Original Article

Determination of the effective of chemical mutagenesis using sodium azide to improvement of vegetative growth and flowering characteristics in Helichrysum bracteatum L. plant

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Abstract

This study were carried out on Helichrysum bracteatum L. plant at the Experimental nursery of the Ornamental Hort. Dept., Fac. Agric., Cairo Univ., Giza, Egypt, during the two successive seasons (2014/15 and 2015/16). The aim of this study was to investigate the effect of on some vegetative and flowering parameters and anatomical structure of leaves and flowers. Seeds of H. bracteatum L. were exposed to four concentrations of (0.05, 0.1, 0.2 and 0.3%) of sodium azide (NaN3) solution for six hours; one set of seeds was kept without sodium azide treatment (untreated) to act as control. The obtained data revealed that the low concentration of sodium azide (SA) increased plant height, number of leaves/ plant, stem diameter (cm) number of flowers, flowers fresh weight (g)/plant and flowers dry weight (g)/plant in M1 and M2. The ratio of chlorophyll (a and b) showed a markedly decrease as a result of increasing sodium azide concentrations. On contrary, in case of carotenoids showed a markedly increase as a result of decreasing sodium azide concentrations. Were obtained on many morphological mutants in flowers through two generations. Some parameters of anatomical structure of leaves and flowers and to confirm the stability of some of the mutations were obtained during the second generations.

Keywords: sodium azide, Helichrysum bracteatum, vegetative, flowering characters, photosynthetic pigments, anatomical structure.

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INTRODUCTION

Helichrysum bracteatum L. is recognized as strawflower or the golden everlasting, it is a blooming plant, belongs to the family Asteraceae. The strawflower is a flowering plant that is indigenous to Australia. It has big, deep-green leaves and spectacular flowers. Depending on the type, plants can reach heights of three to four feet. The flower's papery appearance has earned it the nickname "paper daisy." The heart of the flower is composed of a group of tiny individual blossoms, just like the daisy. Bracts, not petals, encircle the cluster of flowers. From March to October, the plant blooms or produces flowers. The daisy-like flowers come in a variety of hues, including white, red, wine, purple, yellow, orange, pink, deep rose, and pink blooms. For many years, it belonged to the genus Helichrysum bracteatum before being moved to the new genus Xerochrysum in 1990. Worldwide production of fresh and dried helichrysum flowers. The flowers are harvested for drying before fully opening and used in dried arrangements (Sharman, et al. (1989 a, b) and Nishikawa, et al. (2008).

A mutation is an abrupt genetic alteration that takes place in an organism. It may occur naturally or through deliberate induction, and the resulting mutant exhibits changes to the chromosomes or genes (De and Bhattacharjee, 2011). Around the world, induced mutagenesis techniques have been used to successfully develop and market a significant number of novels, promising types in many crops, including decorative plants. For enhancing the desirable traits of floral and ornamental crops including amaryllis, asiatic hybrid lily, bougainvillaea, chrysanthemum, dahlia, gladiolus, hibiscus, Lantana depressa Naud, marigold, rose, tuberose, gerbera, and narcissus, among others, both physical and chemical mutagens were used. Physiological traits in ornamentals include altered photoperiodic response, early flowering, free flowering, keeping quality, and tolerance to biotic and abiotic stresses. Induced mutations in ornamentals also include traits such as altered flower characters (colour, size, morphology, fragrance), leaf characters (form, size, pigmentation), growth habit (compact, climbing, branching), and growth pattern. The main benefit of mutation breeding is the ability to change one or more characteristics of an

otherwise excellent variety without changing the distinctive aspect of the genotype (Datta, 2014).

Another technique for introducing mutations into plants to enhance their agronomic properties is chemical mutagenesis. One of the most potent chemical mutagens for crop plants has been sodium azide (NaN3) (Owias, et al., 1983). Because it is a potent mutagen for plants, it interferes with the metabolic process and has an impact on the growth and development of the various components of the plants. Where they are easy to process uncomplicated and fast do not need a long time. Unlike other education programs (hybridization) it has been consider that the use of chemical mutagens is designed to improve the way of ornamental plants and thus play a major role in the development of many colors of flowers and plant forms of mutant varieties and production plants new decorations.

One of the most potent mutagens in agricultural plants, sodium azide has been demonstrated to have mutagenic potential in numerous screening assays. NaN3, a mutagenic chemical that must be digested by plant cells into azideoalanine, is frequently employed with seeds to cause mutation (Owais et al., 1983). The permeability of the seed coat and the type of mutagen determine how they affect organisms. Chemical mutagens offer a strong opportunity for selection as a technique for changing the genotype to increase character diversity. In order to increase genetic diversity in ornamental plants throughout the past few years, many researchers have used a variety of mutagens, El-Fadaly (2003) on Dimorphotica; El-Nashar (2012) on Calendula officinalis; El-feky et al. 2014 on Helianthus annus; Mostafa et al. (2014) on Celosia argentea; El-Nashar and Asrar (2016) on calendula plant.

This study was conducted to investigate the response of Helichrysum bracteatum L. to sodium azide treatments and to evaluate the effects of sodium azide on quantitative and qualitative traits of growth and flowering, photosynthetic pigments and anatomical structure of leaves and flowers.

