J. of Plant Production, Mansoura Univ., Vol. 14 (7): 331 - 335, 2023

Journal of Plant Production

Journal homepage & Available online at: www.jpp.journals.ekb.eg

Effect of Chemical and Physical Mutagens on Vegetative Growth and Flowering of *Hibiscus Rosa-sinensis* L Plant

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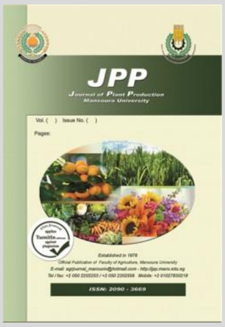

ABSTRACT

The experiment was conducted at the Experimental Farm of Horti. Depar., of Faculty of Agric., Moshtohor, Banha Univ, Egypt in two seasons 2020/2021 or 2021/2022 to evaluate the effect of mutagens (gamma at 100, 200, 300 Gy) and chemical mutagens (EMS) is a mutagenic, carcinogeni with formula C3H8SO3,(DEMS) formula (CH3O)2SO. at 0.1%, 0.2% and 0.3%. Hibiscus plant is considered one of the very important ornamental plants, The effect of mutagens on the growth and flowering of Hibiscus was studied, The ISSR-PCR technique was also used to determine the genetic variation among the hibiscus plants. Hibiscus were irradiated with four different levels of gamma radiation (control, 100, 200, 300 Gy) or EMS and DEMS) at a concentration of (0.00, 0.1%, 0.2% and 0.3%) However, the irradiation results showed that gamma rays at 100Gy treatment significantly increased plant height and number of leaves in the two seasons. The best treatments was achieved with Gy at 100 Gray, followed by Ethyle methane sulphonate at 0.1% gamma rays at 200 Gray treatments gave the next results, but gamma rays at 300 Gray caused a significant decrease in plant height in both seasons, and the optimal dose of radiation per plant was calculated between treatments because of the determination obtained in dry weight, and this factor used to determine is generally high. Gamma ray doses had adverse effects on the growth of hibiscus, and this study showed that high doses of irradiation (200 to 300 Gy) of hibiscus inhibited their growth and significantly decreased them.

Keywords: *Hibiscus rosa-sinensis*, Gamma, ISSR-PCR molecular marker technique.

INTRODUCTION

The use of mutagens is a rapid method used to improve the quantitative and qualitative traits of many plants. Mutagens can affect the chemical, cellular, physiological, and morphological properties of plant tissues and cells. (Adamu and Aliyu, 2007).

(Hibiscus rosa-sinensis L). (Malvaceae) is one of the perennial ornamental flowering shrub widely cultivated in Egypt due to the beauty of its flowers and the multiplicity of colors it is used to make fences in gardens commercial value as a landscaping plant Hibiscus flowers contain medicinal substances used to treat skin diseases (Nevid et al., 2011)

Gamma rays is part of the electromagnetic spectrum, which belongs to ionizing radiation. The biological effect of gamma rays depends on the ionization of plant cell atoms, especially in the presence of water, which works to produce free radicals in cells, and these chemical products can damage DNA molecules or chromosomes or may It affects a physiological or chemical effect that depends on the level of radiation. These effects include changes in metabolism, photosynthesis, accumulation of compounds inside the cell, and chromosomal composition, and accumulation of phenolic compounds in the plant cell. (Hamiduddin and Hussin, 2014).

Ethyl methanesulfonate (EMS) and Diethyl methanesulfonate (DEMS) is a mutagenic, teratogenic, and carcinogenic organic compounds with formula C3H8SO3. It produces random mutations in genetic material by nucleotide substitution; particularly through transitions induced by guanine alkylation. EMS typically produces only point mutations. Due to its potency and well-understood mutational spectrum, EMS is the most commonly used chemical mutagen in experimental genetics Kutscher and Shaham, (2014), Mutations induced by EMS exposure can then be studied in genetic screens or other assays. The experiment was conducted to study the effect of the mutagens on the vegetative, flowering, physiological, and chemical characteristics of Hibiscus (Ali et al., 2015).

