g):

The content of chlorophyll a was diminished across all levels of sodium azide in the M_1-generation. Plants treated with 1500 and 4500 ppm gave the lowest chlorophyll a content with significant differences to the control (Fig., 2A). In the M_2-generation, plants treated with 1500 ppm SA gave the highest chlorophyll a content with no significant difference to the control. While the lowest chlorophyll a content was recorded in plants subjected to 4500 ppm, with a significant difference from the control.

Comparable findings were documented by El-Nashar and Shetta (2015) on *Leucaena leucocephala* and El-Khateeb *et al.* (2022a) on *Borago officinalis*.

Chlorophyll b (mg/g):

Fig. (2A) demonstrated that there were no discernible differences between the various SA treatments in terms of the chlorophyll b content in M_1-generation.

Within the M_2-generation, chlorophyll b content exhibited a significant decrease across all tested concentrations of sodium azide. Specifically, plants subjected to the 6000 ppm treatment displayed the lowest chlorophyll b levels, which were significantly different, compared to the control plants. El-Khateeb *et al.* (2022) reported comparable findings regarding *Borago officinalis*.

Total carotenoid content (mg/g):

Using sodium azide at all concentrations in M_1-generation reduced the total carotenoid content. Plants treated with 4500 ppm gave the lowest content with a significant difference from the control.

Fig. (2B) illustrates the relevant data in the M_2-generation; plants treated with 1500 ppm exhibited the highest total carotenoid content, showing no significant difference relative to the control. While the lowest carotenoid content was scored in plants subjected to 3000 ppm SA, with significant difference to the control.

Comparable findings were documented by El-Nashar and Shetta (2015) in *Leucaena leucocephala* and El-Khateeb *et al.* (2022) in *Borago officinalis.*

The occurrence of chlorophyll mutations is attributed to deficiencies in the biosynthesis or accumulation of chlorophylls, carotenoids, or both, often linked to alterations in plastid genes, manifesting phenotypically as variegation (Kirk and Tilney-Bassett, 1978). Comparable detrimental effects associated with mutagen treatment have been documented in *Salvia splendens* (Hussein *et al.,* 1974) and *Amaranthus* species (El-Nashar, 2006). The induction of chlorophyll mutants by DES, as reported in previous studies, may potentially be explained by mechanisms involving altered chloroplast differentiation or other factors previously elucidated.

Total carbohydrates content:

The highest amount of total carbohydrate content was scored at the treatment of 3000 ppm SA, with a significant difference to the control in M_1-generation (Fig., 2C).

Within the M_2-generation, the highest total carbohydrate content was measured in plants treated with 4500 ppm, showing a