Material and methods

A field experiment was conducted during the three successive seasons of 2014/2015, 2015/2016 and 2016/2017 at the experimental nursery of the Ornamental Horticultural Department, Faculty of Agriculture, Cairo University, Egypt.

Plant materials

The seeds of H. bracteatum L. (Local variety) were obtained from Ornamental Hort. Dep., Fac. of Agric., Cairo Univ., Egypt. The seeds were presoaked in distilled water (wet seeds) for one hour and were then subjected to four different concentrations (0.05, 0.1, 0.2 and 0.3%) of sodium azide (NaN3) solution for six hours. One set of seeds was kept without sodium azide treatment (untreated) to act as control. After completion of treatment period of 6 hours, the seeds were thoroughly washed in running tap water to reduce the residual of sodium azide mutagen on the seed coat. Sodium azide: It has the chemical formula of NaN3, F.W. is 65.009 g/M, soluble in water and fusion point 275 °C.

On 5th Nov., 2014; 5th Nov., 2015 and 5 th Nov., 2016 (for M1, M2 and M3 generations, respectively) the treated seeds of both experiments (gamma rays and sodium azide experiments) were sown in plastic trays, filled with a mixture of peat moss, sand and loam (2:1:1) by volume. Seed germination was started after one week of sowing. Six weeks after sowing (16 th Dec., 2014;16 th Dec., 2015 and 16 th Dec., 2016, for M1, M2 and M3 generations, respectively), uniform seedlings (10-12 cm height) of H. bracteatum L. of each treatment were transplanted into hills, in open field of experimental area (clay loam soil), in three rows at 60 cm apart, at 50 cm between the hills within the row (two plants/hill), as every plot (3.5 x 1.8 m) contained 21 hills /plot.

Statistical analysis

A randomized complete block design was used. Data of the experiment were subjected to statistical analysis, according to the procedure of Snedecor and Cochran (1980), where the means separation were carried out using Duncan (1980) multiple range tests and compared using L.S.D test at 0.05 probability levels significance was determined at $P < 0.05$.

Soil analysis

Soil analysis detected that, particle size distribution (%) was: sand: 26.8, silt: 26.1 and clay: 37.1 (texture: clay loam), pH: 7.9, EC ds.m-1: 0.95, soluble cations (meq./l) were Na+:0.71, K+:0.4, Ca++:1.3, Mg++: 0.9and soluble anions (meq./l) were Cl: 1.5, HCO3: 0.75 and SO4: 1.06.

The first mutative generation (M1)

The mass selection of plants of M1-generation was run from April to June, 2015, where plants which survived in each treatment, were evaluated, selected and selfed, in order to obtain the second mutative generation (M2) seeds according to Sinhamahapatra and Rakshit (1990).

Observations were made throughout the periods of vegetative and flowering. At maturity (June), the seeds of all the survived M1 fertile plants were harvested separately.

The second mutative generation (M2)

Field selections were done treated plants in the first season as variants or mutants on some flowering parameters. Anatomical structure of leaves and flowers and to confirm the stability of some of the changes obtained during the first season. The seeds harvested from M1 generation were taken from individual treatments and used to raise M2 generation (seedlings) plants. The M2-generation was done, as seeds were sown on 5th Nov., 2015 in plastic trays, filled with a mixture of peat moss, sand and loam (2:1:1) by volume to raise M2 generation plants. The seedlings were transplanted into open field, as in the first generation.

Agricultural practices

All the recommended cultural practices namely, irrigation and weeding were carried out during the plant growth and flowering period. The fertilizers were supplied for each plot as recommended, using Kristalon mineral fertilizer (19:19:19), the plants were fertilized monthly after month from transplanting.(2 g/hill). Irrigation was done with tap water according the needed amount of water. Weeding was carried out as the soil needed.

Data recorded

a. Vegetative parameters

1. Plant height (cm). 2. Number of leaves/plant. 3. Stem diameter (cm).

b. flowering parameters

1. Number of flowers/plant. 2. Fresh weight of flowers/plant (g). 3. Dry weight of flowers/plant (g).

c. Photosynthetic pigments, according to Lichtenthaler (1987) and Saric et al. (1967).

d. Abnormalities and mutations in inflorescences.

e. Anatomical structure of the leaves and flowers, according to Sass (1951).

Results and discussion

a. Vegetative parameters

1. Plant height (cm)

Data presented in Table (1) concerning the effect of sodium azide treatments on plant height, number of leaves and stem diameter of H. bracteatum L. indicated that the low dose of sodium azide (0.05%) significantly increased these parameters in M1 and M2 compared with the control. The increments were (22.14% and 11.08%) for plant height, (18.09% and 19.54%) for number of leaves and (23.00% and 14.71%) for stem diameter in M1 and M2, respectively compared with untreated plants. On the contrast, increasing sodium azide doses to (0.3%) led to increase the plant height, number of leaves and stem diameter but no significant compared with control plants.

The stimulatory effect of the mutagen may be attributed to the increase in the rate of cell division or cell elongation as well as an activation of auxin as reported by (Zaka et al., 2004 and Joshi et al., 2011).