MATERIALS AND METHODS

The present study was conducted during the two successive seasons of 2020 – 2021 and 2021/2022 at the Experimental Farm of Hortic., Depar., of Fac. of Agric., Moshtohor, Banha Univ., Egypt.

Plant material:

The cuttings of the Hibiscus plant were obtained from the Farm of the Faculty of Agric., Moshtohor, Banha Univ., Egypt. Therefore, investigate the effect of Gamma rays and chemical mutagens on vegetative growth, and flowering, chemical composition. Also, maybe induced mutations and genetic variations in the *Hibiscus rosa-sinensis L plant.*

The layout of the experiment: The experiment was in RCBD with three replicates.

Treatments:

Hibiscus rosa sinensis plants were irradiated before cultivation using gamma cells, at the (N C R T), Cairo, Egypt. Irradiation doses were (0,0,100,200,300 Gray or Ethyl sulfate and Dimethyl sulfate treatments application DMS solutions at 0.1, 0.2, and 0.3%) then the Plantlets were planted on 1/4/2020 and 2021 and 2021 and 2022 for the two summer seasons.

The achieved experiences can be summarized as follows:

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DOI: 10.21608/jpp.2023.218376.1249

Studied character:

Vegetative growth characters:

Plant height (cm) above the surface of the soil, Number of leaves/plant

Leaves fresh and dry weight (g/plant) All leaves of each plant, Number of branches/plant

Roots length(cm), Roots fresh and dry weighs(g) / plant

Flowering growth:

The number of flowers/plants, dry, Fresh weight of the flower.

DNA isolation procedure and Polymerase Chain Reaction:

The bulked DNA extraction was performed using the DNeasy plant Mini Kit (QIAGEN). The isolation protocol of DNA was as follows:

1- Plant culture was ground using liquid nitrogen to a fine powder, then, the powder was transferred to an appropriately sized tube.
2- Then, 400 µl of buffer AP1 and 4 µl of RNase, a stock solution (100 mg/ml) were added to a maximum of 100 mg of ground fungi culture and then vortexed vigorously.
3- The mixture was incubated for 10 min at 65ºC and mixed 2-3 times during incubation by inverting the tube.
4- Then, 130 µl of buffer AP2 was added to the lysate, mixed, and incubated for 5 min on ice.
5- Lysate was applied to the QIA shredder spin column sitting in a 2 ml collection tube and centrifuged for 2 min at maximum speed (10.000 rpm).
6- The supernatant from step 5 was transferred to a new tube without disturbing the cell-debris pellet. Typically, 450 µl of lysate was recovered.
7- Then, 0.5 volume of buffer AP3 and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting.
8- Then, 650 µl of the mixture from step 7 was applied through the DNeasy Mini spin column setting in a 2 ml collection tube. Then, centrifuged for 1 min at 8000 rpm and the flow-through was then discarded.
9- DNeasy column was then placed in a new 2 ml collection tube. Then, 500 µl buffer AW was added onto the DNeasy column and centrifuged for 1 min at 8000 rpm.
10-Then, 500 µl buffer AW was added to the DNeasy column and centrifuged for 2 min at maximum speed (10.000 rpm) to dry the column membrane.
11-DNeasy column was then transferred to a 1.5 ml microfuge tube and 100 µl of preheated (65ºC) buffer AE was pipetted directly onto the DNeasy column membrane. Then, incubated for 5 min at room temperature and centrifuged for 1 min at 8000 rpm to elute.
12-Elution was repeated once as described. A new microfuge can be used for the first elute. Alternatively, the microfuge tube can be reused for the second elution step to combine the elutes. According to Joshi *et al.* (1997); Collard and Mackill, (2009)

4-Gel Electrophoresis:

According to Xiong *et al.* (2011)

5-Gel reading and analysis

Techniques according to Adhikari *et al.* (2015).