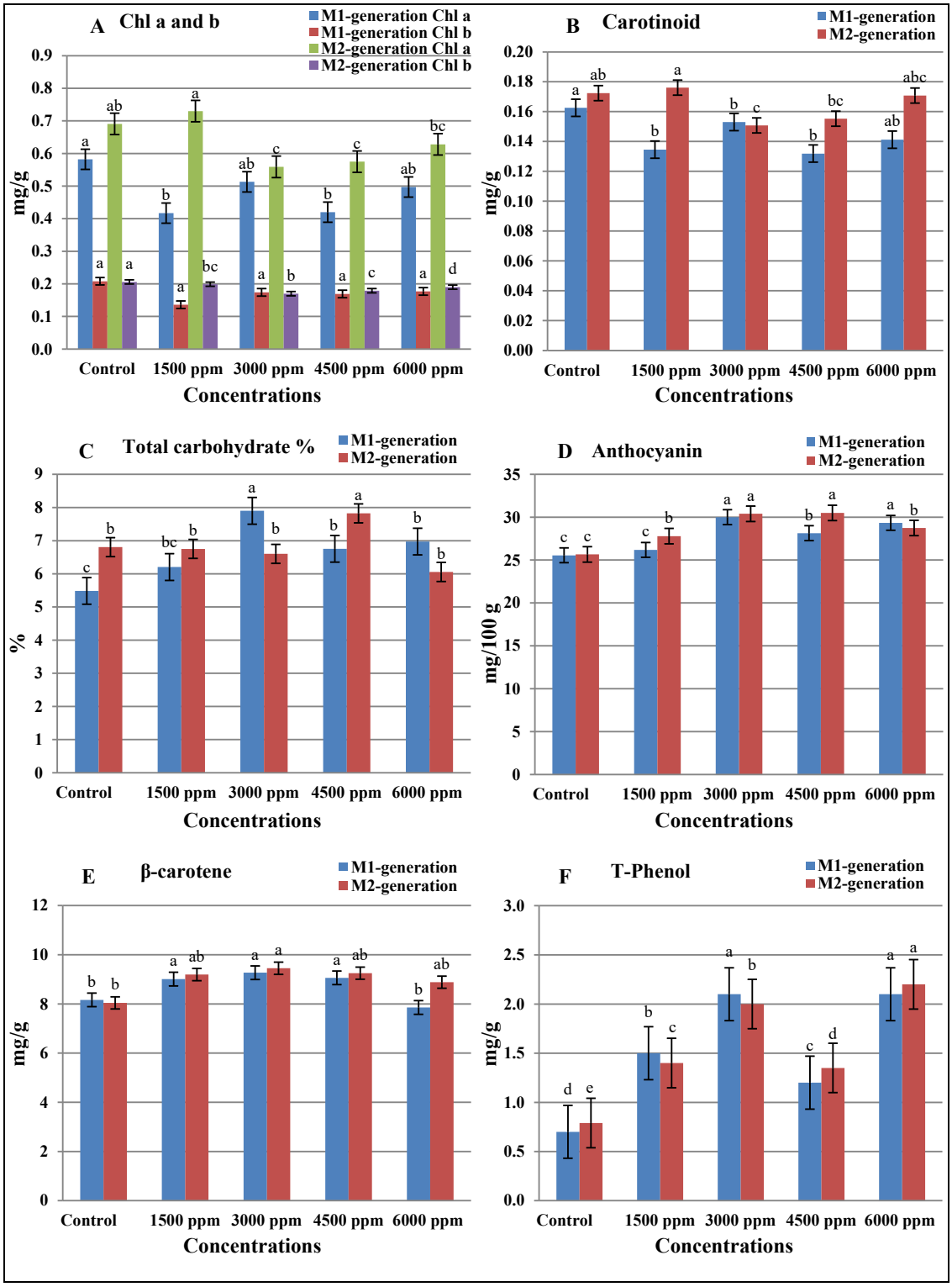

Fig. 2. Chlorophyll a, b (A), carotenoids (B), total carbohydrate % (C), anthocyanin (D), β-carotene (E) and T-phenol (F) of *Dimorphotheca ecklonis* (L.) as affected by sodium azide at the M_1 and M_{2-} generations.

significant difference relative to the control. In contrast, the lowest total carbohydrate content was observed following treatment with 6000 ppm, although this value was not significantly different from the control. Comparable outcomes were reported by El-Khateeb *et al.* (2022) in *Borago officinalis*.

Anthocyanin in the flowers:

In the M_1-generation, the highest level of anthocyanin in the flowers was recorded by 3000 ppm with a notable variation from the control (Fig., 2D).

Within the M_2-generation, the highest anthocyanin level in the flowers was recorded for the treatments of 3000 and 4500 ppm with notable variations from the control.

β-carotene in the flowers:

The concentrations of 1500, 3000 and 4500 ppm gave higher contents. In the M_1-generation, the highest β-carotene content in the flowers realized by 3000 ppm, with notable variations from the control in Fig. (2E).

In the M_2-generation, the highest concentration of β-carotene in the flowers was recorded for the treatment of 3000 ppm, with notable variations from the control.

Total phenols in the leaves,

All concentrations of sodium azide improved leaves total phenol in both generations, as shown in Fig. (2F) and the highest observed value was recorded in plants subjected to 3000 ppm SA in the M_1-generation and 6000 ppm in the M_2-generation.