These results are similar to findings obtained by El-Fadaly (2003) found that treating seeds of Dimorphotica ecklonis with combined treatment from (2x10-3m NaN3 and 1.0% EMS) increased the plant height and number of leaves per plant compared with control; Mensah et al. (2007) treated the seeds of sesame plant by concentrations of sodium azide solutions ranging from 0 - 0.250% (w/v). Results showed that plant heights and dry matter increased with increasing concentrations of sodium azide; Nasare (2011) tested the seeds of Ocimum sanctum with various concentrations of chemical mutagens; sodium azide and EMS at 0.00, 0.001, 0.002 and 0.003% for 18 hrs. He found that two mutagens led to increasing in plant height compared with the control; El-Mokadem and Mostafa (2013) treated stem cuttings of Browallia speciosa with different concentrations of sodium azide, they found that all the concentrations used enhanced number of leaves compared to untreated plants.

Also, Hatlaa et al.(2014) investigated the effect of sodium azide on stem cuttings of Browallia speciosa, and found that sodium increased number of leaves/plant; Mangaiyarkarasi et al. (2014) treated seeds of Catharanthus roseus with EMS at 30, 40, 50, 60 and 70 mM, for 3 hrs. They recorded that the plant height decreased with increasing the concentrations; Mostafa et al. (2014) on Celosia argentea, treated the seeds with 0, 1000, 2000, 3000 and 4000 ppm, dimethyl sulphate (DMS) solution as soil drench. They found that the concentration of 1000 ppm increased stem diameter in both generations, while 3000 ppm significantly decreased stem diameter in both generations; Mostafa (2015)

studied the effect of sodium azide on Khaya senegalensis with concentrations (200, 400, 600, 800 and 1000 ppm) for 15 hrs. He found that plant height decreased with increasing concentrations of sodium azide; El-Nashar and Asrar (2016) who treated seeds of calendula with two chemical mutagens sodium azide and diethyl sulfate, at five different concentrations (1000, 2000, 3000, 4000 and 5000 ppm), they found that lower concentrations of SA mutagen significantly increased plant height.

Table 1. Effect of sodium azide treatments on plant height (cm), number of leaves/ plant, and stem diameter (cm)/plant of Helichrysum bracteatum L. plant, during the M1 and M2 (2014/15 and 2015/16).

	Plant height (cm)		**Number of leaves /plant**		**Stem diameter (cm)**	
	M1	**M2**	**M1**	**M2**	**M1**	**M2**
Control	138.90 bc	153.33 c	294.55 c	305.33 c	1.30 b	1.36 b
0.05% SA	169.66 a	170.33 a	347.83 a	365.00 a	1.60 a	1.56 a
0.1% SA	164.66 a	168.00 a	330.83 b	327.33 b	1.43 ab	1.50 a
0.2% SA	160.50 b	167.50 ab	326.66 b	316.66 bc	1.40 ab	1.41ab
0.3% SA	159.95 b	160.83 bc	321.00 b	315.33 bc	1.40 ab	1.36 b

M1= First generation M2= Second generation SA=Sodium azide

b. flowering parameters

1. Number of flowers /plant

Data presented in Table (2) indicate that treating the seed of H. bracteatum with sodium azide significantly increased the flowers number/plant, flower fresh and dry weight of flowers (g/plant). The most significant increases were recorded when plants were treated with 0.0 5 and 0.1% compared with untreated plants. The increments were (23.82% and 22.14% in M1) and (25.09% and 19.53% in M2) for number of flowers /plant, (36.01% and 34.16% in M1) and (25.10% and 19.54% in M2) for flower fresh weight (g/plant) and (35.04% and 30.94% in M1) and (39.11% and 38.53% in M2) for flower dry weight (g/plant), respectively, compared with control plants. While, treating the seeds with 0.3% (SA) insignificantly increased the number of flowers/plant, flower fresh and dry weight compared with the control plant. The low concentration of sodium azide induced some stimulation effect on flowering, while the higher concentrations decreased it, as found by studies of (Mostafa, 2011 on Helianthus annuus; Mostafa and Alhamd, 2011 on Balanites aegyptiaca; Roychowdhury and Tah, 2011 on Dianthus caryophyllus). This inhibition effect on flowering can be due to physiological damage produced cumulatively by increased chemical mutagen concentrations.

In this regards, it was mentioned by Krupa-Małkiewicz (2010) induced mutation in kalanchoe plant (Kalanchoe hybrida) using different concentrations at 0.0, 0.5, 1.0, 1.5 and 2.0 mM of sodium azide for periods of 60 min, he recorded that the number of flower buds and number of flowers increased with concentration of 1.5 mM; Warghat et al. (2011) investigated the effect of sodium azide on okra plant (Abelmoschus moschatus). Exposed the seeds to varying concentrations of (SA) at 0.05-0.20 % for 18 hrs. They found that sodium azide increased the number of flowers and number of fruits/plants; El-Nashar (2012) who treated the seeds of Calendula officinalis with different concentrations of sodium azide at 1000, 2000, 3000, 4000 and 5000 ppm. He found that the different concentrations had significant effect in M1 generation with respect to number of inflorescences; El-Nashar and Ammar (2016) on Calendula officinalis treated the seeds with concentrations at 0, 400, 800, 1200, 1600, and 2000 ppm of colchicine, in combination with four soaking time treatments (1, 2, 3, and 4 hrs). The results showed that 800 ppm for 4 hrs produced the highest number of flowers; also, they found that lower concentrations of SA mutagen had significant effect on flower fresh and dry weight,

whereas higher consternations decreased flower fresh weight.

