



GENETIC IMPROVEMENT OF *Calendula officinalis* L. THROUGH MUTATION INDUCTION USING GAMMA IRRADIATION AND CHEMICAL MUTAGENS

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ABSTRACT

Calendula is one of the basic plants of ornamentals. It is used in various purposes as cut flowers and in gardens. They are rich with active compounds such as chlorophyll a and b, phenols, flavonoid and carotenoids. The present study focused on mutation induction in order to improve the morphology of the plant which can increase the turnout of its market and to examine which mutagen will make the most noticeable and permanent improvement of the plant. For the mutagens, seeds were treated with three doses of gamma ray (2500, 5000, 7000 Gy) and two chemical mutagens; colchicine and EMS; which were applied at concentrations of 1000, 3000 and 10000 ppm. With the aim to measure the plant improvement, we measured the plant morphology, its active compounds and protein profiling before and after treatments with both γ -radiation and chemical mutagen to see which one has the most effective imprint on the plant. The morphological parameters data showed the highest plant high by the two doses; gamma dose of 7000 Gy and EMS concentration 10000 ppm, while the lowest dose of colchicine (1000 ppm) made the most impact on the plant height. As for the highest number of leaves/plant, it was obtained at 5000 Gy and 10000 ppm of EMS, while 1000 ppm of colchicine had the same effect. But the number of flowers/plant was not affected by the gamma radiation and colchicine but increased significantly by 10000 ppm of EMS. Surprisingly, the flower diameter was not affected

by EMS while decreased at 2500 Gy and 3000 ppm of colchicine. Considering the importance of flower shape, all treatments either gamma radiation or chemical mutagens showed noticeable changes. Whereas, the biochemical parameters measuring the active compounds, both Chl-a, Chl-b and carotenoids contents increased at 5000 Gy, while flavonoid increased at 2500 Gy. Although, all doses of gamma ray eliminated the phenols content. While the lowest used concentration of colchicine (1000 ppm) increased both of Chl-a, Chl-b, flavonoid and carotenoids, even though the 10000 ppm of colchicine increased the phenol. On the other hand, the highest concentration of EMS (10000 ppm) increased Chl-a and Chl-b, while carotenoids, flavonoid and phenol increased at 3000 ppm of EMS. The protein-profile analysis showed fluctuation in the gene expression. The highest performance appeared at 7000 Gy of gamma treatment, 1000, 3000 ppm of colchicine, while the same result was obtained by EMS at 3000 ppm.

Keywords: *Calendula officinalis*, mutation, gamma radiation, EMS, colchicine, protein-profile analysis, Chl-a, Chl-b, flavonoid, phenols, carotenoids.

INTRODUCTION

The floriculture has become a very important industry in last decades. The development of new and novel cultivars of ornamentals is infinite and a breeder always has goals to work towards this

(Datta, 2009). *Calendula officinalis* L. (pot marigold) is a member of Asteraceae family, with $2n=32$ chromosome. The vegetative parts of the plant are green, the stems covered by fine hairs. The flower color varies from yellow to orange (Filipovic et al 2016). There are ray and disc florets in the inflorescence; it could be blossom out in the spring (Jan et al 2017). The wild *Calendula* is native to Egypt and Mediterranean region then grows in the southern, eastern and central Europe and in the north-west of Africa (Nejad and Shakib, 2013). Importance of *Calendula* is due to its use as an economic crop. It is grown as a flowering bedding plant, a cut flower or pot plant. In addition, pot marigold is used in human medicine (Hamzawy 2013), veterinary medicine, cosmetics (Pintea et al 2008) and nutrition (Acikgoz et al 2017). The florets of orange cultivars are used as a source of the spice saffron (Marieschi et al 2012). Phenolic compounds (flavonoids and phenolic acids) are both abundant in marigold (Frum, 2017). It also contains carotenoids (Khalid and Teixeira da Silva, 2012). *Calendula* has medicinal properties used as anti-viral, anti-inflammatory, anti-microbial (Mohammad and Kashani, 2012; Verma et al 2018), anti-tumor (Ashwlayan et al 2018), the genotoxicity and anti-genotoxicity, antioxidant effects and cytotoxic activity (Ashraf et al 2017). *Calendula* is considered one of the important plants in the Egyptian flora that includes more than 2000 plant species. The environment and climate are suitable for its production as ornamental and medicinal plants, which are promising as economic sources; especially there is an increase in the world demand for the Egyptian medicinal plants (Hendawy et al 2019).

Mutation is the most important factor in evolution, and induced mutagenesis has become widespread in the biological sciences, for the genetic base of germplasm for plant breeding and as a tool for functional genomics. Induced mutagenesis was successful in improving many ornamental plants such chrysanthemum, gerbera and gladiolus, they are altered in flower and leaves characters (color, shape, size) and physiological traits (flowering time tolerance to stresses) Kayalvizhi et al (2017).

Genetic markers such as morphological, biochemical traits (isozymes and protein profiles) and DNA based molecular markers are powerful tools for the analysis of genetic diversity and relatedness among genotypes, species and large populations of plants. SDS-protein polymorphism is served as genetic markers as they are direct products of gene expression (active genes) quite polymorphic and generally heritable (Mukhlesur et al 2004). SDS-

protein polymorphism profiles reflect the changes in the active part of the genome. Polyacrylamide gel electrophoresis (PAGE) is generally favored technique for rapid analysis (Raymond et al 1991) due to its validity and simplicity for describing genetic variations (Ahmed and Slinkard, 1992). This technique has been used effectively to decipher genetic diversity among/ between genotypes in different plant species (Mukherjee and Datta, 2008).

The objective of this research is to improve *calendula* plant using different levels of gamma irradiation, different concentrations of colchicine and EMS as mutation induction techniques on morphological and biochemical traits (active components and protein).