Effect of sodium azide (SA) on the induction of variations (Abnormal characters):

Habit of growth:

Certain treatments changed the way some plants grew, giving them different shapes. Fig. (3) showing the changes in the growth habit of *Dimorphotheca ecklonis* (L.), from normal to conical form as a result of the treatment of 1500 ppm of sodium azide in the M_1-generation (A), heavy branches (middle) and compact and heavy branches (right) growth as a result of the treatments of 3000 and 6000 ppm, respectively in the M_1-generation (B), heavy branches growth as a result of the treatment of 1500 ppm in the M_2-generation (C), greater (middle) and snake (right) growth as a result of the treatment of 6000 ppm in the M_2-generation (D) and giant plant growth as a result of the treatment of 3000 ppm in the M_2-generation (E). As shown in Fig. (4), there were different variations in flowers which varied from normal to big flower growth as a result of the treatments of 3000, 4500 and 6000 ppm in the M_1-generation.

Abnormal leaves (color and form):

The treatments of 1500, 3000, 4500 and 6000 ppm SA caused changes in the leaf relative to the control in the M_1-generation (Fig., 5). These results align with previous findings reported by Al-Halawany (1992) concerning *Catharanthus roseus*, as well as those documented by El-Nashar (2006) for *Amaranthus caudatus* and *A. hypochondriacus*. The chromosomal disturbances that may have caused the changes in leaf morphology or shape observed in the M1 and M2 plants. Additionally, such alterations may potentially be ascribed to the rearrangement of tissue layers resulting from the action of chemical mutagens. (Abd El-Maksoud, 1988, and El-Nashar, 2006).

Abnormal inflorescences (color and form):

As shown in Fig. (6), flowers with deformed petals (A, B, C and D) in the M_1-generation, (E) in the M_2-generation of *Dimorphotheca ecklonis* (L.), were observed as a result of the treatment of 1500, 3000, 4500 and 6000 ppm SA compared with the control. These findings align with those documented by El-Nashar (2006) on *Amaranthus,* and Mostafa, 2009 on *Balanites aegyptiaca*. Aberrations in inflorescence shape could be ascribed to the impact of low and high concentrations of chemical mutagens on the cell number and cell length.