Table 2. Effect of sodium azide treatments on number of flowers /plant, flowers fresh weight (g)/plant and flowers dry weight (g)/plant of Helichrysum bracteatum L. plant, during the M1 and M2 (2014/15 and 2015/16).

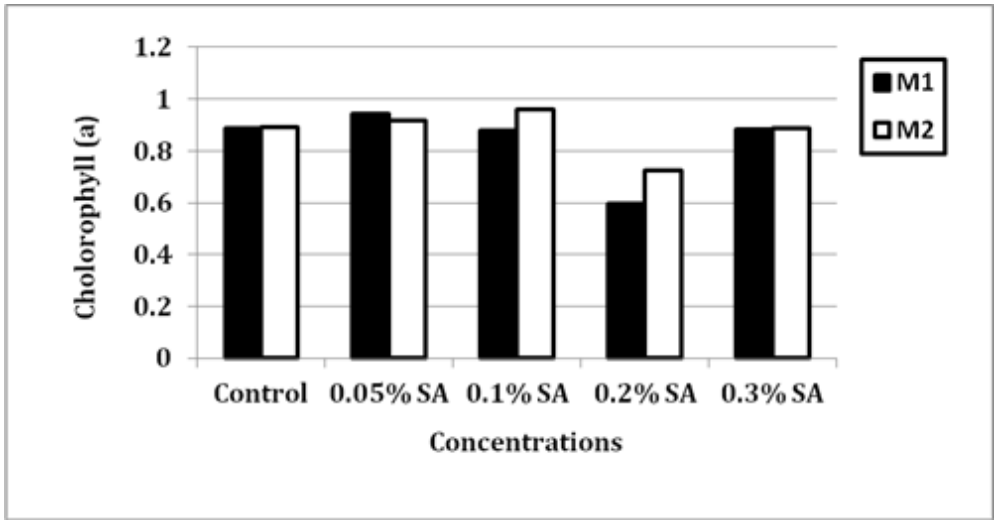
	Number of flowers /plant		**Flowers fresh weight (g)/plant**		**Flowers dry weight (g)/plant**	
	M1	**M2**	**M1**	**M2**	**M1**	**M2**
Control	21.66 c	23.00 c	33.05 c	49.13 d	11.70 c	13.73 d
0.05% SA	29.33 a	33.33 a	44.95 a	61.46 a	15.80 a	19.10 a
0.1% SA	28.66 a	32.00 a	44.34 a	58.73 a	15.32 a	19.02a
0.2% SA	24.33 b	29.66 b	36.30 b	54.51 b	13.19 b	17.90 b
0.3% SA	21.50 c	23.67 c	33.05 c	50.54 c	12.10 d	15.19 c

M1= First generation M2= Second generation SA=Sodium azide

c. Photosynthetic pigments

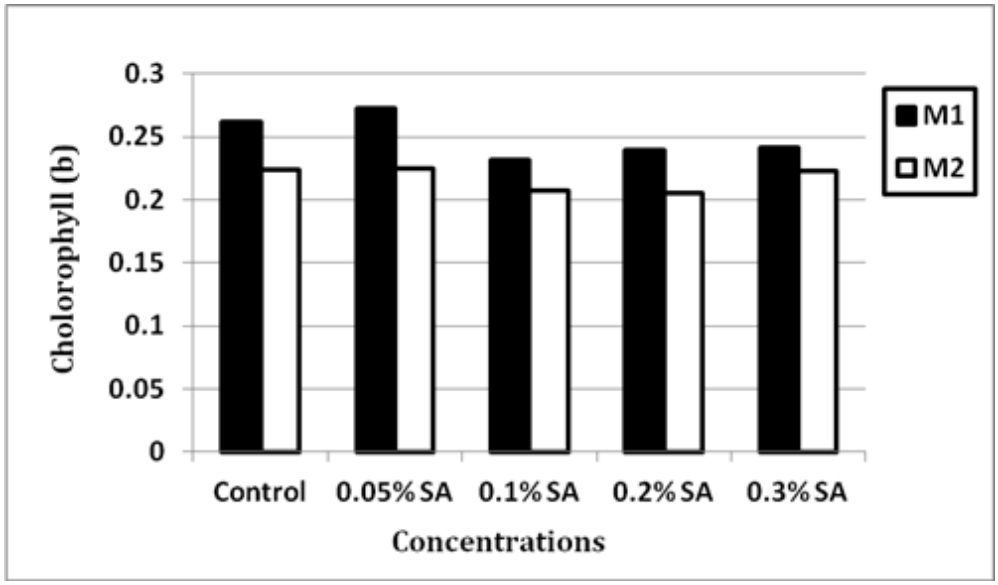
It can be observed from data presented in Figs (1-3) that, using sodium azide gave fluctuated of chlorophyll value during the experimental period and chlorophylls were almost entirely insensitive. Illustrated data in Figs (1-3) indicted that using 0.05% increased the highest values content of chlorophyll a (0.945 mg/g F.W.) for M1, while in M2 using 0.1% sodium azide gave the highest value (0.962 g/g F.W.) as compared with the control. Using sodium azide at 0.05% gave the highest values of chlorophyll (b) (0.272 and 0.225 mg/g F.W.) in M1and M2, respectively, as compared with control (0.262 and 0.224 mg/g F.W.) respectively. The height value of carotenoids were obtained when plants treated with sodium azide at 0.2% in M1 and M2 (0.691 and 0.642 mg/g F.W), respectively, comparing with the control (0.0.597 and 0.570 mg/g F.W) in M1 and M2, respectively.