MATERIALS AND METHODS

This study was carried out in the Department of Genetics, Faculty of Agriculture, Ain Shams University, Shoubra-Elkheima, Egypt and Horticulture Research Institute, Agriculture Research Center, Giza (planting experiment), during the period from 2013 to 2020. For the radiation experiment, seeds were treated in Atomic Energy Authority, Nasr City, Cairo, Egypt. For the SDS-protein profiling analysis was carried out at the Biotechnology lab at Faculty of Agriculture, Cairo University Research Park (CURP).

1. Plant materials

Calendula officinalis L. seeds were provided by Horticulture Research Institute, Agriculture Research Center. These plants have been assessed by morphological, biochemical genetic traits and the seeds were sown under greenhouse conditions.

2. Methods

A. Gamma radiation

Seeds were exposed to gamma rays from the Cobalt-60 source (emitting 1KRD per 30 min. at the time of radiation was used) and kept in dark until grown in the next day. Four doses were applied 0, 2500, 5000 and 7000 Gray (Gy). Seeds were sown individually during the 2013/2014 season for the first generation (M1). Then it was planted during the 2014/2015 season for the second generation (M2).

B. Chemical mutagens

Seeds were soaked in the three different concentrations (1000, 3000 and 10000 ppm) of colchicines or Ethyl Methane Sulfonate (EMS), 6 hours in the dark, while the seeds for the control were soaked in water before they were sown in the green-

house. The survived plants of M1 generation of *Calendula* of each treatment (radiation and chemicals) were selected and self-pollinated, in order to obtain the M2 seeds.

3. Morphological measurement

All measurements were taken on untreated and treated plants. During experimental period, some yield-related morphological traits were measured at flowering time; plants height (cm), flower diameter (cm), number of leaves/plant and number of flowers/plant.

4- Chemical measurements

The quantitative chemical measurements of biologically active substances Chlorophyll-a, Chlorophyll-b, in leaf samples and total carotenoids in flower were colorimetrically determined (mg/100g fresh matter) according to **Dere et al (1998)**. The determination was conducting using acetone (85% v/v) as a blank at wavelengths of 662, 644 and 440 nm, respectively. Total flavonoids content was measured according to **Zhuang et al (1992)** and expressed as mg catechin equivalents per 100 g fresh weight. Total phenolic were analyzed spectrophotometrically using the method described by **Swain and Hillis (1959)**, results were expressed as gallic acid g/100 g FW.

4. Statistical analysis

Data were analyzed based on morphological traits with ANOVA procedure using SAS7. **Duncan (1955)** test was further applied to compare treatments' means at a 5 % level of significance.

5. SDS-PAGE analysis

SDS-protein electrophoresis (SDS-PAGE) was performed according to the method of **Laemmli (1970)**. Seeds were taken from M1 and M2 of treated and untreated plants. They were ground in liquid nitrogen (100 mg) and transferred to Eppendorf tubes with 300 µl saline solution, and then centrifuged at 13,000 xg for 5 min at 4°C. Five hundred µl acetone was added to the supernatant (100 µl) overnight at -20°C, and then centrifuged at 13,000 xg for 3 min 4°C. Mix (1:4) sample: sample buffer (10% SDS, 20% Glycerol, 0.2M tris PH 6.8, 10mM beta-mercapto-ethanol and 0.05% bromophenol blue) and was heated on 95°C for 5 min prior to loading. The resolving gel was 15% acrylamide (w /

v). The protein fractionation was performed at a constant voltage of 80 V, 4°C for 4 hr. After run, the gel was stained in 50% dH₂O, 40% methanol, 10% glacial acetic acid and 0.1% coomassie brilliant blue solution for 20 min with gentle agitation. The gel was distained in 50% dH₂O, 40% methanol and 10% glacial acetic acid) solution until gel background became clear. Gel documentation was with G: Box Syngene model 680XHR.

RESULTS AND DISCUSSION

Calendula officinalis was treated with Gamma radiation and two chemical mutagens: colchicine and EMS. The analysis of variance (ANOVA) for the studied parameters showed significant differences among mutagens concentrations. Data were recorded on the plants at maturity stage (flowering) in the two seasons for M1 and M2 (**Table 1**). M2 results were more preferable than M1 because it includes genetic changes, however, M1 showed physiological changes.

1. Morphological parameters

Morphological parameters in *Calendula* using gamma irradiation

Plant height: plant height increased significantly with 7000 Gy, however, 5000 Gy showed intermediate value of plant height, while the shortest height was observed at 2500 Gy (**Table 1**). On the contrary, these results are disagreed with **Kaur et al (2017)** who found reduction in plant height under all doses of gamma radiation, and with **Tiwari and Kumar (2011)** who found increase in the plant height at 2500 Gy in M1.

Number of leaves/plant: average number of leaves/plant was significantly affected by the different concentrations of gamma radiation. The highest number of leaves/plant (83.30) was detected at 5000 Gy, meanwhile, at 7000 Gy and 2500 Gy the number of leaves per plant decreased to 60.00 and 53.30, respectively. There was a trend towards a gradual increase in the number of leaves/plant as mutagen concentration increased from concentration 2500 to 5000 Gy and reduced in the high concentration of 7000 Gy (**Table 1**).

Number of flowers/plant: the number of flowers/plant was not affected by any gamma radiation doses compared with the control (**Table 1**). These results didn't agree with **Kaur et al (2017)** findings which showed decrease in flowers number from mutant to the control.

Flower diameter: the differences between the mutagen doses were significant compared to the control. In the M2-generations, the lowest mean inflorescence diameter was 3.50 cm that observed at the concentration of 2500 Gy (**Table 1**). This agreed with **Kaur et al (2017)** who showed decrease from the control.