Polymerase chain reaction (PCR) condition for SCoT according to (Nei and Li, 1979)

Gel preparation procedure techniques according to Mohamed *et al*., (2015)

Data analysis

The similarity matrices were done using SPSS (Yang and Quiros, 1993).

Chemical analysis: Plant samples were used for chemical analysis as follows:

Chlorophyll a, b content in fresh leaves, Nitrogen, Phosphorus, Potassium, Calcium, and Sodium content in leaves.

Determination of chlorophyll content: Total chlorophyll content was determined in fresh leaves of plants according to **Wintermans and De Mots (1965)**

The dry matter of leaves was ground, and 0.2 g of each sample was digested with ulphuric acid and hydrogen peroxide to determine the element's content (Guzman and Romero,1988).

Nitrogen was estimated according to the micro Kjeldahl according to (A.O.A.C.,1980) using nitrogen distillation instrument model Buchi323.

Phosphorus was colorimetrically according to (Chapman and Pratt, 1961) using spectro- photometer model (spectronic 21), Potassium, calcium, and sodium was determined by using the flame photometer model (corning 410).

Statistical analysis:

All obtained data in the study were subjected to Analysis of Variance (ANOVA) as a simple experiment RCBD design and comparing between means were achieved by applying the L.S.D. at 5 % according to Snedecor and Cochran (1989).

RESULTS AND DISSCUSION

I- Effect of Gamma rays on the growth of *Hibiscus rosa-sinensis L* plant during the two seasons of 2020/2021 and 2021/2022.

1. Plant height and the number of leaves:

Table (1) shows that gamma rays at 100 Gy treatment significantly increased the plant height, leaves fresh and dry weight, and the number of leaves in the two seasons. The best treatments were achieved with 100 gray, followed by Ethyle methane sulphonate at 0.1% and gamma radiation at 200 gray treatments, but gamma rays at 300 gray caused a significant decrease in plant height in both seasons. These results agreed with those obtained by Ali (2002) on *Polianthes tuberosa*, Also, EL- Noby (2002) on *Delphinium* and *Matthiola* found that the low doses of gamma rays 25-75 gray significantly increased plant height. However, irradiated *Delphinium* and *Matthiola* seeds with high doses of gamma rays 300 grays significantly decreased plant height. Similar trends have been reported by Kong *et al.*, (2007) on beans assuming

5- Number of branches and Roots length:

Table (2) shows that gamma irradiation at doses 100 Gray treatment highly significantly increased number of branches and roots length followed by gamma at 200 gray treatments, but the control produced the next value in this concern, in two seasons. Whereas gamma irradiation at doses of 300 gray significantly decreases the number of branches and roots length in both seasons. Generally, in both seasons, treating *Hibiscus* plant with gamma rays at dose 100 gray increased the number of branches and roots and these results agreed with those obtained by Patel *et al.* (2018) on gladiolus. It was observed that there was a negative correlation between the number of branches and root length and survival percentage with the different treatments.

Table 1. Effect of Gamma rays, Ethyl Methane Sulphonate, and Diethyl Methane Sulphonate on vegetative growth of *Hibiscus rosa-sinensis L* plant during two seasons 2020-2021 and 2021- 2022.