The lowest values were obtained from using (0.2%) treatment of sodium azide, (0.598 and 0.723 mg/g F.W.) for chlorophyll (a) in M1 and M2, respectively compared with (0.888 mg/g and 0.891 mg/g F.W.) for the control plants, using sodium azide at (0.1 and 0.2%) gave the lowest contents for chlorophyll (b) by values (0.232 and 0.205 mg/g F.W.) in M1 and M2 respectively, as compared the control, using (0.3%) sodium azide gave the lowest value for carotenoids in M2 compared with other concentrations of sodium azide.



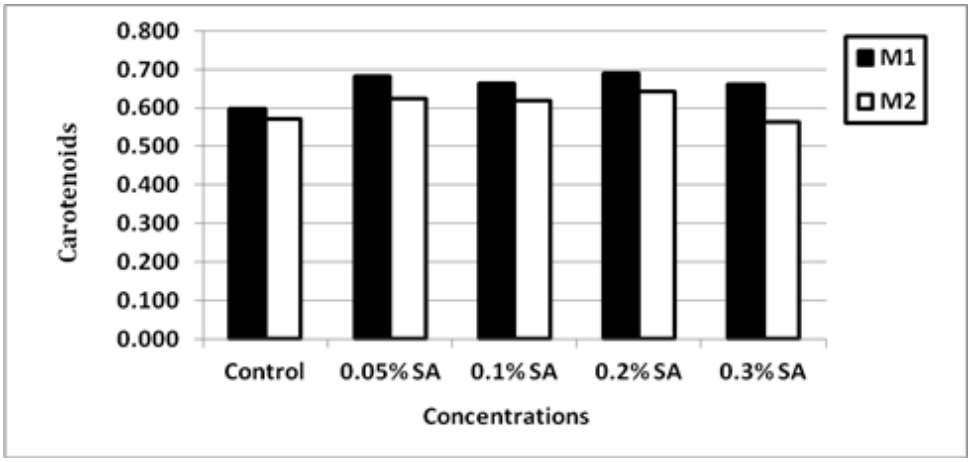
M1= First generation M2= Second generation SA=Sodium azide

Fig. 1. Effect of sodium azide treatments on chlorophyll (a) (mg/g F.W.) of Helichrysum bracteatum L. plant, during the M1 and M2 (2014/15 and 2015/16).



M1= First generation M2= Second generation SA=Sodium azide

Fig. 2. Effect of sodium azide treatments on chlorophyll (b) (mg/g F.W.) of Helichrysum bracteatum L. plant, during the M1 and M2 (2014/15 and 2015/16).



M1= First generation M2= Second generation SA=Sodium azide

Fig. 3. Effect of sodium azide treatments on carotenoids (mg/g F.W.) of Helichrysum bracteatum L. plant, during the M1 and M2 (2014/15 and 2015/16).

In this regard, El-Fadaly (2003) on Catharanthus roseus recorded that 2.0x10-3m NaN3 increased total chlorophyll and carotenoids compared with the control; El-Feky et al. (2014) investigated the effects of various sodium azide concentrations (control, 0.5, 1.0, and 2.0 mM) at various soaking times (30, 60, 90, 120, and 150 min) on Helianthus annuus L.'s photosynthetic pigments (gg-1 f.wt). They discovered that the amount of pigments changed significantly in response to the impact of various sodium azide concentrations at various soaking times. With rising sodium azide concentrations and the soaking time, chlorophyll a and b quantities considerably dropped. On the other hand, the content of carotenoids increased, reaching its maximum level at a sodium azide concentration of 2 mM and a 150-minute soaking time. Numerous writers have noted a decrease in chlorophyll content after treatment with sodium azide (Mahmoud and Al-Twaty, 2006; Al-Qurainy and Khan, 2009), which may be the result of chloroplast injury or a suppression of its biosynthesis. Plants under stress produce more carotenoid pigments to offer defense against the production of free radicals (Ferrat et al., 2003). Additionally, carotenoids can lower lipid peroxidation and may be crucial in protecting chlorophyll and chloroplasts from photooxidative damage (Behera et al., 2002). (Burton and Ingold, 1984).