As for flower shape: A noticeable change in the shape of the flowers after treatment by gamma ray and some of these changes revealed asymmetry disk as compared to the untreated flowers as shown in **Fig. (1)**.

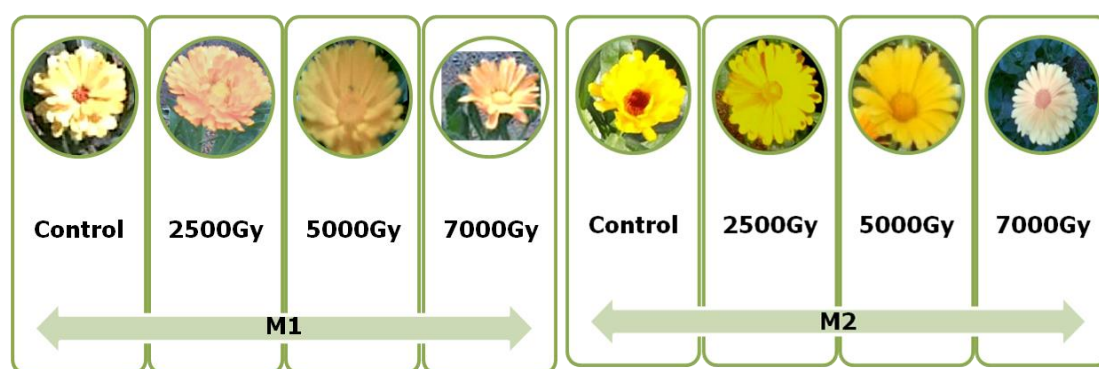


Fig. 1. *Calendula* inflorescences before and after gamma radiation treatments in M1 and M2 generations

Morphological parameters in *Calendula* using colchicine

Plant height: effects were observed at the higher concentrations. Plant height increase were obtained at lower colchicine concentrations (1000 ppm) (**Table 1**), whereas negative at the 3000 ppm colchicine concentration for both M1 (42.60 cm) and M2 (47 cm) generations. This agreed with **El-Nashar and Ammar (2016)** who found that lower colchicine concentrations (400 and 800 ppm) increased plant height while negative effect was obtained at higher concentration (2000 ppm).

Number of leaves/plant: there were significant differences detected among *Calendula* plants. The highest leaf number (52.60) was detected at high colchicine concentration (10000 ppm) (**Table 1**). This result was contradicted with the result of **El-Nashar and Ammar (2016)** that showed that the lower concentration increased the number of leaves.

Number of flowers/plant: the number of inflorescences/plant was not affected by the colchicine treatments (**Table 1**). This result was not similar to **El-Nashar and Ammar (2016)** who found decrease

in flower number by increase the colchicine concentration. While **Roxana et al (2008)** showed increase of flower number using colchicine.

Flower diameter: in the M2-generation, the lowest mean inflorescence diameter was 3 cm at the concentration of 3000 ppm, while the highest diameters were detected at 1000 ppm (5 and 4 cm) for the M1 and M2 generation, respectively, compared with the control (3.33 cm) (**Table 1**). This result disagreed with **El-Nashar and Ammar (2016)** who found increase in the flower diameter over the control.

As for flower shape: there was a noticeable change in the shape of the flowers after the treatment with colchicine and some of them showed asymmetry disk compared to the untreated flowers (**Fig. 2**).

Morphological parameters in *Calendula* using EMS

Plant height: it increased equally at 1000 and 3000 ppm, respectively, in M2 generation (**Table 1**), while the highest height was obtained at 10000 ppm. This was like **El-Nashar (2012)** who found greatest

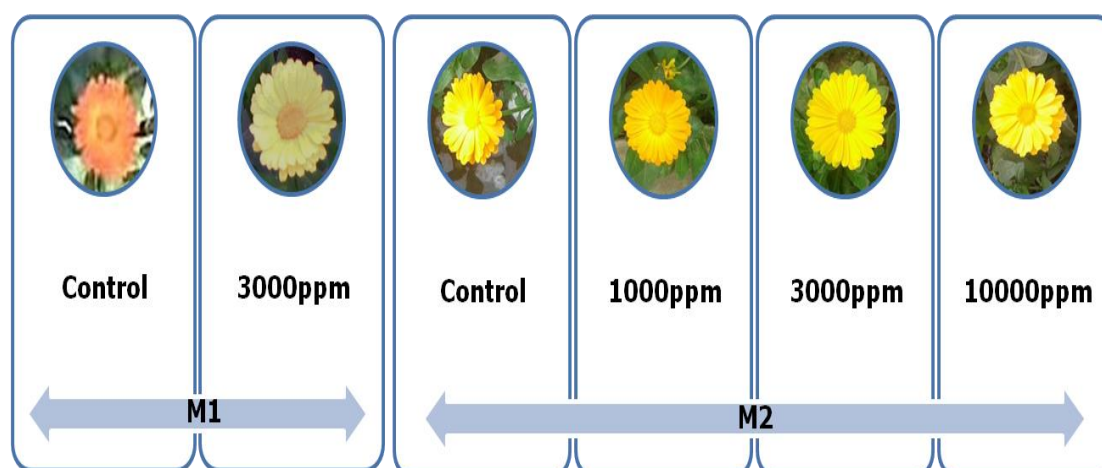


Fig. 2. *Calendula* inflorescences before and after colchicine treatments in M1 and M2 generations

height at 1000 ppm of DES in *Calendula* and Samatadze et al (2019) showed low concentration of EMS (250 ppm) and DES (500 ppm) in *C. officinalis* cv. *zolotoe*.