Character		Plant height (cm)		Number of leaves		Leaves fresh weight g/plant		Leaves dry weight g/plant	
Treatment	**Season**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
Control		195.50g	195.50g	73.5g	75.90 g	62.10f	57.50f	6.88f	7.22f
Gamma rays	100Gy	189.75a	192.05a	57.50a	57.50a	73.60a	75.90a	8.25a	8.33a
	200Gy	166.75b	170.20b	56.35b	56.35b	46.00b	48.30b	6.84b	6.90b
	300Gy	155.25h	152.95h	50.60h	50.60h	33.35h	36.80h	6.52h	6.52h
	mean	170.58	171.73	54.82	54.82	50.98	53.67	7.20	7.25
Ethyl Methane Sulphonate	0.10%	152.95c	154.10c	50.60c	50.60c	29.90b	32.20b	5.71b	5.89b
	0.20%	149.50d	151.80d	43.70d	43.70d	28.75d	32.20d	5.33d	5.44d
	0.30%	143.75e	144.90e	31.05e	31.05e	25.30e	29.60e	4.83e	4.85e
	mean	148.73	150.27	41.78	41.78	27.98	31.33	5.29	5.39
Diethyl Methane Sulphonate	0.10%	138.00d	139.15d	31.05d	31.05d	20.70c	26.45c	4.80b	4.95b
	0.20%	135.70f	134.55f	26.45f	26.45f	17.25c	19.70c	4.57c	5.11c
	0.30%	80.50g	82.80 g	25.55g	25.55g	13.80g	17.25g	3.41e	3.61e
	mean	118.07	118.83	27.68	27.68	17.25	21.13	4.26	4.56

Means in each column have the same letter are not significant at 5%.

Effect of Gamma rays, Ethyl Methane Sulphonate, and Diethyl Methane Sulphonate on number of flowers, Fresh and dry weight of flower of Hibiscus plant of *Hibiscus rosa-sinensis* L plant during the two seasons 2020 -2021 and 2021- 2022.

Gamma rays at a dose of 100 gray significantly increased the number of flowers, fresh and dry weight of the flower of the Hibiscus plant, compared with the control in Table (3) in both seasons, respectively. Gamma rays at a dose of 200 gray gave the next results, but Diethyl Methane Sulphonate at 0.10% produced the next value in this concern, in both seasons. Whereas gamma rays at a dose of 300 gray caused a significant decrease in the number of flowers, fresh and dry weight of the flower of Hibiscus plant in this concern in both seasons, respectively.

On the other side, gamma rays at a dose of 100 gray produced the best value in the connection in both seasons. These results agree with those reported by Karki and Srivastava (2010). This may be because Diethyl Methane Sulphonate at 0.1% or 0.2% is more suitable for growth-promoting hormones which enhance the growth of flowering (Sudha, 2016). It was found that the stimulating effect of gamma rays on growth may be due to an increase in cell elongation or an increase in the number and size of cells and an increase in the metabolic rate that enhances the stimulating effect of plant hormones.

Table 2. Effect of Gamma, EMS, and DMS on vegetative growth of *Hibiscus rosa-sinensis L* plant during two seasons 2020-2021 and 2021- 2022.

Character		Number of branches		Roots length		number of flower		Fresh weight of flower		dry weight of flower	
Treatment	**Season**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
Control		6.05c	6.90c	16.30e	16.10e	10.35d	10.35d	1.22h	1.23h	0.55h	0.56h
Gamma rays	100Gy	10.33a	10.35a	45.25a	46.00a	16.10a	14.95a	2.55a	2.90a	1.16a	1.22a
	200Gy	8.05b	9.20b	25.45b	24.15b	12.65b	13.80b	2.50b	2.90b	1.14b	1.13b
	300Gy	2.88 h	3.45 h	7.75 i	8.05 i	3.45 i	4.60 i	0.79 i	0.84 i	0.35 i	0.38 i
Ethyl Methane Sulphonate	0.10%	6.90 c	6.90 c	18.25 c	18.20 c	8.05 f	9.20 f	2.05 c	2.09 c	1.11 c	1.12 c
	0.20%	8.75 d	9.20 d	13.80 f	14.95 f	9.20 e	10.35 e	1.97 d	2.50 d	0.89 c	1.13 c
	0.30%	4.60 g	4.60 g	9.20 h	11.50 h	6.90 g	6.90 g	1.74 e	1.80 e	0.79 e	0.81 e
Diethyl Methane Sulphonate	0.10%	5.75 d	5.35 d	18.30 d	18.95 d	11.50 c	12.65 c	1.69 f	1.78 f	0.76 e	0.80 e
	0.20%	4.60 e	4.20 e	11.50 e	13.80 e	6.90 e	8.05 e	1.32 e	1.40 e	0.6 e	0.63 e
	0.30%	6.90 f	6.75 f	10.35 h	10.35 h	5.75 h	4.60 h	1.25 h	1.35 h	0.56 g	0.61 g

Means in each column have the same letter are not significant at 5%.