Abd El-Maksoud and El- Mahrouk (1992) on Asparagus densiflorus and El-Nashar (2006) on Amaranthus both mentioned the same detrimental effects. This discovery suggests that an increase in chloroplast differentiation is the cause of the effects of SA and DES, which led to chlorophyll mutants. The yield and pattern of distribution of EMS and SA-induced chromatid aberration along the chromosomes were significantly altered as a result of the repositioning of the chromosome segments. Despite the lack of a direct correlation between specific reconstructions of karyotypes and the response of specific genetic loci, Hordeum vulgare L. provided strong evidence for the chromosome-induced gene mutation in plants (Gecheffe, 1998), El-Nashar (2012) on Calendula officinalis seeds were treated with different SA and DES concentrations 1000, 2000, 3000, 4000 and 5000 ppm. He recorded that the concentration of 5000 ppm in the M1significantly reduced the total chlorophyll content, as compared with other treatments

d. Abnormalities and mutations in inflorescences

Data in Fig (4) showed that sodium azide at concentration of 0.2% gave big-flower mutants in M1 and M2 generations, As it contains a number of petals much more and a large size, comparing the control flowers, also gave fascination flowers in M2, whereas 0.3% SA (light yellow color of petals and deep yellow of disk flower with small No. petals) in M1 and M2, generations. These alterations could also refer to the layer rearrangement as a result of the chemical mutagens effect, as reported by Abd El-Maksoud and El-Nashar (1988). These changes could be caused by chromosomal disruptions (2006). These anomalies could be the result of a gene mutation that caused the floral meristem to be replaced by meristems that exhibit part or all of the traits of an inflorescence. According to Coen and Carpenter (1993), sodium azide in this situation causes a failure or delay in the development of flowers and a proliferation of inflorescence-like structures to take their place.

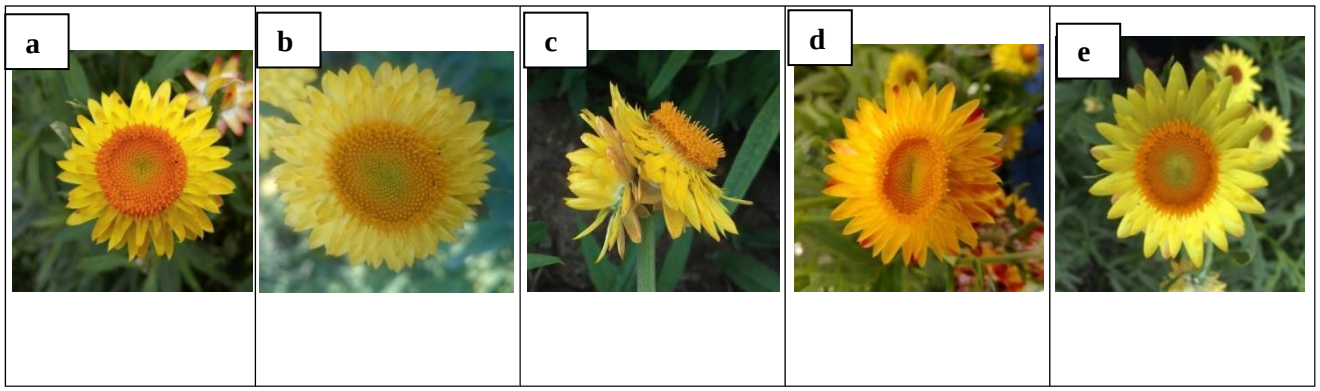


Fig. 4. Showing different shapes of flowers of H. bracteatum as results of the treatments with sodium azide in M1 and M2 generations.

a) control (yellow color of control plants); b) 0.2% SA (big flower)in M1 and M2; c) 0.2% SA two flowers in the same stalk; d) 0.2% SA (fascinated flower) in M2; e) 0.3% SA (light yellow color of petals and deep yellow of disk flower) in M1 and M2.

In this regards, Montalván and Ando (1998) mentioned that sodium azide increased the variance of number of days to flowering (NDF) and tiller number (TN), whereas treating seeds of rice cultivar IAC-1246 with 0.5, 1.0 mM for 8 hrs found that 0.5% EMS gave large flower diameter compared with the control. Also, Fang and Traore (2011) on Saintpaulia exposed leaf sections to various EMS treatments at 0%, 0.2%, 0.4%, and 0.6% for 30, 60, 120, and 240 min, and obtained some flower mutants from the 0.4% to 30 min and 0.4% to 120 min EMS treatments, differed in petal color and presence/absence of white fringe around the lobes and changes in flower shape.; Krupa-Małkiewicz (2010) induced mutation in kalanchoe (Kalanchoe hybrida) plant by using sodium aside at 0.0, 0.5, 1.0, 1.5 and 2.0 mM for periods of 60 min, sodium azide induced mutations with changes in flowers shape with 1.5 mM; El-Mokadem and Mostafa (2013) used the following sodium azide concentrations as a soil drench on rooted cuttings of Browallia: 0, 200, 400, 600, and 800 ppm (10 ml for each pot). They discovered that flower forms were altered at concentrations of 400, 600, and 800 ppm, resulting in flowers with four petals, with deformed shapes, or with stamens converted to petals. Additionally, some flowers' stamens turned into petals as a result of sodium azide exposure at 800 ppm. On some plants in both generations, all sodium azide treatments resulted in the formation of distorted leaves; Wang et al. (2014) studied the effect of EMS on Cucumis sativus L. cv. “Shannong No. 5”, they soaked seeds in 1% EMS solution for 12, 24 and 48 hrs. They obtained big-flower mutants as compared to the control; Prabhukumar et al. (2015) soaked the rhizomes of three flowering plants Boesenber Curcuma inodora, Boesenbergia siphonantha and Larsenianthus careyanus. in three chemical mutagens (1, 2 and 4% EMS, 0.5, 1 and 2% acridine and 100, 250 and 500 ppm colchicine) for 12 hrs. They recorded that Larsenianthus careyanus treated with both acridine and colchicine showed increased size in floral attributes.

e. Anatomical structure of the leaves and flowers.