Number of leaves/plant: at high EMS concentrations (3000 and 10000 ppm), an increase in the number of leaves/plant was observed; the highest leaf number/plant was 65 cm (Table 1). These results contradict with EI-Nashar (2012) who showed decrease in the leaves number by increase the concentration of DMS.

Number of flowers/plant: it was affected by EMS treatment only at 10000 ppm in M2 (Table 1). This result agrees with EI-Nashar (2012) found that higher concentration of DMS (4000 ppm) gave higher number of flowers/plant. Samatadze et al (2019) found increase in the flower number by all treatment of DMS in various degrees.

Flower diameter: there were no significant differences between EMS used concentrations in both M1 and M2 generations compared to the control (Table 1). The result disagrees with EI-Nashar and Asrar (2016) showed lowest flower diameter at 5000 ppm of DMS and heights diameter at low concentration 2000 ppm. Samatadze et al (2019) found increasing in the flower diameter by all treatment of DMS in various degrees.

Flower shape: there was a noticeable change in the shape of the flowers after the treatment with EMS and some of the treated plants showed asymmetry disk compared to the untreated flowers (Fig. 3).

2. Statistical analysis of the morphological parameters of *Calendula* under the treatments

Table (1) combined the results of all treatments on the morphological traits in M1 and M2 generation to detect the preferable treatment to obtain a good plant in M2 which exposure genetic changes, while M1 had physiological changes.

As shown in Table (1), the significant differences in plant height was showed at 2500 Gy that give the short plant (30.67 cm) in M2, while the highest plant was induced by EMS 10000 ppm in M2 (69.6 cm). For the number of leaves per plant the gamma irradiation at 5000 Gy showed the significant differences that gave the highest number of leaves per plant (83.3) in M2 between all treatments. The number of flowers per plant showed Significant differences at 10000 ppm of EMS that the highest number was (21.3) in M2. The lowest mean of flowers diameter (3 cm) showed a significant differences at the concentration of 3000 ppm of colchicine in M2.

3. Biochemical parameters

Biochemical Parameters in *Calendula* using gamma irradiation

Effect of different gamma radiation doses on Chl-a, Chl-b content, phenol compounds, flavonoid and carotenoids is shown in Table (2) and Fig. (4). The three treatments (2500, 5000 and 7000 Gy) increased Chl-a and Chl-b content compared to the

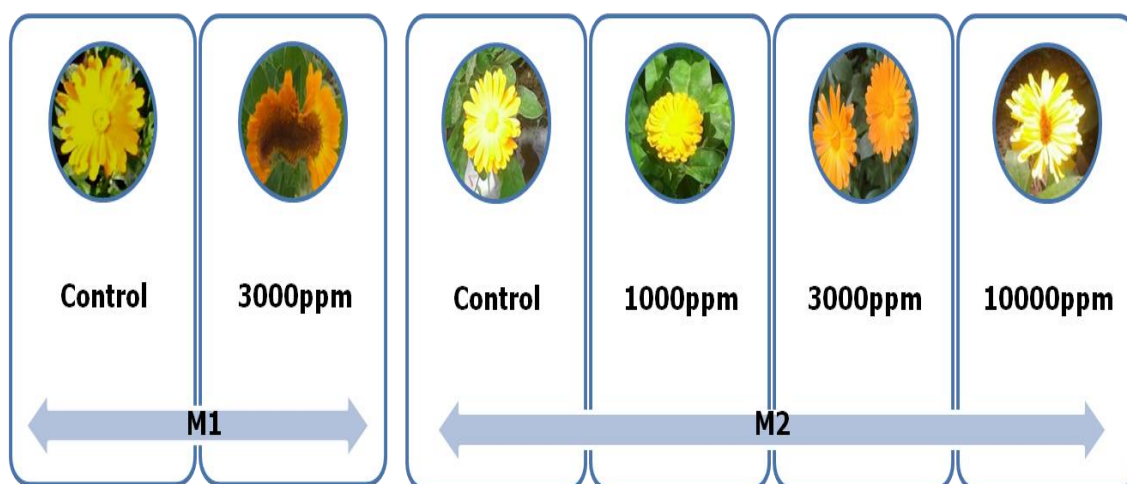


Fig. 3. *Calendula* inflorescences before and after EMS treatments in M1 and M2 generations

control. Obvious increase of Chl-a and Chl-b content was detected with the increase of radiation dose up to (5000) Gy. **Kaur et al (2017)** recorded decrease in both chlorophyll a & b by using of gamma radiation. Seeds treated with gamma radiation imposed a high impact on total carotenoids. The treatment with 2500 Gy and 5000 Gy showed increase in carotenoids contents, while plants exposed to 7000 Gy showed decrease in it. **Kaur et al (2017)** demonstrated that carotene decreased by increasing the dose of gamma ray. A slight increase in flavonoid content was observed with 2500 Gy dose compared to the control plants, while 5000 and 7000 Gy doses showed decrease in the flavonoid levels. The application of gamma radiation at the three doses (2500, 5000 and 7000 Gy) caused decrease in the phenolic content compared to the control ones.

Biochemical parameters in *Calendula* using colchicine (chemical mutagen)

Effect of different doses of colchicine on Chl-a, Chl-b content, phenolic compounds, flavonoid and

carotenoids are shown in **Table (2) and Fig. (5)**. The treatment with 1000 ppm dose of colchicine showed enhanced of Chl-a, Chl-b, carotenoids and flavonoids content compared to the control, but treatment with 3000 or 10000 ppm decreased the content of all these compounds except phenol content was higher at 10000 ppm then at 1000 ppm. **EI-Nashar and Ammar (2016)** found no significant differences in Chl-a and Chl-b contents by various concentration of colchicine and the control. On the other hand, **Robu et al (2012)** found increase in carotenoids, flavonoids and phenols by colchicine 400 ppm treatment.