Table 3. Effect of Gamm, EMS, and DMS on the chemical composition of *Hibiscus rosa-sinensis L* plant during two seasons 2020 - 2021 and 2022.

Character		Nitrogen%		Phosphorus %		Potassium%		Calcium%	
Treatment	**Season**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
Control		2.91c	2.81c	0.87b	0.83b	2.01h	1.88h	1.03h	1.08h
Gamma rays	100Gy	3.41a	3.25a	0.91a	0.93a	2.03a	2.09a	1.91a	1.92a
	200Gy	2.88c	2.89c	0.73c	0.76c	1.89b	2.04b	1.84c	1.85c
	300Gy	2.18e	2.17e	0.27g	0.28g	1.69i	1.71i	0.99i	0.84i
	mean	1.18	1.17	0.63	0.66	1.87	1.95	1.86	1.87
Ethyl Methane Sulphonate	0.10%	2.91b	2.87b	0.84b	0.83b	1.86c	1.85c	1.87c	1.86b
	0.20%	2.38d	2.1d	0.71c	0.72c	1.51g	1.53g	1.11g	1.13g
	0.30%	2.38d	2.1d	0.5h	0.53h	1.38h	1.45h	1.01h	1.06h
	mean	2.56	2.36	0.64	0.66	62.97	1.64	1.18	1.24
Diethyl Methane Sulphonate	0.10%	2.1e	2.1e	0.70d	0.71d	1.80d	1.82d	1.50d	1.54d
	0.20%	1.5g	1.45g	0.35e	0.36e	1.74e	1.75e	1.41e	1.45e
	0.30%	1.81f	1.84f	0.30f	0.31f	1.53f	1.58f	1.16f	1.21f
	mean	1.80	1.80	0.62	0.66	1.66	1.71	1.08	1.13

Means in each column with the same letter are insignificant at 5%.

Effect of Gamma Gamma, EMS, and DMS on the chemical composition of *Hibiscus rosa-sinensis L* plant during two seasons 2020-2021 and 2021- 2022.

Phosphorus percentage:

The obtained results of the P% of Hibiscus are tabulated in Table (3). These data revealed that in both seasons, control and gamma rays at 100 gray treatments gave the most promising effect in increasing the percentage of P% in the first and second seasons. Also, Diethyle methane sulphonate at 0.2% showed an increase in P% in leaves and ranked third in this concern in the two seasons. These results agree with those obtained by EL-

Esawy (1995) obtained an increase in the percentage of N, P, K, in the shoots.

3. Potassium, Calcium, and Sodium percentages

Table (3) cleared that, using gamma rays at 100 Gy showed to be the best effect for increasing leaf nitrogen, potassium, calcium, and sodium, while gamma rays at 200 gray gave second resulted in the first and second seasons, the untreated plants and diethyle methane sulphonate at 0.2% had the lowest percentage of nitrogen, potassium, calcium and sodium in the same Table. However, treating *Hibiscus* with gamma rays at 100 gray gave the maximum Ca% and Sodium% when compared with other treatments, while gamma rays at 200 gray and Ethyl Methane Sulphonate gave the next result. in both seasons, agreeing with those obtained by EL-Noby (2002) found that irradiated *Delphinium* and *Matthiola* seeds with low doses of gamma rays 25-75 gray and 25 and 50 gray significantly increased P and K and N% and P % in the plants with a peak at 25 gray. However, using low doses of gamma rays 25 gray aand 100gray significantly increased N contents. Also, with high doses of gamma rays, 100 gray significantly decreased K contents in the plants.