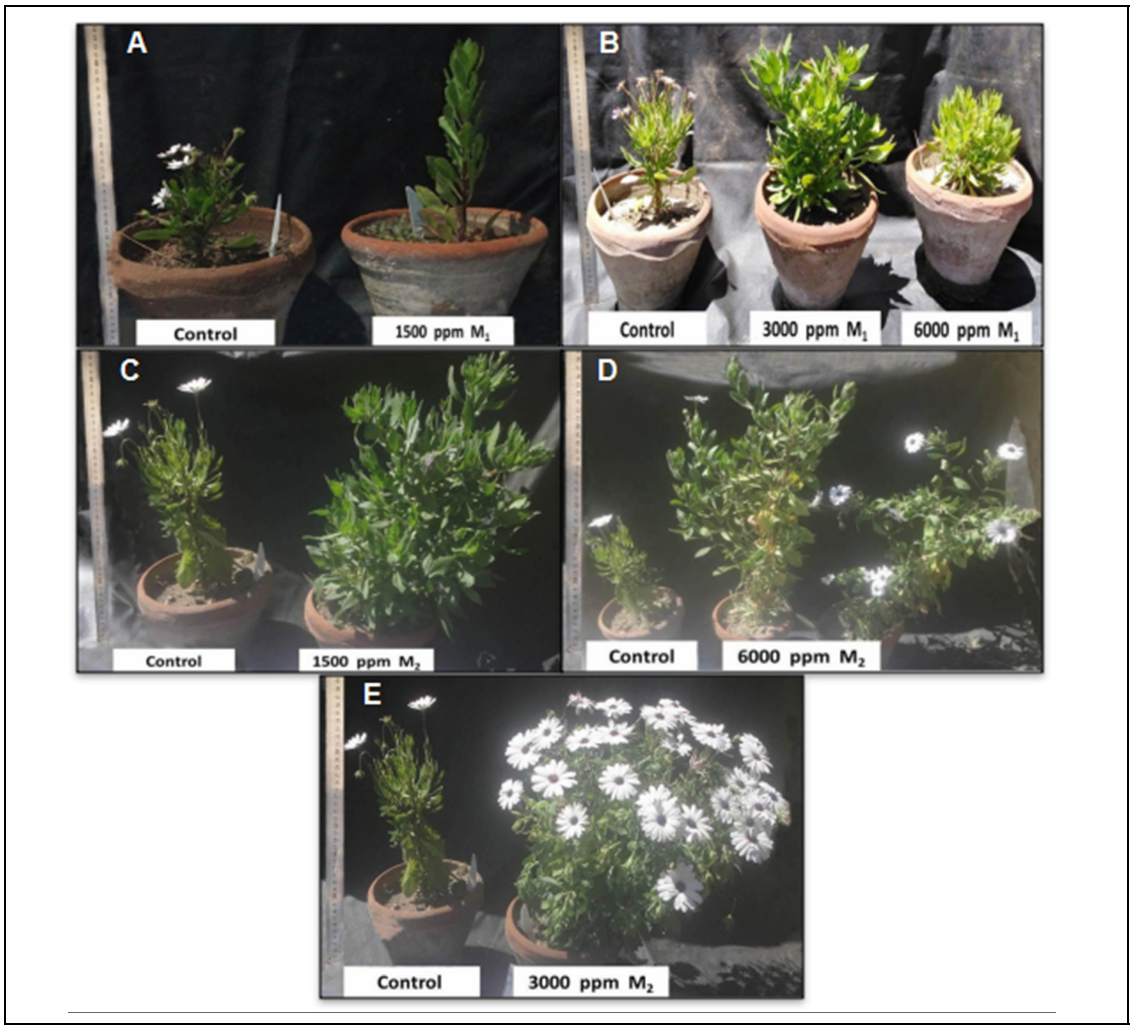

Fig. 3. The changes in the growth habit of *Dimorphotheca ecklonis* (L.) as a result of different sodium azide concentrations in M_1 and M_2-generations.

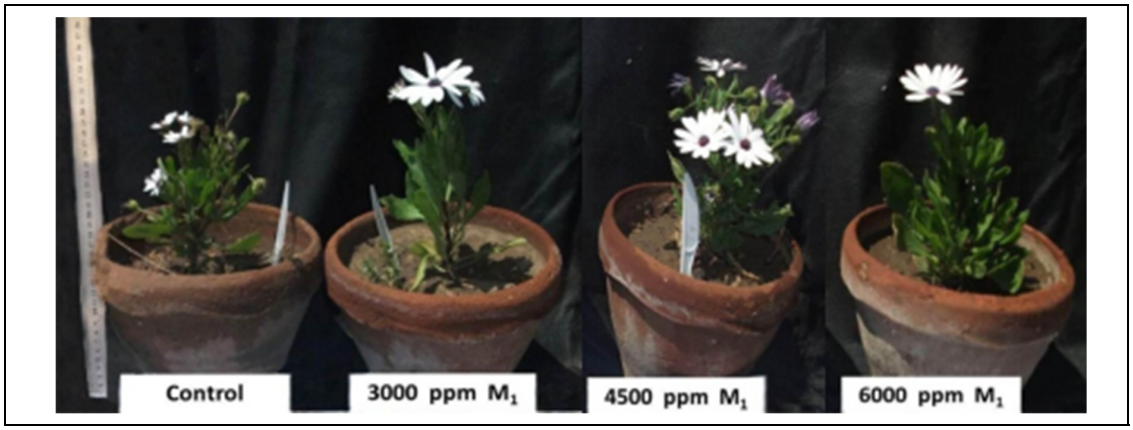

Fig. 4. The changes in the flowers of *Dimorphotheca ecklonis* (L.) as a result of different sodium azide concentrations in M_1-generation.