1. Anatomical structure of the leaves

Data in Table (3) and Fig (5) indicated that azide sodium treatment at the dose of 0.05% decreased the No. of xylem rows of leaf to value (10 rows), as compared with the other azide sodium treatments and the control. On the contrary, the lowest value (9 rows) was obtained with concentration 0.1%. Also, Obtained results in Table (3 and Fig (5) showed that all treatments (0.05, 0.1, and 0.2%) of azide sodium decreased number of xylem vessels (56, 54 and 63) than the control (80). It was revealed from the data in Table (3) and Fig (5) that all treatments of azide sodium caused increase in thickness of lamina compared to control (115.76µm) exposure with azide sodium at 0.2% gave the highest values (205.87µm).

Table 3. Effect of sodium azide treatments on number of xylem rows, number of xylem vessels, thickness of lamina (µm), thickness of midvein (µm), length of vascular bundle (µm) and width of vascular bundle (µm) of H. bracteatum L. plant.

Treatment	No. of xylem rows	No. of xylem vessels	Thickness of lamina (blade) (µm)	Thickness of midvein (µm)	Dimension of bundle	
					Length of vascular bundle (µm)	Width of vascular bundle (µm)
Control	12	80	115.76	493.58	236.90	194.86
0.05%	10	56	143.26	450.04	221.66	200.78
0.1%	9	54	205.87	522.46	209.02	232.62
0.2%	11	63	138.18	722.34	215.40	189.10

SA=Sodium azide µm=Micrometer

Also, Data in Table (3) and Fig (5) concerning the effect of azide sodium treatments on thickness of midvein, the data indicated that treatment with azide sodium at 0.3% gave the highest value (722.34 µm) as compared with the control (493.58µm). Data presented in Table (3) and Fig (5) revealed that the treatment of azide sodium at 0.05% gave the maximum length of vascular bundle (221.66 µm) compared with the other treatments of azide sodium, while treatment of azide sodium at 0.1% gave the lowest value of length of vascular (209.02 µm) compared with the control plant (236.9 µm).The wider width of bundle was obtained from treated seeds with azide sodium at 0.1% (232.62 µm) ,compared with other treatments and control (194.86 µm).