Effect of Gamma, EMS, and DMS on ISSR PCR genomic DNA of *Hibiscus rosa-sinensis L* plant during two seasons 2020 - 2021 and 2022.

ISSR PCR genomic DNA ISSR PCR was used to investigate the extent of changes in DNA due to treatments (0.0, 100,200, and 300 Gy). Six primers out of twenty-eight random primers successfully amplified DNA fragments from *Hibiscus rosa sinensis L* DNA samples (Table 1 and Figure 1).

The results indicated changes in the structure of DNA molecules using different treatments compared to control using six primers (SC0T 1, SC0T 4, SC0T 6, SC0T 8, SC0T 9, SC0T 13, SC0T 15) (Table 6, Figure 1). A total of 54 fragments were visualized across the six primers (Table 4). Genomic DNA polymorphisms due to the treatments are shown in Figure (1). The percentage of polymorphisms was (50%, 66.66%, 33.33%, 33.33%,66.66%, 50%, and 0% respectively) (Table 4).

The ISSR results showed that the polymorphic molecules structural changes DNA with gamma rays and ethyl nissan sulfate treatment at high concentrations of gamma rays 300 Gy, and the dotted bands disappeared above 1500 bpt and appeared starting from 500 bpt in the molecular range 500 bpt due to Ganapathi *et al.* (2008) studied the effect of gamma irradiation on bananas using RAPD-DNA analysis. They noted the changes in the DNA bands, with the main changes in the RAPD profiles of the current investigation being the appearance or disappearance of different bands with variations in their intensity. These effects may be due to structural rearrangements in DNA caused by different types of DNA damage. The emergence of new domains usually results from various structural changes in DNA (breaks, translocations, deletions, etc.). (Danylchenko and Sorochinsky, 2005).

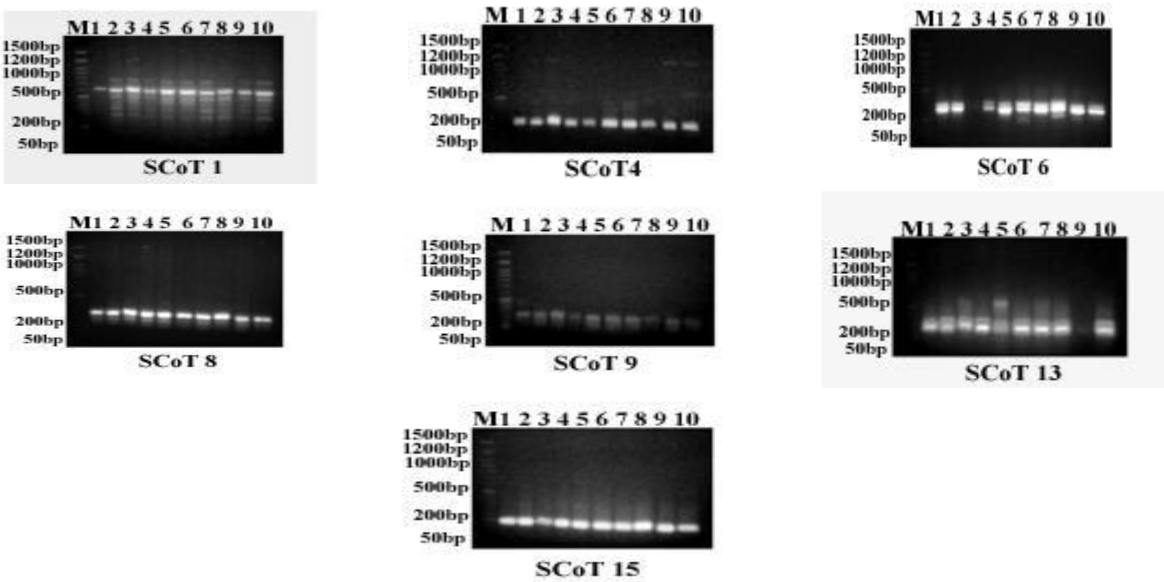

Fig. 1. DNA Inter Simple Sequence Repeats (ISSR) for *Hibiscus rosa-sinensis* treated with different gamma, EMS, and DMS doses during two seasons 2020 - 2021 and 2022.