Biochemical parameters in *Calendula* using EMS (Chemical mutagen)

Effect of different doses of EMS on Chl-a, Chl-b content, phenol compounds, flavonoid and carotenoids is shown in **Table (2) and Fig. (6)**. The treatment 10000 ppm dose of EMS showed enhance in Chl-a and Chl-b content compared to the control plants. Also, there was an increase in the carotene,

Table 1. Mean performance of the morphological parameters *Calendula officinalis* with gamma radiation and two chemical mutagenesis colchicine and EMS in M1 and M2 generations respectively

Treatment		Plant height (cm)		No. of leaves/plant		No. of flowers/plant		Flower diameter (cm)		
		M1	M2	M1	M2	M1	M2	M1	M2	
Gamma radiation	Control	25.71 ^{def}	49.33 ^{abcd}	52.6 ^{cdef}	37.6 ^{def}	11.0 ^{ab}	5.00 ^b	4.8 ^{abc}	5.1 ^{ab}	
	2500Gy	16.33 ^f	30.67 ^{cdef}	27.33 ^f	53.3 ^{cdef}	5.3 ^b	3.3 ^b	3.6 ^{abc}	3.5 ^{abc}	
	5000Gy	18.47 ^f	49.33 ^{abcd}	49.0 ^{cdef}	83.3 ^{bc}	8.6 ^{ab}	7.6 ^b	5.3 ^a	4.7 ^{abc}	
	7000Gy	20.35 ^{ef}	52.6 ^{abc}	31.6 ^{ef}	60.0 ^{bcdef}	6.3 ^b	4.3 ^b	4.3 ^{abc}	5.3 ^a	
Chemical mutagens	Colchicine	Control	49.0 ^{abcd}	47 ^{abcd}	70.0 ^{bcd}	37.3 ^{def}	3.0 ^b	2.0 ^b	4.1 ^{abc}	3.3 ^{bc}
		1000	62.0 ^{ab}	62 ^{ab}	94.0 ^{ab}	52.0 ^{cdef}	6.3 ^b	3.3 ^b	5.0 ^{ab}	4.0 ^{abc}
		3000	42.6 ^{bcd}	47 ^{abcd}	47.6 ^{cdef}	40.0 ^{def}	5.0 ^b	2.0 ^b	5.0 ^{ab}	3.0 ^c
		10000	48.0 ^{abcd}	50.6 ^{abc}	122.6 ^a	52.6 ^{cdef}	7.0 ^b	3.3 ^b	4.1 ^{abc}	3.3 ^{bc}
	EMS	Control	48.6 ^{abcd}	47 ^{abcd}	59.3 ^{cdef}	37.3 ^{def}	2.6 ^b	2.0 ^b	4.5 ^{abc}	3.3 ^{bc}
		1000	54.0 ^{abc}	61.6 ^{ab}	51.6 ^{cdef}	59.0 ^{cdef}	3.3 ^b	2.0 ^b	4.0 ^{abc}	3.7 ^{abc}
		3000	55.0 ^{abc}	60.6 ^{ab}	53.3 ^{cdef}	65.0 ^{bcde}	3.6 ^b	6.0 ^b	4.2 ^{abc}	3.5 ^{ab}
		10000	35.33 ^{cdef}	69.6 ^a	68.0 ^{bcd}	65.0 ^{bcde}	5.3 ^b	21.3 ^a	5.3 ^a	4.1 ^{abc}
Mean of season		39.6 ^b	52.3 ^a	60.6 ^a	53.5 ^a	5.6 ^a	5.1 ^a	4.54 ^a	3.93 ^b	

Letters (a, b, c, d, f) indicates different values- if the values has the same letter, it is not significantly different from each other with $p \leq 0.05$.

Table 2. Effect of gamma radiation and two chemical mutagenesis colchicine and EMS on the active compounds of *Calendula officinalis* in M2 generation.

Treatment		Chl-a µg/100gf	Chl-b µg/100gf	Carotenoids mg/100g	Flavonoid mg/100g	Phenol g/100g	
Gamma radiation	Control	6.843	2.9397	0.532	0.068	0.356	
	2500Gy	14.660	6.2937	0.832	0.222	0.249	
	5000Gy	18.740	8.6482	1.077	0.039	0.184	
	7000Gy	15.480	7.08316	0.507	0.020	0.261	
Chemical Mutagens	Colchicine	Control	0.797	0.776	0.122	0.068	0.122
		1000	1.150	1.913	3.781	0.096	0.147
		3000	0.242	0.096	0.576	0.012	0.114
		10000	0.789	0.633	2.341	0.039	0.159
	EMS	Control	0.797	0.776	0.123	0.068	0.123
		1000	0.562	0.096	0.569	0.094	0.129
		3000	0.515	0.744	3.180	0.105	0.172
		10000	0.805	0.977	0.413	0.100	0.149

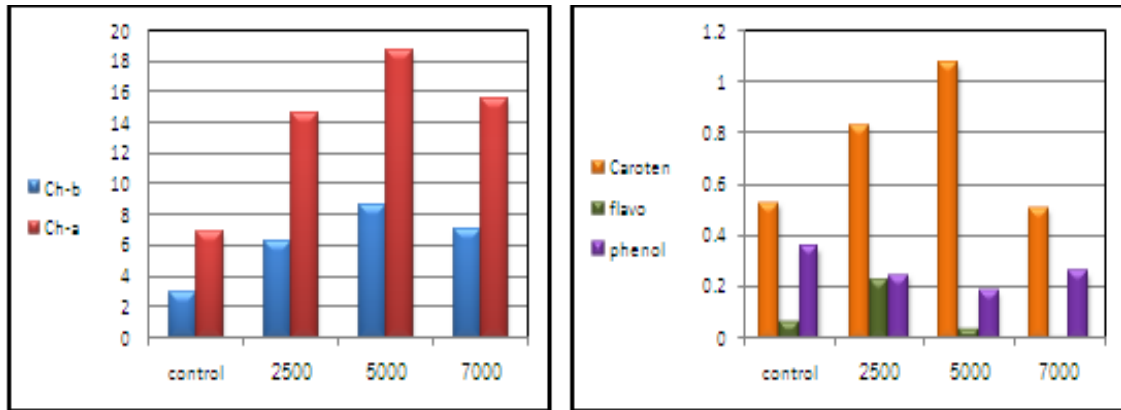


Fig. 4. The active compounds of *Calendula officinalis* extracts as affected by gamma irradiation in M2 generation