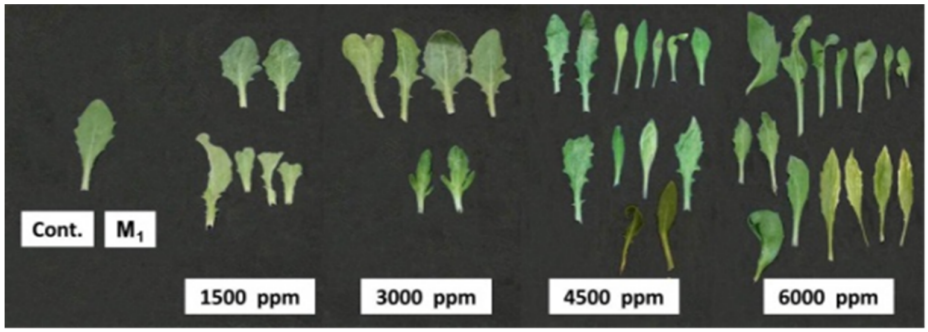

Fig. 5. Leaf changes in the M_1-generation of *Dimorphotheca ecklonis* (L.) as a result of sodium azide at different concentrations compared with the control.

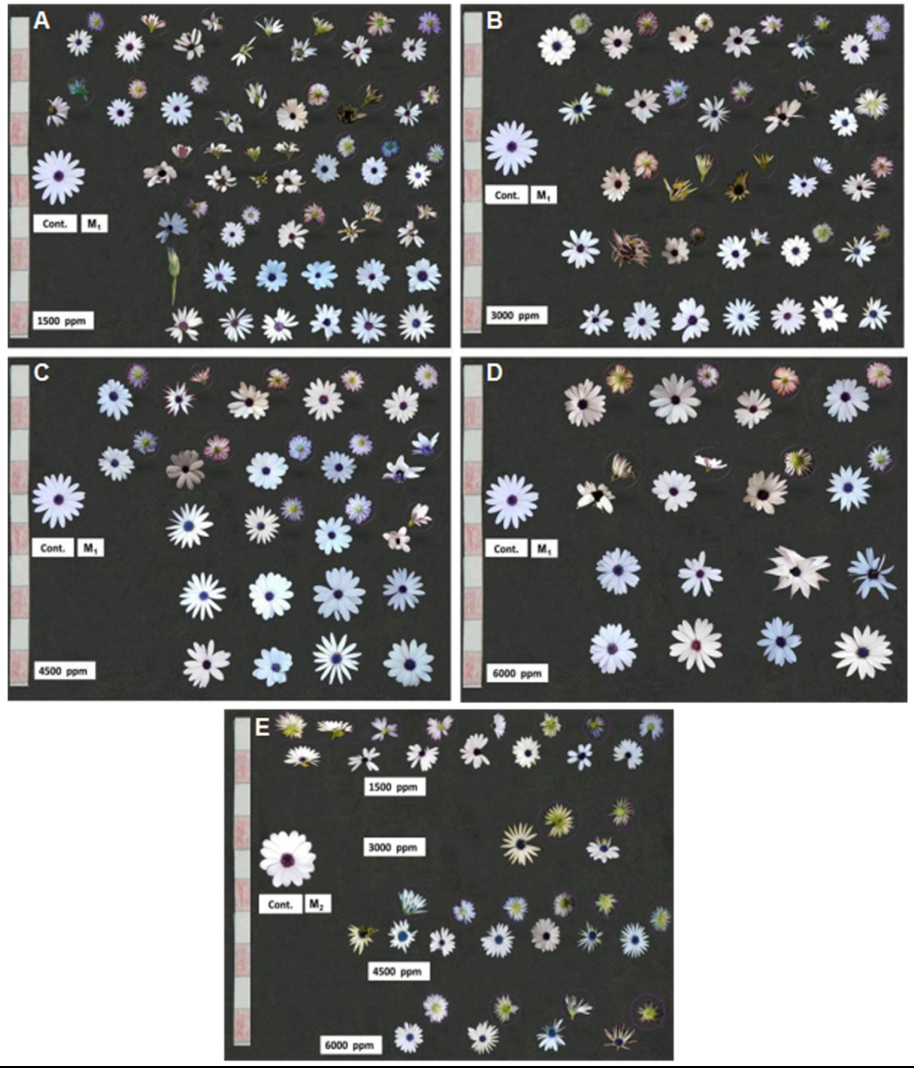

Fig. 6. A Photograph showing flowers with deformed petals (A, B, C and D) in the M_1-generation, (E) in the M_2-generation of *Dimorphotheca ecklonis* (L.), as a result of the treatment of 1500, 3000, 4500 and 6000 ppm SA compared with the control.