Table 4. DNA Inter Simple Sequence Repeats (ISSR) for Hibiscus plants during 2020 – 2021 and 2021 -2022 seasons.

Primer Name	Sequences	Total Band	Monomorphic Band	Polymorphic band	Unique Band	Polymorphic %
SC0T 1	ACGAG ACCA TGG CGA CCCAG CGC	6	3	3	1	50%
SC0T 4	ACGC ATG GCACT ACC ACCC GACA	6	2	4	1	66.66%
SC0T 6	CAA TCGG CTAG TCCACA CTA CAG	3	2	1	-	33.33%
SC0T 8	ACAA CATGG GCT ACACC ACT GAG	3	2	1	1	33.33%
SC0T 9	ACA AATG GCCT ACCCC ACGT GCC	3	1	2	1	66.66%
SC0T 13	ACC ATAG GCT ACCC ACGGA GCGA	4	2	2	-	50%
SC0T 15	CTCA TGCGCTG CTA CCA CGCG GCT	2	2	-	-	-
Total		27	14	13	4	48.14%

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تأثير أشعة جاما علي تحسين بعض الصفات الإقتصادية والتغيرات علي المستوي الجزيئي علي نبات الهبسكس.

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الملخص

تهدف هذه الدراسة إلى معرفة تأثير أشعة جاما وكذلك دراسة تأثير الإيثايل ميثان سولفانات وداي ميثايل سولفانات على النمو الخضري والزهري والمحتوى الكيماوي وكذلك إنتاج بعض الطفرات على نبات الهبسكس . أجريت هذه الدراسة بمزرعة الزينة- بقسم البساتين-كلية الزراعة بمشتهر جامعة بنها في الفترة من 2020 / 2021 و2021 / 2022 في اصص تحتوي علي تربة طينية ورملية بنسبة 2:1 وتم تصميم التجربة بنظام القطاعات كاملة العشوائية حيث تم تعريض شتلات الهبسكس لأشعة جاما بمركز بحوث الإشعاع بمحطة الطاقة النووية بجرعات 100و 200 و300 جراي , كما تم رش باقي المعاملات بإستخدام إيثايل ميثان سولفانات وداي ميثايل سولفانات بتركيز 0.1 ,0.2 و 0.3% لكلا المادتين علي نبات الهبسكس. كما تم إستخدام تقنية ISSR – PCR وذلك للوقوف علي هل هناك طفرات على المستوى الخلوي أم لا وإثبات حدوث الطفرات من عدمه. النتائج التي تم الحصول عليها تشير إلى أن المعاملة بأشعة جاما 100 جراي أدت إلى زيادة كل صفات النمو الخضري والزهري والمحتوي الكيماوي لنبات الهبسكس في كلا الموسمين. كما أن كل المعاملات باستخدام المواد الكيماوية بكل تركيزاتها أدت لحدوث زيادة معنوية في جميع صفات النمو الخضري والزهري والتركيب الكيماوي المدروسة ماعدا أشعة جاما 300 جراي أدت لحدوث نقص معنوي في الصفات سابقة الذكر وذلك مقارنة بالكنترول ولكن أفضل المعاملات كانت أشعة جاما عند 100 جراي ثم 200 جراي. وعلي ذلك فإنه لكي يتم للحصول علي نباتات هبسكس جيدة جيد وسريع نمو خضري وزهري قوي ومبكر يمكن التوصية بالآتي: تعريض نبات الهبسكس لأشعة جاما لجرعات 100 و200 جراي ,ورش الهبسكس بالايثايل والداي ايثايل سلفات لتركيز 0.1 ،0.2%