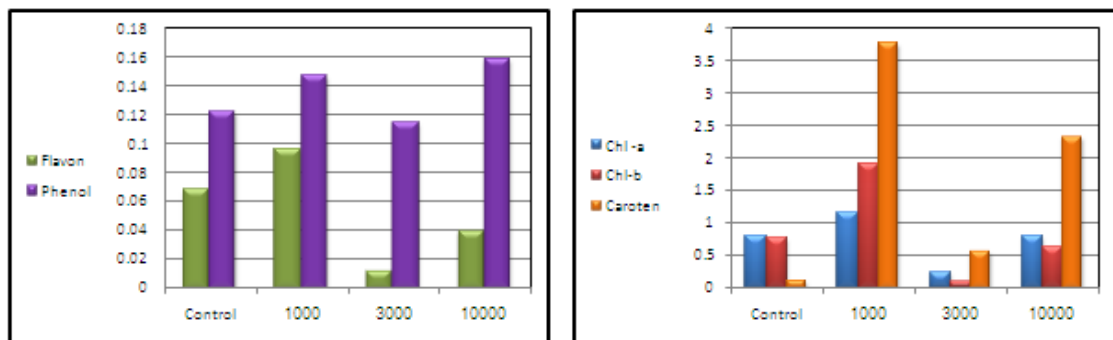


Fig. 5. The active compounds of *Calendula officinalis* extracts as affected by colchicine doses in M2 generation

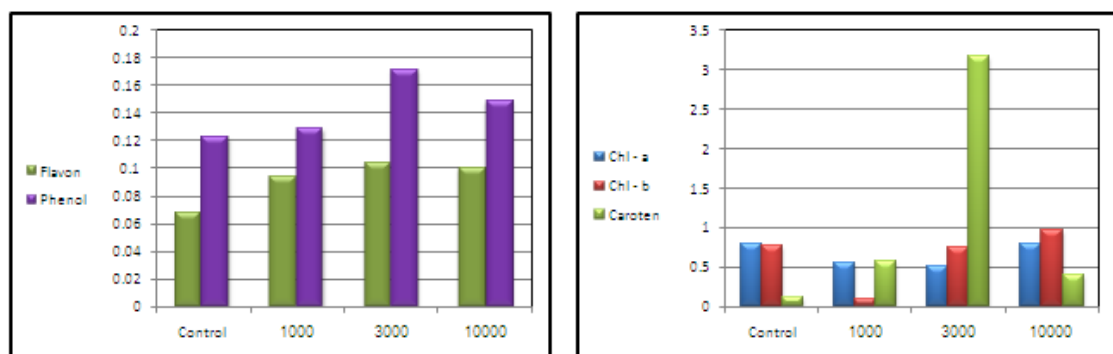


Fig. 6. The active compounds of *Calendula officinalis* extracts as affected by EMS doses in M2 generation

flavonoid and phenol contents with a dose of 3000 ppm of EMS compared to the control plants, whereas the 1000 or 10000 ppm doses caused decrease in their contents. **EI-Nashar and Asrar (2016)** found that high concentration of DES (5000 ppm) reduced the total chlorophyll in *Calendula*.

4. Molecular genetic analysis – water soluble protein content in *Calendula* Gamma irradiation experiment

In **M1 generation**, *Calendula* plants that has been treated with 2500 Gy dose showed the lowest band intensity in the SDS-protein PAGE which cause reduction of expression that correlate with the reduction of the plant height as well. While the highest SDS-protein reading was with the seeds treated with 7000 Gy dose as shown in **Fig. (7a)**.

As for **the M2 generation**, the 2500 Gy treatment remained the lowest in the SDS- protein which means there were no significant differences between M1 and M2 generation in this dose of radiation. Furthermore, the 5000 and 7000 Gy doses have the same effect on M2 generation and showed more stability comparing to the M1 generation as shown in **Fig. (7b)**.

Colchicine treatment

In **M1 generation**, *Calendula* plants that has been treated with 1000 and 3000 ppm of colchicine showed the highest in the SDS-protein which

caused increase in the expression, while the excessive dose of 10000 ppm of colchicine didn't cause any increase or decrease in the expression the same as the control as shown in **Fig. (8a)**.

As for **the M2 generation**, it seems that all colchicine doses (1000, 3000, and 10000 ppm) caused drop in the protein expression to its lowest compared to the untreated **Fig. (8b)**.

EMS treatment

In **M1 generation**, *Calendula* plants that were treated with 3000 ppm dose of EMS showed the highest in the SDS- protein as shown in **Fig. (9a)**.

Meanwhile, **in the M2 generation**, the SDS-protein decreased in all EMS doses comparing with the control untreated *Calendula* seeds and even than the M1 generation as shown in **Fig. (9b)**.