Cell number and cell length may be altered in the inflorescence of treated plants. Large inflorescence had larger florets with a rise in the number of cells and/or cell size. Small inflorescence had smaller florets with a decrease in cell number and/or cell size (Badr and Etman, 1976).

Polymerase chain reaction (PCR) analysis:

The treatments which induced the mutation in *Dimorphotheca ecklonis* (L.) plants and the description of the produced mutants are shown in Table (4). On the other hand, the genetic similarity of the produced eight mutants of *Dimorphotheca ecklonis* (L.) plants as treated by sodium azide (SA) using the SRAP-PCR technique is shown in Table (5).

Sequence-related amplified polymorphism (SRAP) technique was used to identify eight mutants and control plants of *Dimorphotheca ecklonis* (L.), which produced from sodium azide treatments. Six primers were used and generated ninety-eight bands, with an average of 19.6 bands per primer, and ranging from 100 to 950 bp (Table, 6 and Fig., 7).

The primer designated E obtained the largest number of unique, polymorphic and total bands (10, 22, 32, respectively) compared with other primers. The primer A gave the lowest number for these parameters (5, 8 and 14, respectively). Polymorphism percentage ranged from 57.1% for primer A to 84.2 % for primer B.

The dendrogram constructed based on the analysis of the SRAP (Fig., 8) demonstrated that all mutants were genetically distinct from the control.

Mutants four and six were genetically closer the row each other with a 19.4 genetic distance, more distinct from the control and designated in one group. The mutants were two and three, also with 46.2 genetic distances.

SRAP technique could identify sequence, non-coding flanking region and the interval between genes (Elsayed *et al*., 2020).

In this study, the SRAP technique was used successfully to distinguish the obtained mutants of *Dimorphotheca* plants after sodium azide treatments as mutagenic compounds.

Table 4. The treatments which induced the mutation and description of the mutants of *Dimorphotheca ecklonis* (L.) plants.

Number of plants	Treatment	Change
C	**0000 ppm**	Control
1	**1500 ppm**	No morphological characters
2	**3000 ppm**	No morphological characters
3	**4500 ppm**	No morphological characters
4	**6000 ppm**	No morphological characters
5	**1500 ppm**	Mutant morphological characters (colored flower) (Purple flowers of disk flower)
6	**3000 ppm**	Mutant morphological characters (colored flower) (Yellow flowers of disk flower)
7	**4500 ppm**	Mutant morphological characters (colored leaves) (leaf shape abnormalities)
8	**6000 ppm**	Mutant morphological characters (colored leaves) (Small leaves)

Table 5. Genetic similarity of eight mutants and control of *Dimorphotheca ecklonis* (L.) plants treated by sodium azide (SA) using the SRAP-PCR technique.

	C	1	2	3	4	5	6	7	8
C	100								
1	30.8	100							
2	27.6	50.3	100						
3	26.3	49.3	53.8	100					
4	28.1	39.5	40.3	51.8	100				
5	26.6	52.9	44.5	42.2	41.1	100			
6	20.4	25.4	28.2	32.6	29.6	36.8	100		
7	15.1	23.1	24.0	22.3	28.9	38.6	30.9	100	
8	22.2	27.7	27.7	35.5	34.8	36.6	33.7	37.3	100

Table 6. Number of amplified, unique, polymorphic primers of band and polymorphism percentages generating by SRAP technique to distinguish the mutants of *Dimorphotheca ecklonis* (L.) from control plants.

Primers	Number of polymorphic bands	Number of unique bands	Number of polymorphic bands	Polymorphism %
A	14	8	5	57.1
B	19	16	3	84.2
C	18	12	6	66.6
D	15	9	6	60.0
E	32	22	10	68.0
Total	98	67	30	
Average	19.6	13.4	6	67.18