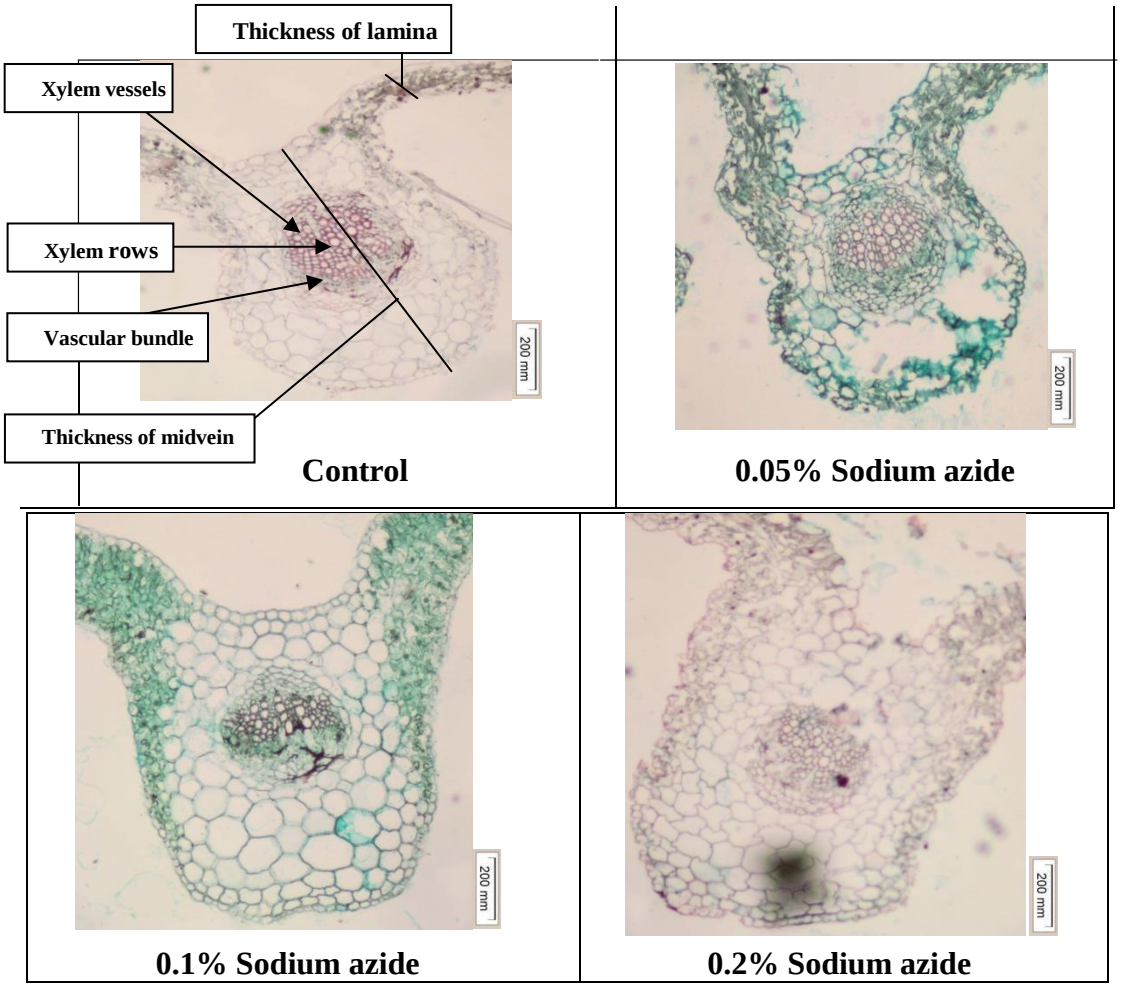


Fig 5. Anatomical structure of leaves in H. bracteatum as affected by sodium azide concentrations compared the control, (Transverse section through the leaves of the third developed leaf from the third branch of the upper part of the main stem of the plant (x=80)).

2. Anatomical structure of the flowers

Obtained results in Table (4) and Fig (6) showed that treated plants with sodium azide at 0.2% gave the large flowering bud diameter (6750 µm) compared the control plant (4725 µm), while the treatment that received sodium azide at 0.2% recorded clearly increase, the percentage increase reached to 42.85% compared with untreated plants. Also, receptacle diameter (µm) gave the largest diameter at 0.2% treated with sodium azide. Where the percentage of increase to 73.33% over the untreated plants.

Table 4. Effect of sodium azide treatments on flowering bud diameter (FBD) (µm) and receptacle diameter (µm) of H. bracteatum L. plant.

Treatments	Flowering bud diameter (FBD) (µm)	Receptacle diameter (µm)
Control	4725	2250
0.2% SA	6750	3900

SA=Sodium azide µm=Micromete

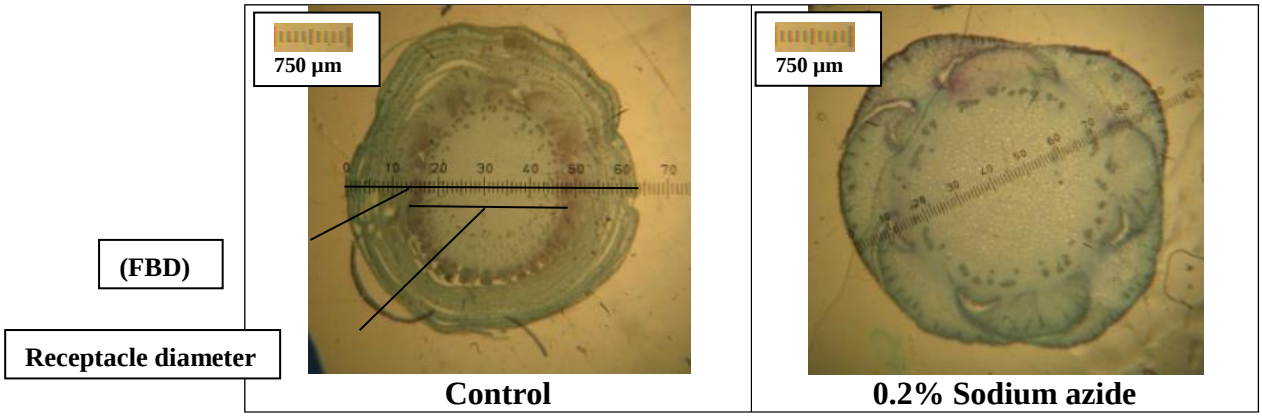


Fig. 6. Anatomical structure of flowers in H. bracteatum as affected by sodium azide concentration 0.2% compared the control, (Transverse section through the flowering bud (x = 40)).

In this regard, Krupa-Małkiewicz et al. (2015) induced chemical mutation in Liatris ligulistylis and Liatris pycnostachya using ethyl methane sulfonate (EMS) the seeds treated by two concentrations (2.0 and 5.0 mM) for 15 min, led to changes in the anatomy of leaves and inflorescence; Azmi et al. (2016) used various concentrations of 0, 50, 500, 1000, and 2000 mg L-1 to assess the efficacy of colchicine treatment on Phalaenopsis amabilis. For three or five days, treated colchicine flowers were nested under each concentration level and covered with aluminum foil to prevent evaporation. There were 40 plants used in the experiment, and colchicine treatments were given to 1-2 flowers per plant and repeated four times. At 500 mg L-1colchicine, they observed alterations in the stomata size of the abaxial leaf (5 days colchicine application).

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