EI-Nasher and Asrar (2016) found that SDS-protein showed low content with SA and DES at 5000 ppm, while high content was at 1000, 2000 ppm of SA and DES, respectively. SA mutagen showed high average of SDS-protein content except at 4000 ppm of DES. **Soliman et al (2008)** studied the karyotype of *calendula* seeds. Eleven bands were detected, 4 bands are common in all types of seeds. The SDS-protein ranged from 2.95 to 8.43. At the same time, **EI-Hadary et al (2018)** showed a reduction in the quantitative level of protein biosynthesis as revealed by the band intensities with PEG treatments in soybeans genotypes.

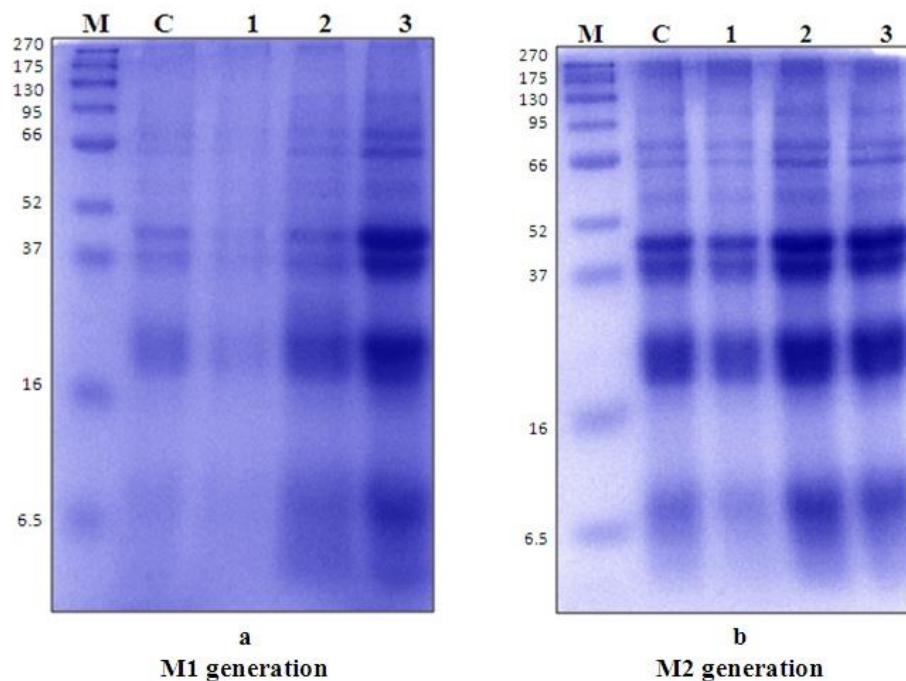


Fig. 7a and b. Polyacrylamide gel electrophoresis for the effect of gamma radiation on the SDS- protein in *Calendula* seeds, M= Marker, C= Control, 1= 2500 Gy, 2= 5000 Gy, 3= 7000 Gy.

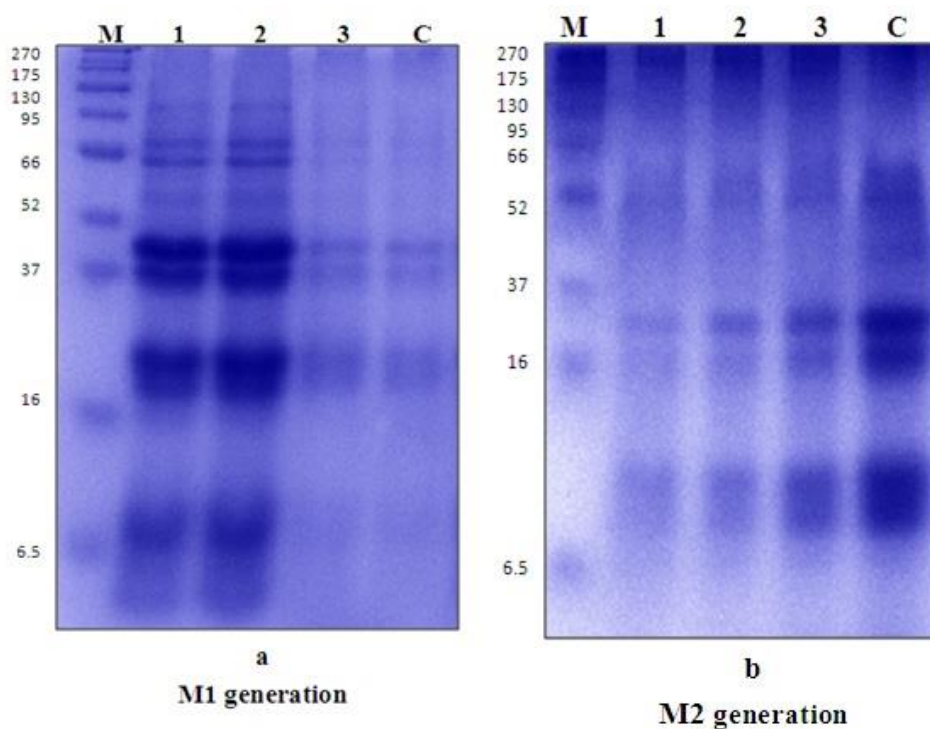


Fig. 8a and b. Polyacrylamide gel electrophoresis for the effect of colchicine on the SDS- protein in *Calendula* seeds, M= Marker, C= Control, 1= 1000 ppm, 2= 3000 ppm, 3= 10000 ppm.