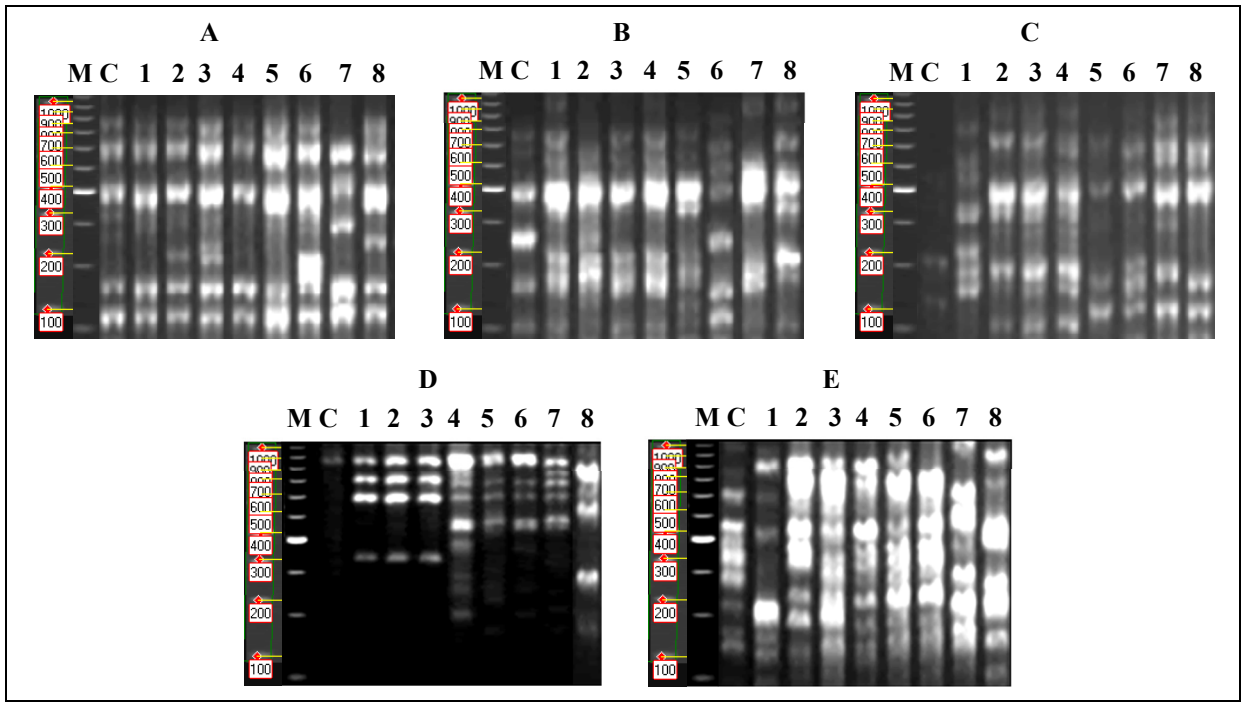

Fig. 7. Photograph showing PCR amplified fragments for the control (original parent) and variant plants of *Dimorphotheca ecklonis* (L.) after amplification with the primer SRAP (A-E).