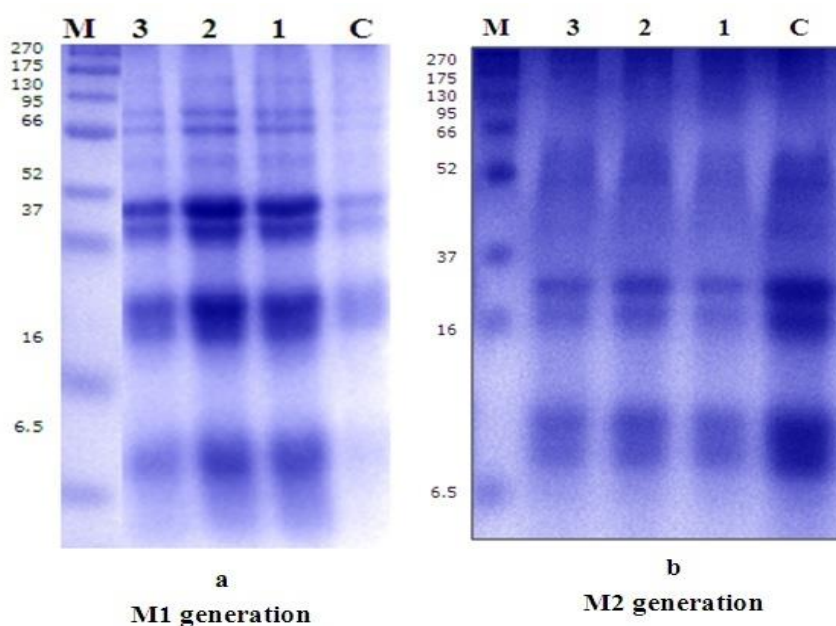


Fig. 9a and b. Polyacrylamide gel electrophoresis for the effect of EMS on the SDS- protein in *Calendula* seeds, M= Marker, C= Control, 1= 1000 ppm, 2= 3000 ppm, 3= 10000 ppm.

CONCLUSION

It was clear from this study that the use of gamma rays had a clear effect on the size of the plant, as the dose of 7000 Gy led to an increase in the length of the plant, and the dose 2500 Gy led to the small size of the plant from a short length and reducing the diameter of the flowers with increase in the carotenoids and flavonoid in a manner suitable for use as pot plants. The treatment with chemical mutagens was unique by changing the shape of flowers, so that the concentration of 3000 ppm of colchicine and 1000 and 3000 ppm of EMS led to the existence of mutations in the form of flowers, which were used to modify and renew the form of flowers and use them as cut flowers. These changes were stable and present in both M1 and M2.

Conflict of interest

The authors declare no conflict of interest.

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التحسين الوراثي لنبات الاقحوان من خلال إستحداث الطفرات بإستخدام الإشعاع والمطفرات الكيماوية

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الكيميائية تغييرات ملحوظة. أظهرت القياسات الحيوية ارتفاع محتوى كلوروفيل أ و ب والكاروتينات عند 5000 جرای، بينما زادت الفلافونويد عند 2500 جرای. وخفضت جميع جرعات أشعة جاما محتوى الفينولات. أدى تركيز الكولشيسين 1000 جزء في المليون إلى زيادة كل من كلوروفيل أ و ب والفلافونويدات والكاروتينات، وعند 10000 جزء في المليون من الكولشيسين زاد الفينول. زاد من كل من كلوروفيل أ و ب عند تركيز EMS (10000 جزء في المليون) بينما زادت الكاروتينات والفلافونويد والفينول عند 3000 جزء في المليون من EMS. أظهر تحليل البروتين تغييراً في التعبير الجيني فظهرت أعلى نسبة عند 7000 جرای ، 1000، 3000 جزء في المليون من الكولشيسين، EMS عند 3000 جزء في المليون. واتضح من هذه الدراسة أن أشعة جاما كان لها تأثيراً واضحاً على حجم النبات وزيادة محتوى الكاروتين والفلافونويد بما يناسب الإستخدام كنباتات أصص. وانفردت المعاملة بالمطفرات الكيماوية بوجود تحورات في شكل الأزهار مما يمكن من استخدامها كأزهار قطف. وقد تميزت هذه التغيرات بالثبات والاستمرارية خلال الجيلين الأول والثاني.

الكلمات المفتاحية: الاقحوان، الطفرات، أشعة جاما، EMS، الكولشيسين، تحليل البروتين كلوروفيل أ، كلوروفيل ب، الفلافونويد، الفينول، الكاروتينات.

الموجز

يعتبر نبات الأقحوان *Calendula* من نباتات الزينة الهامة فيستخدم كأزهار قطف وفي الحدائق. وملئته بالمواد الفعالة مثل الكلوروفيل أ و ب والمركبات الفينولية والفلافونيدات والكاروتينات. ركزت هذه الدراسة على إحداث طفرات في النبات للوصول إلى تغيير ملحوظ ودائم. تمت معاملة البذور باستخدام ثلاث جرعات من أشعة جاما 2500 ، 5000 ، 7000 جرای واثنين من المطفرات الكيماوية : الكولشيسين و EMS بتركيزات من 1000، 3000، 10000 جزء في المليون. أخذت قياسات مورفولوجية (طول النبات، عدد الأوراق للنبات، عدد الأزهار للنبات، قطر الأزهار، شكل الأزهار)، والمركبات الفعالة والبروتين على كلٍ من النباتات المعاملة وغير المعاملة. وأظهرت النتائج أعلى طول نبات عند جرعة 7000 جرای و EMS بتركيز 10000 جزء في المليون وتركيز الكولشيسين 1000 جزء في المليون. تم الحصول على أعلى عدد أوراق عند 5000 Gy و 10000 جزء في المليون من EMS وتركيز 1000 جزء من المليون من الكولشيسين. لم تتأثر عدد الأزهار / النبات بإشعاع جاما والكولشيسين ولكنه زاد عند تركيز 10000 جزء في المليون من EMS. لم يتأثر قطر الزهرة ب EMS بينما انخفض عند 2500 جرای و 3000 جزء في المليون من الكولشيسين. بالنسبة لشكل الزهرة أظهرت أشعة جاما والمطفرات

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