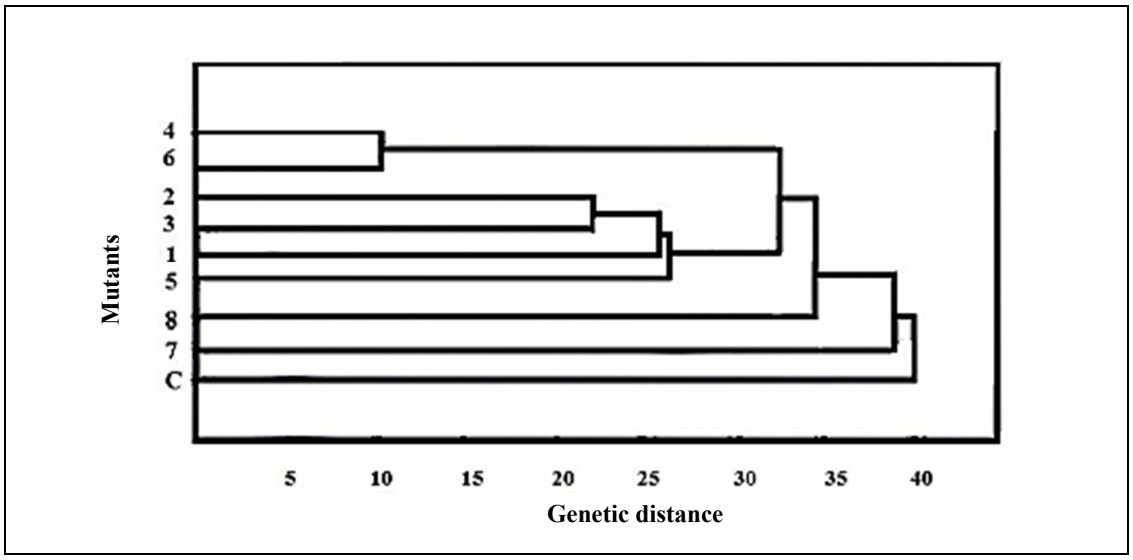

Fig. 8. Tree diagram for M_2 generation of *Dimorphotheca ecklonis* (L.) plants treated by sodium azide on the basis of SRAP using PCR technique.

CONCLUSION

The data obtained showed that various sodium azide concentrations induced some changes in the vegetative and blooming growth's morphology, as well as increased chemical composition of *Dimorphotheca ecklonis* (L.) plants. The SRAP-PCR technique was able to detect some mutations in the plant.

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إستخدام أزيد الصوديوم (NaN_3) لتحفيز حدوث الطفرات في نباتات الديمورفوتيكا والمعلمات الجزيئية SRAP الوراثية لتحديد التباين الوراثي

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نبات الديمورفوتيكا، المعروف باسم الأقحوان الأفريقي، نبات حولي شبه قوي، يُنتج أزهارًا كبيرة .تتميز أزهاره بتباين مذهل، حيث تكون بيضاء اللون، بينما تُضفي أزهاره القرصية لونًا أزرق أو بنفسجيًا غامقًا. إنه نبات متعدد الاستخدامات، يُمكن استخدامه في مجموعة متنوعة لتصميم اللاندسكيب. يُستخدم غالبًا كنبات زينة، مُضيفًا ألوانًا زاهية لأحواض الزهور والحدود. كما أنه ينمو في الحدائق الصخرية والأصص، مما يُتيح خيارات وضع مرنة. قدرته على تحمل ظروف الجفاف تجعله خيارًا ممتازًا للحدائق قليلة المياه، مما يدعم ممارسات تنسيق الحدائق المستدامة. أُجريت هذه الدراسة خلال موسمين متتاليين ٢٠٢٢/٢٠٢٣ و ٢٠٢٣/٢٠٢٤ في مشتل الزهور و نباتات الزينة، كلية الزراعة، جامعة الإسكندرية، جمهورية مصر العربية. تم رش شتلات النبات بالصوديوم أزيد بتركيزات ١٥٠٠، ٣٠٠٠، ٤٥٠٠، ٦٠٠٠ جزء في المليون والنباتات غير المعاملة (ماء مقطر) لتقييم تأثيره على النمو الخضري والنمو الزهري والتركيب الكيميائى للنبات، وتحفيز الطفرات والتغيرالجينى الحادث فى النبات، وقيمت الطفرات المستحثة بتفنية SARP. أبدت النتائج زيادة معنوية فى طول النبات والوزن الجاف للمجموع الخضرى عند المعاملة بجرعة ٦٠٠٠ جزء فى المليون في الجيل الثاني، أما في كلا الجيلين تم تسجيل زيادة في عدد الأفرع وعدد الأوراق، كما أدت المعاملة بجرعة ٦٠٠٠ جزء فى المليون الى زيادة كبيرة في المساحة الورقية للنبات في كلا الجيلين، وسجلت المعاملة بالتركيزين ٦٠٠٠ و ٣٠٠٠ جزء في المليون على التوالى في الجيلين تأخير معنوي في عملية الازهار. أدت المعاملة بتركيز ٣٠٠٠ جزء في المليون في كلا الجيلين إلى زيادة معنوية فى الأنثوسيانين وبيتا كاروتين . لوحظت بعض الاختلافات في طبيعة النمو وشكل الأوراق وخصائص الأزهار في كلا الجيلين. كما تم الكشف عن حدوث تغيرات جينية في النبات مقارنة بالنباتات غير المعاملة.